

## Complement in basic processes of the cell

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### ABSTRACT

The complement system is reemerging in the last few years not only as key element of innate immunity against pathogens, but also as a main regulator of local adaptive responses, affecting dendritic cells as well as T and B lymphocytes. We review data showing that leucocytes are capable of significant autocrine synthesis of complement proteins, and express a large range of complement receptors, which in turn regulate their differentiation and effector functions while cross talking with other innate receptors such as Toll-like receptors. Other unconventional roles of complement proteins are reviewed, including their impact in non-leukocytes and their intracellular cleavage by vesicular proteases, which generate critical cues required for T cell function. Thus, leucocytes are very much aware of complement-derived information, both extracellular and intracellular, to elaborate their responses, offering rich avenues for therapeutic intervention and new hypothesis for conserved major histocompatibility complex complotypes.

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### 1. Introduction

The complement system classically exerts three main activities: host innate defense against infection – through antigen opsonization, driving chemotaxis and leukocyte activation and lysing bacteria and cells –, bridging innate and adaptive immunity – by augmenting antibody responses and enhancing immunologic memory –, and disposing of immune complexes and inflammatory products – through the clearance of tissue immune complexes and apoptotic cells – (Walport, 2001). In the last few years, however, complement has re-emerged as a key regulator of cellular and immunological responses, both innate and adaptive, by pro-

viding costimulation and proliferation signals. Such signals act extracellularly through specific cell receptors of classical fragments (Table 1), some still undefined, but also intracellularly after new cleavage schemes and proteases through specific membrane receptors that probe vesicular structures. Some of those vesicles contain pathogens and thus complement has clear defensive roles, but other vesicles are sterile and likely involved rather in leukocyte regulation. The recent success of complement-targeted therapeutics such as anti-C5 for transplant rejection and hemolytic uremic syndrome or anti-factor I for age-related macular degeneration have fueled heightened interest in the complement system among scientists, clinicians and the pharmaceutical industry. As new unconventional roles may suggest new therapies, we summarize unexpected contributions of hepatic and extrahepatic extracellular and intracellular complement soluble proteins, fragments and receptors to physiopathology of a wide range of cells and tissues.

### 2. Hepatic versus extrahepatic complement

In mammals most complement proteins are of hepatic origin under normal conditions, with some exceptions such as C1q, C7, properdin and factor D (Morgan and Gasque, 1997), which are predominantly produced by myeloid cells, notably monocytes and macrophages (or adipocytes for factor D, also called adipsin). Under inflammatory conditions, however, both hepatic and extrahepatic production are enhanced (Laufer et al., 2001), and extrahepatic sources may become critical for local protection (or damage), particularly for larger proteins such as C1q (400 kD) or for those that are rapidly cleaved such as C3. For instance, C3 from

**Abbreviations:** AM, adrenomedullin; AMD, age-related macular degeneration; APC, antigen-presenting cells; BCR, B-cell receptor; bm, bone marrow cells; bmDC, bone marrow dendritic cells; cDC, conventional dendritic cells; CLR, calcitonin receptor-like receptor; CR1, complement receptor 1; CR2, complement receptor 2; CR3, complement receptor 3; CR4, complement receptor 4; DC, dendritic cells; eC3, extracellular C3; FDC, follicular dendritic cells; FH, factor H; GM-CSF, granulocyte macrophage colony-stimulating factor; iC3, intracellular C3; iC5, intracellular C5; IFN- $\gamma$ , interferon- $\gamma$ ; IL-12, interleukin 12; IL-2, interleukin 2; IL-4, interleukin 4; KD, knock-down; KO, knock-out; LPS, lipopolysaccharide; MCP, membrane cofactor protein; MHC, major histocompatibility complex; Mo, monocytes; moDC, monocyte-derived dendritic cells; NLRP3, nucleotide-binding domain leucine-rich repeat family pyrin domain containing 3; pDC, plasmacytoid dendritic cells; RAMP2, receptor activity-modifying protein 2; TCR, T-cell receptor; TLR, Toll-like receptors.

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**Table 1**

Surface complement receptor expression in B or T cells, monocytes, monocyte-derived dendritic cells and follicular dendritic cells.

Receptor	Alternative names	Ligand					Cell type					
		C3a	C3b	iC3b	C3dg	C3c	C5a	B	T	Mo	moDC	FDC
CR1	CD35	–	+	+	–	–	–	+	+	+	+	+
CR2	CD21	–	–	+	+	–	–	+	+	–	–	+
CR3	CD11b/CD18; $\alpha_M\beta_2$ ; Mac-1	–	–	+	–	–	–	+	+	+	+	+
CR4	CD11c/CD18; $\alpha_X\beta_2$ ; p150/95	–	–	+	–	–	–	+	+	+	+	+
CR1g	Z931g; VSIG4	–	+	+	–	+	–	–	–	+	+	–
C3aR	–	+	–	–	–	–	–	+	+	+	+	+
C5aR1	CD88	–	–	–	–	–	+	–	+	+	+	+
C5L2	C5aR2; GPR77	+	–	–	–	–	+	–	+	+	–	–
MCP	CD46	–	+	–	–	–	–	+	+	+	+	+
DAF	CD55	–	+	–	–	–	–	+	+	+	+	+

Monocytes (Mo), monocyte-derived dendritic cells (moDC) and follicular dendritic cells (FDC). (Arbore et al., 2016; Kremlitzka et al., 2014; Li et al., 2011; Ohno et al., 2000; Qualai et al., 2016; Ricklin et al., 2010; Rubtsov et al., 2011; Török et al., 2015; Zipfel and Skerka, 2009).

bone marrow-derived cells can restore normal lymphoid organ-dependent antibody responses in mice lacking C3 (Fischer et al., 1998). Conversely, the renal contribution to the recipient's plasma C3 pool can increase from the normal 5% to 16% in case of allograft rejection (Tang et al., 1999), and can shorten graft survival time almost ten-fold (Pratt et al., 2002). In other species such as fish the hepatic/extrahepatic complement ratio is reversed (Zhang and Cui, 2014), suggesting that locally synthesized complement may have been the rule rather than the exception earlier in evolution. While most of these studies addressed extracellular complement, they now become relevant in light of the new intracellular roles ascribed to several complement proteins (see below).

### 3. Extracellular complement

#### 3.1. Complement polymorphisms and tissue damage

The C3 Fast/Slow polymorphism (p.G102R) came to stage because of a report of improved long-term survival of C3F<sup>+</sup> kidneys transplanted into C3S<sup>+</sup> recipients (Brown et al., 2006), although it could not be reproduced in a larger study (Varaganam et al., 2009). More recently, C4 polymorphism has been shown to influence renal allograft outcome (Bay et al., 2013). Nevertheless, the C3F allele was reported as detrimental in several disorders where extracellular complement is likely involved in tissue damage, such as several nephropathies, age-related macular degeneration (AMD), or systemic vasculitis, but also in other diseases where the conventional role of complement is more controversial (Table 2). Our contribution to this controversy was the unexpected finding of a strong and apparently primary association of chronic renal failure and C3F in a small sample of Spanish patients (Regueiro and Arnaiz-Villena, 1984). Such associations suggested some sort of functional C3 variation associated with the C3 genotype, which has recently been shown biochemically to be caused by a lower affinity of factor H for C3F as compared to C3S (Harris et al., 2012). Studying the combination of interacting common risk vs protection variants for factor H, factor B and C3 in AMD has defined functional clonotypes that clearly increase disease susceptibility by drastically facilitating complement activation and thus inflammation. The unconventional roles have not been explored under this new light. In this regard, the reported association of C3 gene variants with asthma and Th2-dependent responses suggests a potential unconventional pathogenic mechanism involving T cells (Barnes et al., 2006), perhaps including clonotypes.

The clonotype concept was first coined to define combinations of complement alleles within the major histocompatibility complex, MHC (C2, C4 and factor B), and indeed C2/factor B MHC clonotypes have been reported in connection with AMD protec-

tion (Sun et al., 2012). The conserved linkage of three complement genes within the MHC (class III genes) is still a matter of debate, as classical MHC molecules (class I and class II) are critical for T cell selection and function, seemingly unrelated to the conventional role of complement. It is thus tempting to speculate that the unconventional role of complement in T cells (see 4.1) may explain the conserved mapping of complement genes within ancestral MHC haplotypes (Candore et al., 2002) to ensure balanced protection from pathogens and inflammation.

#### 3.2. Regulation of dendritic cells

The classical role of complement components in dendritic cells (DC) includes inducing migration toward inflamed tissues via the C3a or C5a receptors, or facilitating detection and internalization of opsonized pathogens or immune complexes using complement receptor 3 or 4 (CR3 or CR4, see Table 1). Beyond such roles, several complement components have been shown to impact the differentiation, maturation, cytokine production, Th1/Th2 promotion, and phagocytic capacity of antigen-presenting cells (APC), including DC.

Due to the scarcity of blood DC, the best-studied *in vitro* model for human APC is that of monocyte-derived dendritic cells (moDC) (Fig. 1). The procedure to generate human moDC begins with isolation of monocytes (Mo) from peripheral blood mononuclear cells either by sorting as CD14<sup>+</sup> cells or by adherence to plastic microtiter plates at 37 °C. Isolated Mo are subsequently cultured for 5 to 7 days with granulocyte macrophage colony-stimulating factor (GM-CSF) and interleukin 4 (IL-4) to yield immature moDC, which may be fully matured by adding pro-inflammatory mediators such as lipopolysaccharide (LPS) for 24 to 48 h (Castiello et al., 2011). Immature moDC are characterized by low to moderate surface expression of antigen presenting molecules such as MHC-II and CD1a and of T cell activating co-receptors such as CD80 and CD86. Mature moDC express high levels of MHC-II, CD80 and CD86, but not CD1a (Merad et al., 2013). moDC are distinct from conventional or plasmacytoid DC (cDC and pDC, respectively) (Qu et al., 2014), but their easy availability and handling have made them the standard in the field. In mice, by contrast, APC are modelled by a similar strategy but starting with bone marrow cells (bm) cultured in the presence of GM-CSF and isolated as CD11c<sup>+</sup> cells, which are then termed bmDC. Most of the findings that follow, however, should be confirmed in primary cDC and pDC in both species.

Human Mo and moDC express a wide range of surface complement receptors (Table 1) capable of binding both hepatic and extrahepatic (including autologous) soluble complement proteins. Indeed, both cell types produce autologous complement soluble proteins belonging to all three activation pathways, including C1q,

**Table 2**  
Diseases classified by their likely C3-dependent pathological mechanism.

Disease group	Likely C3-dependent mechanism	
	Conventional	Unconventional/Unknown
Renal	Mesangiocapillary nephritis Glomerulosclerosis Atypical haemolytic uraemic syndrome Dense deposit disease Renal failure Allograft rejection	
Ophthalmological	Age-related macular degeneration	
Rheumatological	Systemic vasculitis Systemic lupus erythematosus	
Dermatological	Atopic dermatitis	Partial lipodystrophy
Pulmonary	Tuberculosis	Asthma Lung cancer Ischaemic stroke
Neurological		
Cardiovascular	Chagas disease cardiomyopathy	Hypertension Myocardial infarction Metabolic syndrome Crohn's disease Non-Hodgkin lymphoma
Endocrinological		
Gastrointestinal		
Haematological		

Adapted from Delanghe et al. (2014).

C3 and C5 (Li et al., 2011; Reis et al., 2006), as shown in mouse bmDC (Peng et al., 2008).

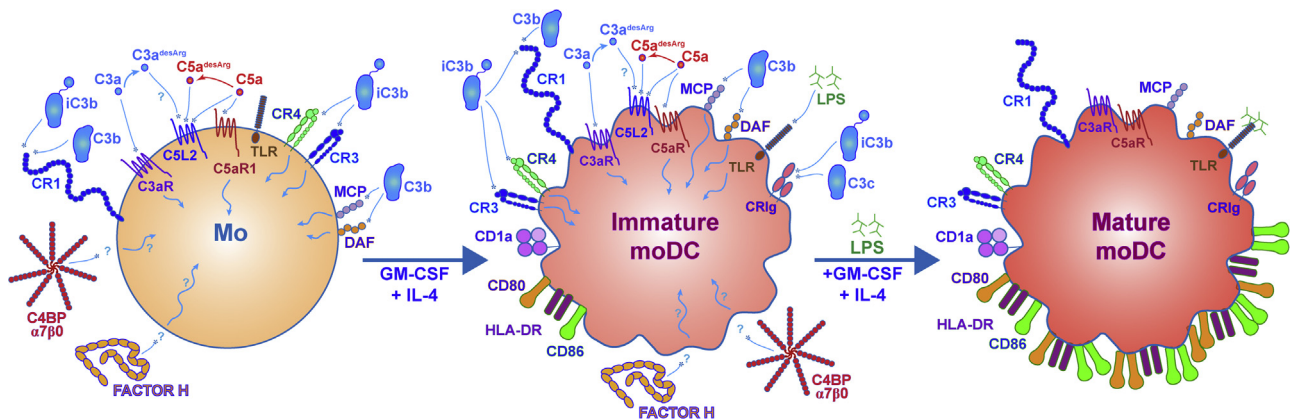
C1q has been shown to enhance DC maturation, achieving higher levels of CD86, MHC-II and interleukin 12 (IL-12), which promote Th1-biased responses (reviewed by Kouser et al., 2015). However, C1q also inhibits the differentiation of Mo into moDC with GM-CSF and IL-4, likely through the inhibitory role of the leukocyte-associated Ig-like receptor, which is known to bind C1q.

Several studies have shown that DC require autologous extracellular C3 (eC3) to reach their full APC potential (Fig. 1). Indeed, murine C3 knock-out (KO) bmDC showed reduced cell surface expression of MHC-II and CD86, reduced LPS-induced synthesis of Th1-polarizing cytokines such as IL-12 and reduced capacity to stimulate alloreactive CD4<sup>+</sup> T cell proliferation and synthesis of Th1 cytokines such as interferon-γ (IFN-γ) and interleukin 2 (IL-2), and this was confirmed in C3 knock-down (KD) bmDC. Th2 cytokines such as IL-4, by contrast, were increased (Peng et al., 2006). The mechanism likely involves eC3a signaling, as both C3aR KO and C3aR antagonist-treated mouse bmDC showed similar features (Peng et al., 2008). Similarly, Mo from human C3-deficient patients showed impaired differentiation to moDC *in vitro* with

reduced number of differentiated moDC, reduced expression of CD1a and MHC-II, reduced LPS-induced synthesis of Th1-polarizing cytokines such as IL-12 and reduced capacity to stimulate alloreactive CD4<sup>+</sup> T cells (Ghannam et al., 2008 and Jiménez-Reinoso, unpublished results).

Similar results have been reported for C5a and its receptors (C5aR in mice and C5aR1 or C5L2 in humans), which are both expressed in normal DC (Fig. 1 and Table 1). Indeed, bmDC from C5aR KO mice showed reduced surface expression of MHC-II and CD86, reduced LPS-induced synthesis of Th1-polarizing cytokines such as IL-12, increased LPS-induced synthesis of Th2-polarizing cytokines such as interleukin 10 (IL-10) and reduced capacity to stimulate alloreactive CD4<sup>+</sup> T cells (Peng et al., 2009).

These data indicate that C3a and C5a anaphylatoxins share signaling pathways that are required by DC to reach their full APC program, including efficient antigen presentation, T cell costimulation and Th1 polarization. When such signals are impaired, as shown in KO mice, a weaker and Th2-skewed DC phenotype is apparent, and thus termed tolerogenic. Enhancement of those signals by adding soluble C3a or C5a in human Mo to moDC differentiation cultures essentially confirmed mouse data, supporting that



**Fig. 1.** Differentiation of Mo (left) by incubation with GM-CSF and IL-4 into immature moDC (center) and, by adding LPS, into mature moDC (right). Hepatic as well as extrahepatic soluble complement proteins affect moDC differentiation through known or unknown (?) receptors, as indicated. C4BP, C4b-binding protein; GM-CSF, granulocyte macrophage colony-stimulating factor; IL-4, interleukin 4; LPS, lipopolysaccharide; ?, unknown surface receptor.

it is a general mechanism connecting innate and adaptive immunity (Li et al., 2012).

Recently, other soluble complement proteins have been shown to regulate human DC maturation. For instance, addition of factor H (FH) to human Mo to moDC differentiation cultures down-regulated moDC surface costimulatory proteins expression and shifted their cytokines toward a Th2 rather than Th1 pattern (Olivar et al., 2016), resulting in a tolerogenic and anti-inflammatory phenotype, very much like C3a or C5a. The authors further showed that FH-dependent DC modulation was unrelated to its complement regulation role and independent of CR3 or CR4 or surface glycosaminoglycans, but mapped to the 19–20 CCP surface-binding FH region, although the exact mechanism remains elusive. Similar results were reported for the C4-binding protein  $\alpha\beta 0$  isoform (C4BP lacking the  $\beta$ -chain), which induces semimature and tolerogenic moDC (Olivar et al., 2013).

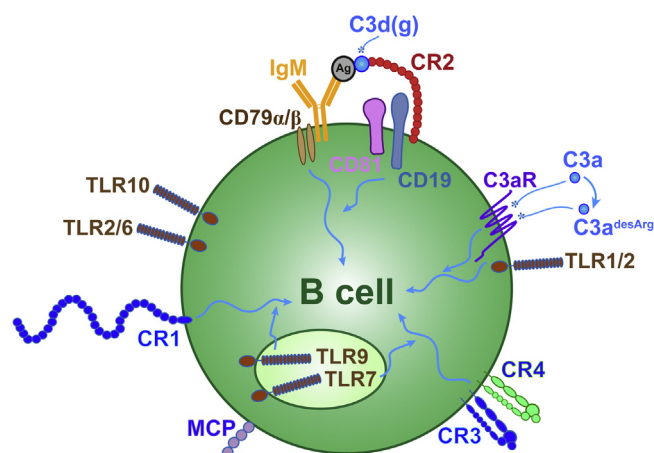
In summary, DC differentiation in mice or human can be regulated by complement components such as C1q, C3, C5, and inhibitors such as FH and C4BP, all of which can be produced in response to inflammatory signals, to become tolerogenic and anti-inflammatory, thus amenable to exploitation for the treatment of inflammatory disorders such as transplant rejection, hypersensitivity or autoimmune disorders.

### 3.3. Regulation of B lymphocytes

B lymphocytes can also respond to hepatic and extrahepatic complement (Table 1). However, the regulation of B cells by complement is connected to its opsonizing activity, rather than to inflammation as shown in DC. More than 40 years ago it was demonstrated that thymus-dependent antibody production was impaired when C3 was depleted by cobra venom factor (Pepys, 1974). The mechanism was later shown to involve the interaction between C3 fragments C3d(g) and their receptor, complement receptor 2 (CR2, van den Elsen and Isenman, 2011), which belongs to the B cell co-receptor (CR2/CD19/CD81, Matsumoto et al., 1993). CR2 is also expressed in the surface of follicular dendritic cells (FDC) in order to accumulate opsonized antigens in germinal centers. Binding of C3d(g)-opsonized antigen to CR2 on B cells strongly lowers the threshold for B cell activation (Dempsey et al., 1996), thus improving antibody formation and facilitating the differentiation of naïve B cells into effector and memory B cells (Fang et al., 1998). Recent findings in CR2-deficient patients indeed showed that hypogammaglobulinemia in such patients is due to sub-optimal B-cell receptor (BCR) costimulation (Thiel et al., 2012).

Toll-like receptors (TLR) also interplay with CR to modulate B cell functions. Several self-damaged or microbial products (nucleic acids, zymosan, LPS) quickly trigger the complement cascade as well as TLR signaling (Kremlitzka et al., 2016). *Staphylococcus aureus* Cowan strain I + IL-2-activated B cells express C3aR, and C3a as well as C3a(desArg) have been shown to have a direct suppressive effect in antibody and cytokine production via TLR2 (Bekeredjian-Ding et al., 2007; Fischer and Hugli, 1997). Further, it has recently been shown that co-engagement of complement receptor 1 (CR1) and TLR9 inhibits BCR-triggered B cell proliferation, and antibody and cytokine production (Kremlitzka et al., 2015).

CR and TLR may also be involved in the unresolved issue of gender-biased autoimmunity, as TLR7-induced age-associated CR3<sup>+</sup>CR4<sup>+</sup>CR2<sup>-</sup> B cells, believed to be involved in the production of autoantibodies, were higher in aged female mice than in young females or in males of any age. Similar CR4<sup>+</sup>CR2<sup>-</sup> age-associated B cells were present in peripheral blood of elderly women with rheumatoid arthritis or systemic sclerosis, but not in healthy control women (Rubtsov et al., 2011).



**Fig. 2.** Complement receptors, C3-derived cleavage fragments and Toll-like receptors (TLRs) expressed on B cells. TLR ligands: TLR1/2: lipoproteins/lipopeptides; TLR2/6: lipopeptides; TLR9: RNA; TLR10: unknown (adapted from Kremlitzka et al., 2016).

In summary, extracellular complement regulates B cell function through CR and TLR crosstalk (Fig. 2).

### 3.4. Regulation of T lymphocytes

In mice models, extracellular C3a and C5a generated from T and APC endogenous production interact with C3aR and C5aR on both cell types and these engagements participate in maintaining their viability, activation and cytokine production by both partners (Strainic et al., 2008). Impairing these interactions reduced MHC-II and costimulatory molecules expression (CD28 and CD40L) dramatically diminishing T cell responses, and in the opposite direction, costimulatory molecules engagement upregulate complement production. Thus both axis, C3a/C3aR and C5a/C5aR1, exhibit overlapping but not fully redundant function because inhibition or deficiency of both has a significantly more profound effect than the absence or blockade of either alone.

### 3.5. Unconventional roles of extracellular complement proteins

The chronic activation of the alternative pathway of complement causing low-level inflammation has been associated with metabolic disorders (reviewed in Moreno-Navarrete et al., 2010). Indeed, several complement proteins can be secreted by the adipose tissue, some exclusively such as factor D (adipsin), but also factor B and FH, which are strongly associated with obesity and regulate the alternative pathway C3 convertase. They may thus generate C3a and C3a(desArg) (also called acylation-stimulating protein), which have been shown to bind C5aR2 and stimulate triglyceride synthesis in adipocytes. When absent, as in C3 KO mice, lipid abnormalities ensue. In humans, C3a(desArg) levels are high in metabolic disorders including obesity and type 2 diabetes and in individuals at risk of arterial disease, and low in association with exercise and weight loss, which connects with the unconventional role of FH discussed above.

FH has an unconventional role as adrenomedullin-binding protein 1 (Sim et al., 2015). Adrenomedullin (AM) is a constitutively secreted but short-lived pleiotropic peptide, expressed widely, including leucocytes, but particularly high in the vascular endothelium. Through its main vascular receptor AM1, a dimer of calcitonin receptor-like receptor (CLR) and receptor activity-modifying protein 2 (RAMP2), AM is involved in the regulation of vascular integrity, including the maintenance of vascular structure, the regulation of angiogenesis and the protection of vascular injury, and

is strongly increased in septic shock and cardiovascular diseases in humans. Therefore, AM has many potential clinical applications, but its short half-life makes it unpractical for chronic diseases. FH, which is 105-fold more abundant than AM, appears to protect it from degradation while preserving its biological activity, thus they could potentially be used together to treat vascular disorders or to prevent cancer angiogenesis.

In addition to its role as recognition molecule of the complement classical pathway, C1q plays a large number of unconventional roles unrelated to complement, including clearance of apoptotic cells and modulation of disparate cell types such as dendritic cells (see 3.2) and microglia (reviewed by Kouser et al., 2015). Numerous reports also suggest that C1q plays an important role in normal pregnancy through its local role in fetal trophoblast invasion of the decidua, as its deficiency can lead to implantation failure. C1q is also produced locally in the central nervous system, and is believed to exert neuroprotective functions upon interaction with aggregated proteins that cause neurodegenerative diseases, such as  $\beta$ -amyloid in Alzheimer's disease, and to participate in synaptic pruning during neuronal development. Indeed, microglia, astrocytes, and neurons are known to secrete complement proteins, especially under stress, injury, ischemia, or infection. Lastly, C1q has been shown to promote angiogenesis during wound healing through local deposition in endothelial cells, which respond by increasing permeability, proliferation and tube formation.

Considerable evidence has been published supporting communication between the complement and coagulation systems (Kenawy et al., 2015). Indeed, disseminated coagulation due to trauma or blood loss cause strong complement activation, which in turn accelerates coagulation.

#### 4. Intracellular complement

Although a more specific review will address this topic in this series, we will include a brief outline of the emerging unconventional role of intracellular complement components, which may help to understand some of the unresolved issues.

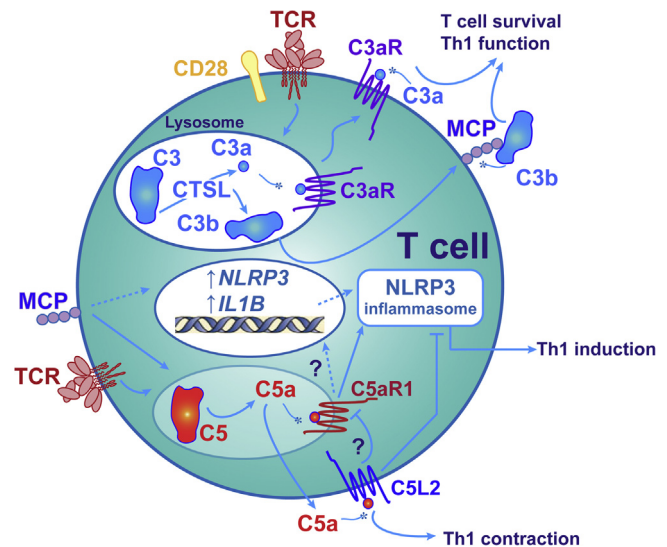
##### 4.1. Regulation of T lymphocytes

Conventional extracellular C3 and C5 cleavage during microbial infection leads to induction of an inflammatory response, including the mobilization of phagocytes toward their powerful chemoattractant fragments C3a and C5a (Ricklin et al., 2010).

Several years ago PHA-activated T cells and HTLV-1-transformed T cell lines were shown to synthesize and release C3 (Pantazis et al., 1990), which at that time was believed to increase extracellular C3 for its conventional cleavage. More recently it was demonstrated that TCR-activated T cells synthesize iC3b that appears on the cell surface (Török et al., 2012). Unexpectedly, the intracellular C3-cleaving enzyme was shown to be cathepsin L (CTSL) rather than the conventional plasma convertases (Liszewski et al., 2013). In addition, cleaving of intracellular C3 (iC3) was shown by the same authors to be essential for T cell homeostasis through intracellular binding of C3a to C3aR (Fig. 3). They further showed that intracellular C3a and C3b shuttle to the cell surface following TCR activation, where they stimulate C3aR and membrane cofactor protein (MCP), respectively, for full Th1 induction.

Thus, autocrine iC3 has a crucial role in the induction and modulation of T cell function (Kolev et al., 2014).

Very recently it has been shown that this unconventional mechanism is also functional in apoptotic cells, which actively internalize FH to enhance CTSL-mediated iC3 cleavage and thus increased surface iC3b opsonization (Martin et al., 2016).



**Fig. 3.** iC3 and iC5 activation mechanisms. iC3 activation by cathepsin L (CTSL) is crucial for T cell survival and for T cell differentiation after T-cell receptor (TCR)-activation. Secreted intracellular C3a and C3b promote Th1 functions through their surface receptors. TCR and MCP signaling generates iC5a that binds to intracellular C5aR1, leading to nucleotide-binding domain, leucine-rich repeat family, pyrin domain containing 3 (NLRP3) assembly and Th1 induction, whereas secreted intracellular C5a binds to surface C5L2 negatively regulating this mechanism. Adapted from (Arbore et al., 2016; Liszewski et al., 2013).

**Table 3**

Complement proteins and receptors expressed by T lymphocytes as tested with different techniques.

Protein	Technique			
	FC	CM	WB	RT-PCR
C3	+	+	+	+
C3a	+	+	+	+
C3aR	+	+	ND	+
C5a	+	+	ND	+
C5aR1	+	+	+	+
C5aR2	+	+	+	+

Data from Liszewski et al. (2013) and Arbore et al. (2016). FC: flow cytometry. CM: confocal microscopy. WB: Western blot. RT-PCR: Reverse transcription polymerase chain reaction. +: positive result. ND: not determined.

On the other side of the T/APC synapse iC3 had also been reported to regulate the generation of T cell ligands through its putative role as a chaperone of endocytosed C3-opsonized antigens in the endolysosomal compartment of the MHC class II pathway (Serra et al., 1997), which adds to the reported role of extracellular C3 in DC (see 3.2).

Therefore, iC3 may have wider unconventional roles in multiple cell types, particularly in adaptive immunity, than previously recognized.

The similar extracellular roles of C3 and C5 and the finding of unexpected cellular roles for iC3 stimulated the search of intracellular functions also for C5 (Table 3), which have been very recently reported (Arbore et al., 2016). Indeed, intracellular C5 (iC5) is cleaved within CD4<sup>+</sup> T cells and its activation is required for the assembly of the T-cell's NLRP3 inflammasome, promoting interleukin-1 $\beta$  secretion and, thereby, IFN- $\gamma$  production and autocrine Th1 induction (Fig. 3). Although the protease responsible for iC5 cleavage remains unknown, this finding highlights the important role of intracellular activation of complement components for T cell function, and opens a new door for therapy through the modulation of Th1 responses in autoimmunity and infections.

## Conflict of interest

All authors declare that they have no relevant conflicts of interest.

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