Complement activation in the context of stem cells and tissue repair

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Abstract
The complement pathway is best known for its role in immune surveillance and inflammation. However, its ability of opsonizing and removing not only pathogens, but also necrotic and apoptotic cells, is a phylogenetically ancient means of initiating tissue repair. The means and mechanisms of complement-mediated tissue repair are discussed in this review. There is increasing evidence that complement activation contributes to tissue repair at several levels. These range from the chemo-attraction of stem and progenitor cells to areas of complement activation, to increased survival of various cell types in the presence of split products of complement, and to the production of trophic factors by cells activated by the anaphylatoxins C3a and C5a. This repair aspect of complement biology has not found sufficient appreciation until recently. The following will examine this aspect of complement biology with an emphasis on the anaphylatoxins C3a and C5a.

Key words: Complement activation; Embryonic and adult stem cells; C3a; C5a; Tissue repair

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Core tip: This review article provides an overview over the scenarios, where complement activation contributes to tissue repair and regeneration through its effect on stem and progenitor cells, which is an area that needs further investigation.

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INTRODUCTION

Complement activation cascade
Complement is an effector system present in blood
consisting of about 30 soluble proteins and 15 cellular receptors. Although it has been known for over a century that complement is a participant in host immunity, it has recently become generally realized that complement is a contributor to a variety of non-immune functions inclusive of resolution of inflammation, clearance of apoptotic cells, angiogenesis, wound healing, stem cell recruitment and activation, as well as repair processes.

There are three routes of complement activation, the alternative pathway, the lectin pathway, and the classical pathway (Figure 1). All of these converge on the cleavage by complex enzymes referred to as C3 convertases of component C3 (Mr: about 195000) to C3a (Mr: about 85000) and C3b (Mr: about 185000). As a consequence of C3b deposition on C3 convertase, C5 convertase is created that acts similarly producing from component C5 (Mr: about 196000) the activation peptide C5a (Mr: about 11000) and C5b (Mr: about 185000). The anaphylatoxins, C3a and C5a, are important for elaboration of mechanisms of wound healing and regeneration. These small mediators are recognized by their cognate receptors: C3aR and C5aR/CSL2 that are GPCRs found on a diversity of cells inclusive of immune cells, endothelial cells, differentiated repair cells, and stem cells. In addition C3b and its split product iC3b, C3d are recognized by receptors inclusive of CR1-4 that assist clearance of microorganisms, cellular debris, immune complexes, and apoptotic cells. The ultimate outcome of complement activation is the formation of the MAC that is a transmembrane pore (100 Å) assembly that embeds in target cell membranes. In the absence of proximal phospholipid membranes, the terminal components of complement associate into complexes referred to as SC5b-9. These are probable heterogeneous and contain multiple copies of vitronectin and clusterin (apolipoprotein J). Because vitronectin in an oligomeric state can present the canonical tripeptide, Arg-Gly-Asp, to integrins on a variety of restorative cells, such as fibroblasts and keratinocytes, SC5b-9 may have a wound healing function. MAC: Membrane attack complex; MSC: Mesenchymal stem cells; HSC: Hematopoietic stem cells; PMNs: Polymorphonuclear cells.
immunoglobulins, the collectin, C1q, links to these, along with C1r and C1s to evoke the cleavage of C4 and C2 resulting in the assembly of a C3 convertase (C4b, C2a), which has the same composition as that formed by the lectin pathway[15].

Additional C3b deposition onto either the alternative or classical pathway C3 convertases changes these into C5 convertases (C3b, B6, P or C4b, C3b, C2a)[16,17]. These complex enzymes are now competent to process component C5 (Mr: about 196000) into C5a (Mr: about 11000) and C5b (Mr: about 185000)[18].

**Basic biology of C3a and C5a**
The small activation peptides, C3a and C5a, Figure 1 are in inflammation and germane to this review in wound healing and regeneration[19]. Both C3a and C5a, collectively referred to as anaphylatoxins, cause vasodilation, smooth muscle contraction, and increase vascular permeability[20-22]. Although C3a can be generated in greater abundance than C5a, the latter has greater specific inflammatory potential[21,23]. C5a especially is known for its ability to evoke chemotaxis of immune cells such as neutrophils and eosinophils[24,25]. Both C3a[22,26-28] and C5a[21,24,26,29-31] can stimulate an oxidant burst in granulocytes, but the response of these cells to C3a is considerably weaker and more transient than that to C5a[24,25,30,31]. In particular C3a fails to chemotactically attract circulating leukocytes in vivo[25]. Apart from the weak response of leukocytic C3aRs, the response to C3a in vivo would be expected to be limited largely to the interstitial space, since C3a is inactivated by serum carboxypeptidase N (CPN)[32].

The anaphylatoxins are recognized on target cells by G-protein coupled receptors (GPCRs)[33-35] coupled primarily to Gi. Unusually, C3aR has a long second extracellular loop that is important for binding C3a[33,34].

C5a is recognized by two distinct GPCRs, C5aR (CD88) and C5L2, but only the former is coupled to Gi proteins, whereas the latter is enigmatic because it is not connected to a signal transduction pathway, and its biological role has not been established[36]. Several investigations have assigned roles for C5L2 inclusive of an anti-inflammatory function[37] and as a decoy-scavenger receptor[38], but it has also been argued from studies using C5L2 knockout mice that this receptor is important for C5a-mediated signal transduction in neutrophils, macrophages and fibroblasts[39]. Thus the true biological roles of C5L2 to date are not established[40].

The receptors for the anaphylatoxins are not restricted to immune cells as C3aR and C5aR are found on a variety of non-immune cells[44]. These include differentiated cells that can be important for wound healing and regeneration: mast cells[45], benocytes[46,47], chondrocytes[48,49], synoviocytes[50], smooth muscle cells[51], endothelial cells[52-54], alveolar epithelial cells[55], mesangial cells[56,57], and regenerating hepatocytes[58]. In addition various stem and progenitor cells express the C3aR and C5aR[2,59-61] including HSC, mesenchymal stem cells (MSC)[61], NSC[2], and dental pulp progenitor cells[62]. Table 1 shows a list of the cell types that express C3aR and C5aR and their function.

**Late-acting components of the complement system**
While the C3b portion of C3 binds to the surface of pathogens leading to greater internalization by phagocytic cells, C5b, the remaining split product of C5, assembles with complement C6, C7, C8, and polymeric C9 to form the membrane-spanning membrane attack complex (MAC), which lyses bacteria, but which can also damage eukaryotic cells. Finally, a C3b cleavage product, iC3b can bind to the β2-integrins CR3 (CD11b/CD18) and CR4 (CD11c/CD18) on phagocytic cells facilitates the clearance of apoptotic cells.

There are multiple modalities which inhibit complement activation or the formation of the MAC; these include the plasma proteins factor H and C4b-binding protein and the membrane-anchored complement receptor 1 (CR1/CD35), membrane cofactor protein (CD46), decay accelerating factor or CD55, and MAC-inhibitory protein (CD59). As the plethora of inhibiting factors indicates complement activation has to be fine-tuned to provide optimal protection from infection without causing inflammatory tissue injury.

**Sites of complement synthesis and activation**
While complement proteins in the circulation are primarily produced by the liver except for the late acting complement components in particular C7 which are produced by monocytes/macrophages[63,64], it has become apparent that production and activation of complement proteins can happen in a localized fashion in many different parts of the body[65-68], and one would expect prolonged activation by the anaphylatoxins C3a and C5a under such conditions because of the absence of CPN in the interstitial space.

**Role of complement activation in inflammation**
The important role of complement in the defense against infection comes, however at a price: excessive complement activation plays a role in numerous disease processes ranging from ischemic reperfusion injury[69-71] to asthma[69], acute lung injury[72,73], glomerulonephritis[74], rheumatoid arthritis[75], Alzheimer's disease[76], multiple sclerosis and demyelination in general[77,79], and age-related and genetic macular degeneration[80-83]. In some instances the specific injurious complement pathway components have not been distinguished[75,76], in others C5a[69-71,77,80] or the MAC are the clear culprits[79].
Table 1 Cell types expressing the C3aR and the C5aR and their function

| Cells expressing C3aR | Function of C3aR | Cells expressing C5aR | Function of C5aR |
|-----------------------|-----------------|----------------------|-----------------|
| Neutrophils[39]       | Respiratory burst[23], bone marrow retention in vivo[19] | Neutrophils[28] | Respiratory burst[23], chemotaxis[28], enzyme release[20] |
| Eosinophils[44]       | Chemotaxis[61], in vivo but not in vivo[27] | Eosinophils[26] | Respiratory burst[27], chemotaxis |
| Monocytes/macrophages[51] | Chemotaxis[47], cytokine/chemokine production[14] | Monocytes/macrophages | Chemotaxis[61,62], cytokine/chemokine production[94] |
| Mast cell             | Mediator release[101], chemokine production[100], chemotaxis | Mast cell | Mediator release[100], chemokine production[94], chemotaxis[91,101] |
| Small fraction of lymphocytes[20,20,21] | Complex in vivo functions[52] | Small fraction of lymphocytes[20,20] | Complex in vivo functions |
| Osteoblasts[20,172,254] | Chemotaxis, accelerated osteogenesis, improved bone healing in vivo[174] | Osteoblasts[90,271] | Chemotaxis[90], accelerated osteogenesis[270], improved bone healing in vivo[274] |
| Chondrocytes[87]      | Osteogenic differentiation (?) | Chondrocytes[72] | Osteogenic differentiation (?) |
| Tenocytes[86]         | Not clear | Tenocytes[86] | Not clear |
| Smooth muscle cells[51] | Increased mediator release from mast cells[205] | Smooth muscle cells[181,81] | Not clear |
| Endothelial cells[52] | Transient ERK and rho activation[22], cytokine production[51] | Endothelial cells[52] | Chemotaxis[52], increased permeability[52], cytokine production[52], proliferation[126] |
| Hepatocytes[96]       | Protection from apoptosis[96], liver regeneration in vivo[52,94] | Hepatocytes[94] | Proliferation[94], protection from apoptosis liver regeneration in vivo[94] |
| Renal epithelial cells[204] | Chemokine production[205], EMT under stress conditions[206] | Renal epithelial cells | EMT under stress conditions[209] |
| Neurons[94]           | Protection from cell death[141,198], indirect neuroprotection[97], NGF expression[198] | Neurons[294] | Protection from cell death[141,145], Cytokine and NGF expression[18,82,26] |
| Astrocytes[202]       | Indirect neuroprotection[97], production of angiogenic factors[91] | Astrocytes[261] | Chemotaxis[91], protection from apoptosis[91], production of angiogenic factors[91] |
| MSC[20,86]            | Chemotaxis[91], protection from apoptosis[91], production of angiogenic factors[91] | MSC[91,92] | Chemotaxis[91], protection from apoptosis[91], production of angiogenic factors[91] |
| HSPC[52]              | Enhanced effects of SDF-1α[192], improved bone marrow engraftment[208,209] | Not expressed | Indirect: decreased mobilization[192]; direct: improved bone marrow engraftment[208,209] |
| CSPC[152]             | Chemotaxis[91], proliferation[159] | CSPC[152] | Chemotaxis[91], proliferation[159], cardiac dysfunction in C5/C5aR-/- mice[184] |
| NSPC[2]               | Increased neurogenesis[5], chemotaxis and differentiation[9] | NSPC[2] | Increased neurogenesis[5] |
| ESC                   | Not expressed | ESC | Prevents differentiation[94] |

ERK: Extracellular signal-regulated kinase; EMT: Epithelial-to-mesenchymal transformation; NGF: Nerve growth factor; MSC: Mesenchymal stem cells; SDF: Stromal-cell derived factor; HSPC: Hematopoietic stem and progenitor cells; CSPC: Cardiac stem and progenitor cells; NSPC: Neural stem and progenitor cells; ESC: Embryonic stem cells; MAC: Membrane attack complex.

for C3a was only seen in a mouse asthma model[22] and a mouse model of laser-induced macular degeneration, where the presence of the C3aR was associated with increased angiogenesis[90], which is detrimental in the retina, but which could support repair following ischemic insults in other tissues.

It should be noted here that C5a appears to be a major culprit responsible for most of the observed pathologies, and that specific C5/C5a inhibition preserving the early steps of complement activation could be highly advantageous in some circumstances.

ROLE OF COMPLEMENT ACTIVATION IN TISSUE REPAIR

Complement mediated inflammation leading to resolution and clearance

While the inflammatory aspect of complement activation has long been emphasized, it has been largely ignored that complement activation contributes also to resolution of inflammation and tissue repair with few reviews covering this aspect[4,84-86].

In particular, C3a has anti-inflammatory and regenerative effects[21,57,90-91]. In fact the regenerative potential of C3/C3a dates way back phylogenetically, as its expression is prominently up-regulated in mesenchymal cells in the regeneration zone in amphibians undergoing limb regeneration[92]. Furthermore, recent findings indicate that the C3aR on mesenchymal cells plays an important migration-directing role during early vertebrate development in zebrafish[93]. Neural crest cells mutually attract each other via C3a and the C3aR forming clusters of migratory mesenchymal cells. Such collective cell migration is a phenomenon crucial for morphogenesis. It remains to be seen, whether C3a and the C3aR play the same role during mammalian embryonic development.

While C5a also has regenerative effects for instance by its effects on the liver[88,99], neurons[88], osteoblasts[97], and dental pulp progenitors[82], these properties are often overshadowed by the strong inflammatory reaction caused by the activation of leukocytic C5a receptors,
which are involved in most of the pathologic conditions described above.

However, it should also be considered that inflammation itself constitutes a first step in wound healing. C3a and C5a can lead to an increase in vascular permeability[21,98], which is important for wound healing as it aids the flow of chemical and cellular entities necessary for repair and regeneration while facilitating waste removal[99].

Although swelling is traditionally seen as a characteristic of inflammation, edema is also necessary for the resolution of inflammation and restoration of functional tissue because an increase in vascular permeability facilitates entry of repair and restorative cells. Specific to this theme is the function of histamine. C3a and C5a both are chemotactic for mast cells and both are inducers from these cells of histamine release[100-102]. Histamine due to its potent vasodilatation activity can induce swelling, but histamine is also required for skin wound healing as demonstrated using Kit mutant mice that are mast cell deficient. These animals are unable to secrete mast cell derived histamine, and the animals were found to have a defective response to cutaneous wound healing[103].

The increase in vascular permeability facilitates the recruitment of monocytes that can respond to C5a mediated chemotaxis gradients[104], and these cells are crucial for “cleanup” functions. Today it is understood that clearance of debris and apoptotic cells is an important activity necessary for subsequent wound healing, and complement along with pentraxins have been shown to participate in this activity[105,106]. Indeed the clearance function was probably the original function of the complement system dating all the way back to metazoans[107].

The collects C1q and MBL are important for enhanced phagocytosis by monocytes and macrophages of modified lipoprotein complexes, immune complexes, and apoptotic cells[108-111]. Apoptotic cells present exteriorized phosphatidyl serine that can be recognized at an early stage by the lectin domains of members of the collectin family[112-114]. Apoptotic cells, debris or immune complexes tagged by C1q or MBL are identified by monocytes and macrophages bearing CD91 that can be in complex with a collectin receptor, calreticulin[115-117]. The facilitated uptake of these “disposables” has been referred to as macropinocytosis[118].

In addition to recognition of pathogens, debris and dead cells by members of the collectin family, fragments of C3 are important for clearance functions. C3b is susceptible to processing by Factor H and I to iC3b that can be cleaved further into C3d and C3c[119]. C3 fragments are recognized by receptors such as CR1 (CD35), CR2 (CD21), CR3 (CD11b/CD18), CR4 (CD11c/CD18), and CR1G found on Kupffer cells, monocytes and macrophages, which are immune adherence receptors that facilitate removal of opsonized microorganisms, immune complexes and apoptotic cells[120,121].

Complement and angiogenesis

The importance of angiogenesis in wound healing and regeneration has been clearly understood[122]. The process has been categorized in three continuous overlapping phases: inflammatory, proliferative, and remodeling[122].

Some aspects of participation in inflammation inclusive of increase in vascular permeability induced by C3a and C5a have already been discussed, but these mediators have additional functions that indirectly support angiogenesis. C5a but not C3a has been shown to induce an upregulation of gene expression on endothelial cells for adhesion molecules E-selectin, ICAM-1, and VCAM-1[123,124]; the upregulation of these adhesion molecules facilitates extravasation of immune cells inclusive of monocytes that are important for debridement, remodeling and angiogenic mediator secretion[125]. Angiogenesis requires restructuring of the extracellular matrix by controlled proteolysis, and the anaphylatoxins were reported to increase the levels of MMP-1 and MMP-9 in monocytes[126] and to be secretagogues of MMP-9 from granulocytes[127].

Both C3aR and C5aR are found on cultured endothelial cells, but these mediators use different signal transduction pathways and the response to C3a is more transient[127]. Both the anaphylatoxins up-regulates chemokine production in endothelial cells[53], but only C5a is chemotactic for human umbilical vein endothelial cells (HUVECs)[52] and microvascular endothelial cells[54]. Moreover, it was reported that C5a could induce not only migration of cultured microvascular endothelial cells but proliferation and ring formation as well[128].

C3a and C5a were found to increase vascular endothelial cell growth factor (VEGF) in human culture retinal pigment epithelial cells, and when the anaphylatoxins were injected intravitreously into normal mice, an increase in VEGF within the retinal pigment epithelial-choroid layer of the retina was observed[80]. Others found that C5a but not C3a induced VEGF synthesis and secretion from a retinal pigment epithelial cell line[129]. Furthermore, both C3a and C5a were reported to induce production and secretion of VEGF from MSC[91]. Although there is no in vitro evidence that C3a and C5a are directly angiogenic, they have been shown to be angiogenic in in vivo situations[80,130,131], perhaps in response to angiogenic factors that the anaphylatoxins induce in cells in the proximity as just described.

In summary, C3a and C5a can contribute to the inflammatory and proliferative phases of angiogenesis, and thus the anaphylatoxins can be viewed as factors with indirect angiogenic potential; however, it is necessary to mention that one publication is in apparent contradiction to this view, namely investigators studying experimental retinal neovascularization published that C5a is anti-angiogenic[132]; however, these investigators were examining murine models of retinopathy of prematurity and hypoxia induced retinal vascularization, and these observations though correct may not be of a
general nature.

**ROLE OF COMPLEMENT ACTIVATION IN SPECIFIC REPAIR PROCESSES**

*Role of complement activation in liver regeneration*

Although tissue regeneration is very limited in mammals, the mammalian liver has retained an amazing capacity for regeneration following viral infection, exposure to toxins or surgical resection. This regeneration can occur at the hepatocyte level in cases of acute liver injury, although liver stem and progenitor cells appear to contribute in more chronic conditions.

The complement activation products C3a and C5a play an essential role in regeneration of the liver parenchyma\[^{87,95}\]. After experimental CCl\textsubscript{4} induced liver toxicity or partial hepatectomy, mice deficient in C3 or C5 exhibited defective regeneration and a higher frequency of mortality\[^{87}\]. Furthermore, C5a was demonstrated to be a growth factor for regenerating hepatocytes, and blockade of the C5aR in experimental liver regeneration experiments resulted in the inability of hepatocytes to proliferate leading to defective liver restoration\[^{50,94}\].

However, the role of complement activation is a double-sided sword in hepatic regeneration and the MAC was found to be the principle mediator of hepatic ischemia reperfusion injury\[^{133}\], which creates a dilemma, since the early components of complement activation, C3a, and C5a are necessary for liver regeneration. However, targeted inhibition of MAC formation with CR2-CD59 significantly improved survival after partial hepatectomy in mice\[^{133}\], while retaining the benefit of complement activation and anaphylatoxin production.

**EFFECTS OF COMPLEMENT ACTIVATION ON MSC AND OTHER MESENCHYMA L CELLS**

*MSC and tissue repair*

MSC are rare, often perivascular cells found in all tissues that are able to differentiate into all types of connective tissue lineages including osteoblasts, adipocytes and chondrocytes. Furthermore, these cells produce a variety of angiogenic and trophic factors\[^{134,135}\] and possess anti-inflammatory properties\[^{136-138}\]. Owing to the immune-evasive properties of MSC, allogeneic MSC transplantation is generally accepted. Because of all these properties MSC have started to find clinical application in a variety of diseases ranging from myocardial infarction\[^{139}\] to graft vs host disease\[^{140}\] and have found attention in the context of acute lung injury\[^{141}\].

*Limitations of MSC therapies as used today*

However, in the rush to the clinic, survival of the transplanted MSC has not been sufficiently considered, and there have been failed clinical trials using MSC - in spite of promising results in animal models\[^{142-146}\], and the full regenerative potential of these cells has not been harnessed due to poor tissue homing and limited cell survival following transplantation. Successful clinical trials will require additional information about the mechanisms by which MSC repair injured tissues, about the optimal route of administration, and about means of increasing their survival at a site of tissue injury. It is surprising, how little there is known about MSC recruitment and survival in vivo for a cell type that is being investigated in numerous clinical trials. Various means of improving MSC homing\[^{147}\], growth factor production\[^{148,149}\] and survival\[^{150}\] are being pursued as ways to improve the therapeutic efficacy of MSC, but usually different means are used to achieve each one of these goals. It is hypothesized here that C3a can improve all of these functions, since we postulate that the C3a-dependent regenerative capacity of MSC seen in amphibians\[^{92}\] has been preserved in mammalian tissue repair.

*MSC and complement activation*

Although MSC have various anti-inflammatory and immune-evasive properties\[^{151}\] - including the ability to inhibit the proliferation of allogeneic T cells, low levels of expression of MHC class I and II proteins, the ability to convert inflammatory M1-type macrophages to repair-type M2 macrophages, and secretion of the complement-inhibitory factor H\[^{152}\] - they are not fully protected from complement induced injury themselves, and complement activation appears to be involved in the demise of MSC following allogeneic transplantation\[^{151,153}\]. One would wish that such basic complement biology had been considered before using allogeneic MSC in clinical trials. Incubation of MSC with complement active human plasma resulted in the deposition of C3c and iC3b on the cell surface of the MSC and C3a and soluble C5b-9 detection in the supernatant\[^{150}\], indicative of complement activation, which could be prevented by various means of complement inhibition.

In addition, MSC as well as osteoblasts express components of the complement cascade themselves\[^{154}\] including C3, C5\[^{155}\], the C3aR and C5aR\[^{161}\] and the cell surface complement regulators CD46, CD55, and CD59\[^{155}\]. Furthermore, MSC engineered to up-regulate CD46, CD55, and CD59 protected these cells from complement-mediated cell lysis in vitro and in vivo\[^{156}\].

*Effect of C3a and C5a on MSCs*

MSC show tropism for areas of tissue damage\[^{157,158}\], but it is controversial which chemotactic factors are responsible for this. In leukocytes a large degree of cell recruitment to an area of tissue injury depends on chemokines and C5a, but the role of chemokines in trafficking of MSC is unclear with widely contradictory findings\[^{158-162}\]. Since MSC are chemo-attracted by C3a and C5a in vitro\[^{61}\], we hypothesize that complement activation is an important player in attracting MSC to an area of tissue damage in vivo. C3a and C5a can be locally generated at the surface of MSC which contact serum\[^{90}\].

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Role of complement activation on bone formation

Consistent with the role of complement activation during limb regeneration in amphibians\cite{172}, described above, it has been suggested some time ago that complement activation may be important in cartilage-bone transformation during fracture healing and that the alternate complement activation pathway may be involved\cite{172}. Like their MSC precursors, osteoblasts are able to express the key complement proteins C3 and C5\cite{173} and express the C3aR and C5aR, which both mediate osteoblast migration\cite{173}. Expression of the C5aR was highly up-regulated during osteogenic differentiation\cite{173}, but later during osteoblast to osteocyte differentiation complement genes were greatly down-regulated\cite{173}.

Although osteogenic differentiation of MSC can occur in the absence of C3a or C5a, it is accelerated in the presence of C3a or C5a in a C3aR and C5aR-specific fashion as shown with receptor-specific inhibitors in Figure 2A: After two weeks of osteogenic differentiation in the presence of fetal calf serum (FCS) that was not heat-inactivated, i.e., complement proteins had not been inactivated, Alizarin red staining of calcium salt deposits indicated moderate staining in FCS, which was significantly augmented, when C3a or C5a had been added to the media. However, by 3 wk the difference between these groups was largely diminished (results not shown). If heat-inactivated FCS (FCS) replaced the FCS, osteogenic differentiation was still further delayed but the addition of C3 or C5 partially substituted for the presence of serum complement components (Figure 2B) indicating that the differentiating cells themselves must have provided the necessary complement components.

Consistent with these in vitro findings, delayed fracture healing was observed in C3 or C5-deficient mice which received a standardized femur osteotomy\cite{174,175}, C5-deficiency also resulted in poor quality bone\cite{174}, indicating that complement activation plays an important role in fracture healing. However, under chronic conditions the osteogenic effect of complement activation is a double-edged sword, because it can also result in vascular calcification during the atherosclerotic process, where MSC-derived C5aR participation has been shown recently\cite{175}.

Role of complement activation in cardiac repair

Following cardiac infarction extensive necrosis of ischemic cardiomyocytes activates complement. The ensuing infiltration of the infarct zone with neutrophils and monocytes serves to clear the injured site from dead cells and debris, and initiates reparative pathways.

However, there is little doubt that complement activation plays an injurious role in the acute phase of myocardial infarction mostly in the context of C5a-mediated reperfusion injury and neutrophil influx\cite{176-178} but clinical trials inhibiting at the level of C5 have been unsuccessful\cite{179} indicating that even in this early phase, complement activation is not all deleterious.
Furthermore, even C5a appears to be protective in several models of cardiac hypertrophy, where C5/C5aR knockout mice fared worse than wild type mice. The beneficial effect of complement activation becomes more apparent in the more chronic situation, where complement activation contributes to tissue repair. C3-deficiency in C3 knockout mice exacerbated myocardial dysfunction four weeks after coronary artery ligation showing more scar tissue, and decreased cardiac stem/progenitor cells (CSPC) in the infarct zone. Both murine and human CSPC express C3aR and C5aR, are chemo-attracted by C3a...
and C5a, and show greater proliferation in the presence of the anaphylatoxins\(^{182}\). It remains to be seen, whether they also produce more angiogenic factors as described above for MSC stimulated with C3a or C5a, which would be a further advantage in the context of cardiac repair. In CSPC C3a or C5a also induced several genes associated with - unwanted - myofibroblast differentiation \textit{in vitro} \(^{182}\), but it remains to be seen, whether this is relevant \textit{in vivo}.

**Effect of complement activation on HSC**

Like any tissue damage, myeloablation by radiation or chemotherapy activates complement resulting in the generation of the complement activation peptides C3a and C5a \(^{59,60}\). Following bone marrow transplantation fast and efficient homing to and engraftment in the bone marrow is important. In this scenario SDF-1 is the most important chemotactic factor, which chemo-attracts hematopoietic stem and progenitor cells (HSPC) to the bone marrow and retains them there through the CXC chemokine receptor 4 on these cells \(^{183,184}\).

While HSPC express the C3αR, C3α itself does not appear to be a direct chemo-attractant, but it augments the chemotactic responsiveness of HSPC to gradients of SDF-1 as well as to sphingosine-1-phosphat and ceramide-1-phosphate \(^{59,60,185,186}\). \textit{In vivo}, mice deficient in complement C3 exhibited delayed engraftment of HSPC \(^{96}\). This effect was specifically mediated by the C3αR as shown when HSPC from C3αR-/- mice were injected into irradiated wild type mice, which resulted in a significant delay in recovery of leukocytes and platelets and decreased committed progenitors in the bone marrow \(^{187}\). Similarly, engraftment of human CD34\(^+\) cells treated with a C3αR inhibitor showed impeded engraftment in nonobese diabetic/severe combined immune deficiency mice \(^{187}\).

C3α also contributes to the retention of HSPC in the bone-marrow as C3α-/- or C3αR-/- mice showed accelerated mobilization of HSPC into the peripheral blood following administration of granulocyte colony-stimulating factor (G-CSF) \(^{188}\). This retention mechanism is not limited to HSPC, but also applies to their neutrophil progeny, and indeed the C3αR protects from ischemic intestinal injury due to reduced neutrophil mobilization, and increased neutrophil accumulation causes exacerbated injury in C3αR deficient mice \(^{189}\). Indeed, decreased neutrophil mobilization in wild type vs C3αR-/- mice may explain the increased mortality observed in C3αR-/- mice in an endotoxin shock model \(^{190}\), although the mechanism was not reported for this model.

C5-deficient mice also exhibited impaired HSPC engraftment: In this scenario the role of C5 cleavage leading to the formation of soluble MAC resulted in increased adhesion of HSPC to bone marrow stromal cells and augmented secretion of SDF-1 by the bone marrow stroma \(^{191}\). However, HSPC do not express the C5αR themselves, and C5 deficient mice show reduced HSPC mobilization following the administration of G-CSF \(^{192}\), which causes complement activation. Apparently, granulocytes, which are released into the circulation in response to C5α formation, pave the way for HSPC to egress from the bone marrow perhaps due to MMP9 release, which facilitates HSPC mobilization \(^{192}\).

**Effect of complement activation on neurons, neural stem and progenitor cells**

It has been known for some time that neurons express both C3αR \(^{193}\) and C5αR \(^{194}\), and that these two receptors protect from neural cell death \(^{190-195}\). This protective effect is not limited to differentiated neurons, but already functions in neural stem and progenitor cells, which express both C3αR and C5αR. C3α-deficient mice showed deficits in both basal and ischemia-induced neurogenesis \(^{2}\), and C3αR expression was essential for basal neurogenesis \(^{2}\), while C5αR expression made no difference in this respect \(^{196}\). Consistent with these results, C3α protected from ischemic insult-induced memory impairment in neonatal mice \(^{197}\).

\textit{In vitro}, C3α could induce neuronal differentiation of neural progenitor cells \(^{99}\), and increased the chemotactic response to low concentrations of SDF-1 \(^{99}\) similar to the situation with HSPC. In addition, C3α protected from NMDA neurotoxicity, but only in the presence of astrocytes \(^{198}\), which suggests that C3α-stimulated astrocytes, which express the C3αR \(^{199}\), were the primary target, and that they in turn protected through the production of NGF and other neurotrophic factors \(^{200}\). However, in a mouse model of ischemic reperfusion injury, C3αR inhibition had the opposite effect resulting in increased neuroprogenitor proliferation and suppressed T cell infiltration \(^{201}\). The reasons for such opposing results are not clear; although it is possible that the last model includes a larger inflammatory response that may cancel out any direct effect of C3α on neuronal progenitors and/or astrocytes. Specific pathways by which complement activation protect neural stem and progenitor cells await further elucidation.

Interestingly complement C1q, - in the absence of other components of the complement cascade - increased neuron viability and neurite outgrowth and prevented \(\alpha\)-amyloid-induced neuronal death \textit{in vitro} \(^{202}\) and \textit{in vivo} \(^{203}\). Neuroprotection was promoted by activation of the transcription factor cAMP responsive element binding protein and by increasing LRP1B and GPR6 expression \(^{203}\). Furthermore, in retinal neurons, TGF-β signaling regulates C1q expression, which in turn is necessary for synaptic pruning \(^{204}\). Indeed, complement activation plays a role during a process called synaptic elimination in new-born mice \(^{205}\), where either C1q or C3 deficiency resulted in failure of synaptic elimination \(^{205}\), implying the classical complement cascade in this process. Interestingly, C1q/-/- mice presented with signs of epilepsy due to increased excitatory synaptic connectivity \(^{206}\).
Complement involvement during embryonic development

ESC only express a limited number of proteins of the complement cascade including C6, C7, C8, C9, factor I, H, properdin/factor D, and complement component 1r, s and q receptor, and beta polypeptide\(^{207,208}\). However, a recent report indicates that they may also express C5 and the C5a receptor\(^{168}\) and more importantly that C5a promotes survival and maintenance of the pluripotent state of ESC in the absence of bFGF\(^{168}\), the standard addition to maintain human ESC in the undifferentiated state. While this report awaits further validation, it highly suggests that complement activation presumptively with the support of maternal complement components plays a role in embryonic development from the very beginning.

It is known that the maternal complement system plays a crucial role starting early on during fetal development and that it is essential for the maintenance of fetomaternal tolerance. In mice Cr1l/Crry (complement regulatory protein) deficiency is embryonically lethal, but the embryos are rescued in C3/-/- mothers\(^{209}\). Indeed, ESC are more susceptible to complement mediated cell lysis than differentiated cells, and this pathway may contribute protection from teratocarcinoma formation during pregnancy\(^{210}\). Complement activation has, however, to be finely regulated during pregnancy, since excessive activation of this pathway in later pregnancy is associated with miscarriage\(^{211}\) and preeclampsia\(^{212-215}\).

There is limited knowledge about the role of complement in early vertebrate development with much of the information derived from lower vertebrates. While further investigation using mammalian models is surely required, the existence of these complement pathways during amphibian development indicates that complement activation is a phylogenetically preserved ancient process during embryogenesis. In xenopus complement components are extensively expressed during development starting during the gastrula/early neurula stage\(^{216}\) with organ-specific expression patterns during early organogenesis. C1q,A, C3 and C9 are strongly expressed in the early neural plate, while C1qR and C6 are expressed at the periphery of the neural plate presumably in the neural crest\(^{216,217}\) preceding the development of hematopoiesis. At this point C3 and C3aR show a predominantly mesodermal expression. Interestingly, neural crest cells, a multipotent embryonic cell population undergo epithelial to mesenchymal transition (EMT) in xenopus and zebrafish in a fashion reminiscent of metastasizing cancer cells and it is following this EMT transition that they express both C3 and the C3aR\(^{93}\). These cells form cohesive clusters of migrating cells that are co-attracted via C3a and the C3aR and this process is necessary for collective migration of these cells\(^{93}\) suggesting that C3aR/C3a contribute to the intricate mass cell movements of the developing embryo.

In rats C3 derived from the visceral yolk sac is an embryotrophic factor between days 9.5 to 11.5 post conception\(^{218}\), - however no further details have been elucidated.

Evidence for a role of C3a in fetal tissue regeneration comes from studies on embryonic chick retina regeneration. In this model C3a can induce complete regeneration of the ablated chick retina from stem/progenitor cells via Stat3 mediated up-regulation of IL-6, IL-8, and TNF-α\(^{219}\). However, there was an optimal concentration of C3a that induced regeneration, while very high concentrations caused apoptosis, indicating that fine-tuning of the C3a/C3aR axis is necessary, perhaps not surprising since the cytokines produced by C3a stimulation may serve as growth factors at low concentrations, but become highly inflammatory at higher concentrations.

Beyond the early effect of C5a stimulation on ESCs mentioned above, C5a and the C5aR play a continued important role during mammalian development: They are both expressed during the period of neuralulation in mice and humans\(^{220}\), and while C5aR knockout mice show no congenital defects under normal pregnancy conditions, they present with a wide variety of congenital malfunctions due to neural tube defects ranging from anencephaly to scoliosis and anophthalmia, if the mothers are folate deficient\(^{220}\).

Soluble complement C5b to 9: Possible roles for vitronectin and clusterin in wound healing and stem cell biology

Complement evolved to destroy microorganisms, and one effector outcome of complement activation is the assembly within target cell membranes of a multiprotein complex referred to as the MAC. This consists of one molecule each of C5b, C6, C7, C8 and multiple copies of C9 (6 or more). In its complete form the MAC creates a transmembrane pore of 100 Å that destroys the functional integrity of cellular membranes\(^{221,222}\).

In the absence of proximal phospholipid membranes the terminal components of complement form a soluble complex referred to as Soluble complement C5b to 9 (SC5b-9), which was initially described as having a composition of one molecule each of C5b through C8 and three units of C9 and vitronectin\(^{223}\). Later it was also shown to contain clusterin (apolipoprotein J)\(^{224}\), which is known to be a component of a subclass of high-density lipo-protein (HDL) particles\(^{225}\). Although the term “SC5b-9”, as originally conceived designated a soluble form of the terminal complement complexes, it is probable that these assemblies are heterogeneous with some containing vitronectin and others clusterin presumably associated with HDLs. Whether heterogeneous or not, indications exist that these macromolecular composites may be adaptive for recovery from injury.

Vitronectin, a known matrix and adhesive protein, circulates in human plasma in an inactive state in which its heparin linkage region and integrin binding site, containing the canonical Arg-Gly-Asp sequence, are buried\(^{226,227}\); however, as a consequence of
oligomerization and conformational change these regions on the protein can interact with glycosaminoglycans (GAG) and integrins\(^{228,229}\). GAGs are a fundamental constituent of the extracellular matrix that will necessarily become exposed upon tissue damage. Furthermore, vitronectin binding integrins, \(\alpha_\text{IIb}\beta_3\) and \(\alpha_\text{IIb}\beta_1\), are found on a variety of cells responsive to injury inclusive of platelets, fibroblasts, myoblasts, vascular smooth muscle cells, and endothelial cells\(^{220-223}\).

Thus incorporation of plasma derived vitronectin into damaged ECM can be seen as a beneficial response that facilitates wound healing because this arrangement can help dock and anchor restorative cells. Furthermore, because vitronectin is known to bind growth factors such as insulin like growth factor\(^{223}\), it may be speculated that vitronectin in context of SCSb-9 could deliver the growth mediators to a wound site.

It is also conceivable that complexes of SCSb-9 containing clusterin may also contribute to host recovery from injury. Clusterin is found in HDL containing apolipoprotein A-I but not apolipoprotein A-II\(^{222,234-237}\). HDL particles are highly heterogeneous, and whereas HDLs were originally ascribed to function for reverse cholesterol transport, it is now realized that these operate for a diversity of biological roles inclusive of transport of hormones and bioactive lipids, inflammation regulation, clearance, and immune defense against parasites and microorganisms\(^{238-241}\).

Although investigations about the interface of HDLs and stem/progenitor cell biology are just commencing, a few publications suggest that this will be a fruitful topic for future research. For example HDL can promote MSC proliferation by interaction with Scavenger receptor class B member 1\(^{242}\). Also HDL have been shown to advance endothelial cell precursor migration and proliferation\(^{243}\).

We leave it an open question as to whether HDL-associated SCSb-9 can facilitate wound healing through influence on stem and progenitor cells.

CONCLUSION

Although complement is best known for its role in inflammation, increasing evidence has accumulated that emphasizes that complement activation and in particular the complement split products C3a and C5a play a role in many scenarios of tissue repair. Table 1 shows a compilation of cell types expressing C3aR and C5aR and the function of these receptors on any particular cell. However there are still many gaps in our understanding of the role of complement activation outside the inflammatory axis. A more complete understanding of the effects of complement activation in stem cell biology will contribute to improve the therapeutic potential of these cells.

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