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Review

Differential effects of anaphylatoxin C5a on antigen presenting cells, roles for C5aR1 and C5aR2

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ABSTRACT

The anaphylatoxin C5a is well-known for its role as chemoattractant and contributes to immune cell recruitment into inflamed tissue and local inflammation. C5a has recently been implicated in modulation of antigen presenting cell function, such as macrophages and dendritic cells, which are pivotal for T cell activation and final T cell effector function. The published data on the effect of C5a on APC function and subsequent adaptive immune responses are in part conflicting, as both pro and anti-inflammatory effects have been described. In this review the opposing effects of C5a on APC function in mice and human are summarized and discussed in relation to origin of the involved APC subset, being either of the monocyte-derived lineage or dendritic cell lineage. In addition, the current knowledge on the expression of C5aR1 and C5aR2 on the different APC subsets is summarized. Based on the combined data, we propose that the differential effects of C5a on APC function may be attributed to absence or presence of co-expression of C5aR2 and C5aR1 on the specific APC.

1. Introduction

The anaphylatoxins C5a and C3a are well-known for their role as chemoattractants and are formed upon cleavage of C3 and C5 during the process of complement activation. C5a is the most potent chemoattractant and induces recruitment of different immune cells to inflamed tissue, among which are neutrophils [1–3], eosinophils [1,3], monocytes [4], and human dendritic cells (DCs) [5,6]. In addition, release of C5a increases blood vessel permeability, chemokine release from neutrophils, and expression of adhesion molecules on endothelial cells, as reviewed in [7]. All of these process facilitate further immune cell recruitment into inflamed tissue and local inflammation. Compared to C5a, C3a is a weaker chemoattractant, and is mainly involved in the attraction of eosinophils and mast cells [1,8].

C5a has recently been implicated in modulation of mouse macrophage (MQ) and DC function [9–16]. Antigen presenting cells (APCs), like MQs and DCs, are pivotal for T cell activation and polarization [17,18]. These cells are thus essential to link innate and adaptive immunity upon pathogen invasion, but also in autoimmune diseases. Strong crosstalk between activated complement products and APC maturation is implicated by the facts that activation of the complement system and APC maturation occur side by side upon infection or in sterile (auto) inflammatory conditions and that APCs are capable of locally producing complement components, including C5 [10,19–21]. Most research focuses on the modulating effect of anaphylatoxin C5a during DC and MQ maturation by PAMPs [9–11,20,22–25]. Published data on the effect of C5a on APC function and subsequent adaptive immune responses most often consist of data from mice, are in part conflicting and concluding insights are often still lacking. Especially the number of studies on the effect of C5a on human DCs is limited [26–29] and asks for further research.

An additional need to increase our knowledge on overall effects of C5a on the different arms of the immune system comes from the intensified interest in the use and development of C5/C5a interfering compounds for the treatment of various inflammatory diseases, such as atypical hemolytic uremic syndrome, age-related macular

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Abbreviations: AP, Cantigen presenting cell; BMD, Cbone-marrow derived DC; C5a, Complement component 5a; C5a, RC5a receptor; DC, Dendritic cell; GPCR, G protein-coupled receptor; imDC, immature moDC; LPS, lipopolysaccharide; moDC, monocyte-derived dendritic cell; moMQ, monocyte-derived MQ; MQ, macrophage; PAMP, Pathogen-associated molecular patterns; slanDC, 6-sulfo LacNAc dendritic cell; TLR, Toll-like receptor

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Table 1
Stimulatory effect of C5a on antigen presenting cell activation and adaptive immunity.

Treatment	Species	Cell type	Stimulus	Cytokine production	Co-stimulatory molecules	T cell activation	Implications	Ref
C5aR1 deficiency	murine	BMDC	OVA	n.d.	n.d.	↓ T cell response, ↓ IFN-γ	C5aR1$^{-/-}$ reduces Th1 response to *T. gondii* and in EAE and herpes keratitis models	[9]
	murine	BMDC	LPS	↓ IL-12, ↑ IL-10	↓ MHCII, CD80, CD86	↓ T cell response, ↓ IFN-γ	n.d.	[10]
	murine	BMDC	allograft	n.d.	n.d.	↓ T cell response, ↓ IFN-γ	C5aR1$^{-/-}$ prolongs renal allograft survival and decreases cell infiltration	[35]
	murine	peritoneal MQ	no stimulus	↓ IL-12	n.d.	↓ T cell response, ↓ IFN- γ	n.d.	[22]
	murine	peritoneal MQ	OVA	n.d.	↓ CD80, CD40	n.d.	n.d.	[9]
C5aR1 antagonist	murine	BMDC	OVA	↓ IL-12, IL-23	↓ MHCII, CD80, CD86, CD40	↓ T cell response, ↓ IFN-γ	n.d.	[9]
	murine	BMDC	LPS	↓ IL-12, ↑ IL-10	↓ MHCII, CD80, CD86	↓ T cell response, ↓ IFN-γ	n.d.	[10]
C5a	murine	BMDC	LPS	↑ IL-12, TNF-α, ↓ IL-10	↑ MHCII, CD86, CD40	↑ T cell response, ↑ IFN-γ	n.d.	[10]
	murine	BMDC	*P. gingivalis*	↑ IL-12, TNF-α, IL-23	n.d.	n.d.	C5a promotes *P.gingivalis* intracellular killing	[39]
	human	moDC	no stimulus	↑ IL-6, TNF-α, IL-12	↑ HLA-DR, CD86, CD40	↑ IFN-γ, TNF-α, IL-17	n.d.	[26]
	human	moDC	no stimulus	↑IL-6	n.d.	n.d.	n.d.	[28]
	human	moDC	*P. gingivalis*	n.d.	n.d.	n.d.	C5a promotes *P.gingivalis* intracellular killing	[39]
	human	Monocyte*	LPS	↑ IL-6, TNF-α, no effect on IL-10	n.d.	n.d.	n.d.	[37]

Publications investigating the direct involvement of specific antigen presenting cells or monocytes were included in the table. n.d., no data; BMDC, bone marrow derived dendritic cell; MQ, macrpohages; moDC, monocyte derived dendritic cells; OVA, Ovalbumin.

* Monocytes are not defined antigen presenting cells.

degeneration, antiphospholipid syndrome; ANCA vasculitis, sepsis and more [30–32]. Both the fundamental questions and clinical needs together emphasize the importance to understand the intersection between these two arms of innate immunity in immune activation. In this review we will summarize the effects of C5a on APC function and propose an explanation for the reported and seemingly conflicting findings of both pro- and anti-inflammatory modulation of APC function by C5a. Many studies investigated the overall effect of C5a on T cell adaptive immune responses in animal models and have been thoroughly reviewed by Zhou [33]. This review focusses on studies in which the modulatory effect of C5a on specific APC subsets was analyzed.

2. Effects of C5a on APC function are opposing

2.1. Effects of C5a on APCs in mice

In mice, the immune modulatory functions of C5a on APCs have been mainly investigated in bone marrow-derived DCs (BMDCs) and peritoneal MQs (Tables 1 and 2). C5a promotes co-stimulatory molecule expression and enhances IL-12 production in BMDCs when these cells are matured with LPS, resulting in an increased ability of BMDCs to promote Th1 polarization [10]. In line with this, disabled C5aR signaling during BMDC-T cell interaction, either in C5aR1 deficient mice or by use of a C5aR1 antagonist, results in an impaired Th1 immune response [9,10,22]. C5a stimulation also leads to increased production of TNF-α in LPS-stimulated BMDCs, while decreasing IL-10 production [10,34]. During *Toxoplasma gondii* infection, C5aR1-deficient BMDCs are impaired in their capacity to induce Th1 immune responses, resulting in decreased protection during infection [9]. These mice also show impaired Th1 immune responses during experimental autoimmune encephalomyelitis and herpes keratitis [9] (Table 1). In addition, the pro-inflammatory effect of C5a on DCs interferes with successful organ transplantation, as blockage of C5aR signaling reduces graft rejection and allo-reactive T cells responses in mice [35].

In contrast to the stimulatory effects of C5a, inhibitory effects of C5a on APC activation and Th1 immunity have also been reported (Table 2) [11,13–16,22]. Different from the studies described above, all of these studies were performed with mouse peritoneal MQ, which were in addition activated by either LPS or *Porphyromonas gingivalis*. Here, C5a decreased production of many pro-inflammatory cytokines, including TNF-α, IL1-β, IL-6, IL-12, and IL-23, while increasing expression of IL-10 [11,13–16]. Decreased pro-inflammatory cytokine production upon C5a stimulation has been associated with reduced Th1 polarization and attenuation of *Porphyromonas gingivalis* clearance [11–13]. Interestingly, although not the scope of this manuscript, C5a inhibits also LPS-induced TNF-α production in rat neutrophils, while being stimulatory in rat alveolar MQs [36].

Summarizing, the current data show that C5a promotes pro-inflammatory immune activation of mouse BMDCs, whereas it decreases immune activation of mouse peritoneal MQs.

2.2. C5a effects on APCs in human

Knowledge on the regulatory function of C5a on human APCs is mainly limited to analyzed effects on cytokine production by the APCs (Tables 1 and 2) [26–29,37]. C5a stimulation did not affect pro-inflammatory cytokine production of human monocyte-derived DCs (moDCs) when added 1 h prior to stimulation with *Staphylocucss aureus* Cowan I (SAC) and IFN-γ [27]. Our studies, however, show that C5a can inhibit pro-inflammatory cytokine production induced upon TLR ligation in both moDCs and slanDC when added simultaneously [28,29]. In concordance, this inhibition was not observed when C5a was added 1 h before or after LPS stimulation [28]. C5a diminished LPS-induced production of the pro-inflammatory cytokines IL-6, IL-12, IL-23 and TNF-α in human moDCs [28]. Similar results were found using other TLR ligands, namely R848 (TLR7/8), and Pam$_3$CSK$_4$

Table 2
Inhibitory effect of C5a on antigen presenting cell activation and adaptive immunity.

Treatment	Species	Cell type	Stimulus	Cytokine production	Co-stimulatory molecules	T cell activation	Implications	Ref
C5a	murine	peritoneal MQ	LPS	↓ IL-12, IFN-γ, ↓mRNA IL-12 family members	n.d.	↓ Th1, ↓ IFN-γ	n.d.	[11]
	murine	peritoneal MQ	LPS	↓ IL-12, ↓mRNA: p40, p35, TNFα, NOS, IFN-γ, ↑IL-10	n.d.	n.d.	n.d.	[14]
	murine	peritoneal MQ	LPS	↓ IL-12	n.d.	n.d.	n.d.	[22]
	murine	peritoneal MQ	LPS	↓ IL-12	n.d.	n.d.	n.d.	[13]
	murine	peritoneal MQ	LPS	↓ IL-1β	n.d.	n.d.	n.d.	[16]
	murine	peritoneal MQ	LPS	↓ IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-12, IL-13, IL-17, Eotaxin, GM-CSF, IFN-γ, KC, MCP-1, MIP-1α, MIP-1β, RANTES, TNFα ↑ G-CSF, IL-10	n.d.	n.d.	n.d.	[15]
	murine	peritoneal MQ	*P.gingivalis*	n.d.	n.d.	n.d.	C5a reduces *P. gingivalis* intracellular killing	[12]
	murine	peritoneal MQ	*P.gingivalis*	↓ IL-12, ↑ IL-6, TNF-α	n.d.	n.d.	C5a attenuates *P. gingivalis* intracellular clearance	[13]
	human	moMQ	LPS	↓ IL-6, TNF-α, ↑IL-10	n.d.	n.d.	C5a decreases *S. typhimurium* survival in MQ	[37]
	human	moMQ	*P.gingivalis*	n.d.	n.d.	n.d.	C5a decreases *P. gingivalis* intracellular killing	[39]
	human	moDC	LPS	↓ IL-6, TNF-α, IL-12, IL23	no effect on CD40, CD80, CD83, CD86	n.d.	n.d.	[28]
	human	moDC	LPS	↑IL-10	n.d.	↓ IFN-γ (CD4 and CD8)	n.d.	[29]
	human	moDC	Pam3CSK4, R848, or *S. typhimurium*	↓ TNF-α, IL-23, IL-12	n.d.	n.d.	n.d.	[28]
	human	moDC	SAC/IFN-γ[†]	no effect on IL-12[†]	n.d.	n.d.	n.d.	[27]
	human	slanDC	R848	↓ TNF-α, IL12p40, IL12p40 mRNA, IL12p35 mRNA, ↑IL-10, IL-10 mRNA	n.d.	n.d.	n.d.	[29]
	human	Monocyte[*]	LPS/IFN-γ	↓ IL-12, ↓ mRNA IL-12 family members	n.d.	n.d.	n.d.	[40]
	human	Monocyte[*]	LPS/IFN-γ	↓ IL-12	n.d.	n.d.	n.d.	[41]
	human	Monocyte[*]	SAC/IFN-γ	↓ IL-12, mRNA:p40, p35; no effect on TNF-α, IL-10, TGF-β	n.d.	n.d.	n.d.	[27]

Publications investigating the direct involvement of specific antigen presenting cells or monocytes were included in the table. n.d., no data; MQ, macrpohages; moMQ, monocyte derived macrophages; SAC, S. aureus Cowan-I.

[*] monocytes are not defined antigen presenting cells.

[†] C5a was added 1 h prior to TLR stimulus.

(TLR2), or during exposure of moDCs to the gram-negative bacterium *S. typhimurium* [28]. In depth analysis of human blood DC subsets demonstrated that especially slanDCs are prone to regulation by C5a [6,29,38], and revealed that C5a inhibited R848-induced pro-inflammatory cytokine production by human slanDCs. In human monocyte-derived MQs (moMQs), similar results have been found as observed in mouse peritoneal MQ [37,39]. Also here, presence of C5a inhibited LPS-induced IL-6 and TNF-α production [37]. Although not classified as APCs, it is still worthwhile to mention that C5a also inhibits TLR-induced pro-inflammatory cytokine production in three out of four studies performed in human monocytes (Tables 1 and 2) [27,37,40,41].

Interestingly, in absence of TLR stimulation, C5a increases the production of TNF-α, IL-6, and IL-12 and the expression of co-stimulatory molecules by human moDC [26,28]. Also in mouse peritoneal MQ, C5a increased IL-12 production in absence of TLR stimulation, yet the opposite was found in presence of LPS [22].

3. Current insights in APC classification indicate that C5a-induced inhibition is general among monocyte-derived APC subsets

The classification of monocyte, DC and MQ subsets has been a point of debate, and has changed to a great extent during the last decade. Traditionally, classification of APC subsets was based on the expression of a wide variety of cell surface proteins, as well as APC-specific functional properties which are often overlapping between subsets [42,43]. Recently, Guilliams and colleagues [42] proposed a classification to categorize APC subsets based on ontogeny. This resulted in a more robust classification of different APC subsets, as this nomenclature permits overlapping functions or phenotypic properties among APCs. Based on this new nomenclature, monocyte-derived cells are a distinct lineage of APCs, which exists next to DC and MQ lineages [42]. Inflammatory DCs [44], such as moDCs and moMQs, have now been classified to belong to the lineage of monocyte-derived APCs [42,44,45]. In addition, dermal CD14$^+$ DCs, intestinal CD103$^-$CD172$^+$ DCs, and tissue residing CD16$^+$ cells (including slanDC), are suggested to derive from monocytic origins [42,46–48], and are therefore likely part of this lineage as well.

Recent studies revealed that the “traditionally classified” peritoneal MQs are actually comprised of two subsets [49]. One of these subsets is of embryonic origin and now classified as MQ subset, while the other subset is of monocytic origin, and therefore now classified as monocyte-derived APC lineage [49]. Interestingly, the procedure used to collect peritoneal MQ for *in vitro* studies involves injection of thioglycollate several days before isolation of MQs. This injection increases the recruitment of monocytes into the peritoneum, and promotes the development of monocyte-derived peritoneal MQ [49]. This implicates that *in vitro* experiments performed with peritoneal MQs mainly contain monocyte-derived APCs.

The above described new ontogeny of the different APC subsets may explain why the inhibitory effects of C5a on TLR–induced pro-inflammatory cytokine production by human moDCs and slanDCs are in line with previous findings with *in vitro* cultured mouse peritoneal MQs and human monocyte-derived MQs [11–16,28,29]: Since all of these APC subsets are part of the monocyte-derived lineage, the inhibitory effect of C5a during C5aR and TLR crosstalk seems to be general among monocyte-derived APC subsets.

Interestingly, the stimulatory effect of C5a on cytokine production in presence of TLR stimulation has only been observed in BMDCs, which are classified to the DC lineage.

4. Differences in C5aR1 and C5aR2 expression among APC subsets

C5a is recognized via specific receptors [50], C5aR1 (CD88) and C5aR2 (previously called C5L2). These are seven transmembrane-spanning receptors, of which C5aR1 is a G-protein coupled receptor.

In mice, both C5aR1 and C5aR2 are strongly expressed on various MQ subsets, including peritoneal macrophages, whereas expression of both receptors varies among mouse DC subsets. This was nicely demonstrated by generation of C5aR1 and C5aR2 specific reporter mice [50–52]. C5aR1 is expressed on BMDCs, moDC, splenic CD8$^+$-like cDC, lung resident CD11b$^+$ DC, but not on intestinal and pulmonary CD103$^+$ DCs and pDC [51]. Modest C5aR2 expression is observed in lung moDCs (60–65%) and lung CD11b$^+$CD103$^-$ DCs (15–20%), and low C5aR2 expression is observed in intestinal CD11b$^+$ and CD103$^+$ DC subsets. C5aR2 expression is absent on all other DC subsets investigated, including pDC, splenic DCs, and BMDCs [52].

Among human circulating DC subsets, no C5aR1 expression is observed on *ex vivo* isolated myeloid DC [6,29,53]. C5aR1 expression by pDCs correlates with DC activation status, while C5aR2 was not determined in these studies [6,19,54]. Both C5aR1 and C5aR2 are expressed on human moMQs [55], peritoneal MQs [56] and moDCs [19,21,27,29,57]. Whereas for human slanDCs C5aR1 protein expression has been described, C5aR2 has not been studied yet [6,29,38,53].

Since data are very limited regarding C5aR1 and especially C5aR2 protein expression on human APC subsets [50], we analyzed online-available RNA expression data of *C5AR1* and *C5AR2* in human APC subsets from blood, skin, spleen and thymus from several published studies (Fig. 1) [53,58–61]. Overall, the expression of *C5AR1* seems lower on DC populations (CD1c$^+$, CD11c$^+$, CD141$^+$ DC subsets and pDC) than on monocyte-derived APC subsets (monocytes, slanDC, non-classical monocytes) in blood as well as in tissues. This is in agreement with the fact that C5aR1 protein expression has only be detected on monocyte-derived APC subsets. *C5AR2* has roughly the same expression pattern as *C5AR1*. The expression levels are, however, clearly lower than *C5AR1*, in concordance with other reports [57,62,63]. For the data (GSE85305) obtained by McGovern and colleagues it is remarkable that *C5aR1* and *C5aR2* expression profiles are different when comparing DC subsets in skin and spleen [58]. *C5AR2* is expressed less on skin CD1c$^+$ compared to skin CD14$^+$ cells, while *C5AR1* expression was comparable for these subsets. In contrast in the spleen, *C5aR2* is more or less equally expressed on CD14$^+$ and CD1c$^+$ cells, while *C5aR1* is expressed less on CD1c$^+$ compared to CD14$^+$ cells.

Together, these data show that C5aR1 seems to be quite broadly expressed, while C5aR2 is predominantly expressed on monocyte-derived APC subsets, both in mice and human, and is absent on mouse BMDCs. *C5AR2* RNA is, however, expressed in several human DC subsets, for which protein expression needs further confirmation.

Of note, although no C5aR1 expression is observed on circulating conventional DC subsets [6,29,53], protein expression on tissue residing conventional DCs needs further investigation to exclude that under certain circumstances, e.g. inflammatory conditions, these DCs might express C5aR1 and/or C5aR2 and will be responsive to C5a mediated modulation.

4.1. C5a receptor signaling

Interestingly, BMDC, which only express C5aR1, respond differently to C5a than monocyte-derived APC, which likely express both C5aR1 and C5aR2. Stimulation of C5aR1 induces activation of the coupled G-protein, resulting in a Ca^{2+} influx and activation of several different downstream signaling molecules, such as cAMP induction, p38, ERK, JNK, NF-kB, PI3K, and induces recruitment of β-arrestins for receptor desensitization [10–14,26,37,64,65]. C5aR2 is a non-signaling GPCR because it does not contain the motif for G-protein binding, but can directly recruit β-arrestins [65]. For a long time C5aR2 has been postulated as a non-signaling decoy receptor that limits C5a availability to C5aR1, thereby regulating C5aR1-mediated pro-inflammatory immune responses [63,66,67]. More recent publications, however, state that C5aR1-C5aR2 heterodimerisation, via C5aR2 mediated β-arrestin recruitment, is crucial for the modulation of ERK1/2 phosphorylation in human monocyte-derived MQ [55,65,68].

We demonstrated that C5aR and TLR crosstalk in human moDCs and

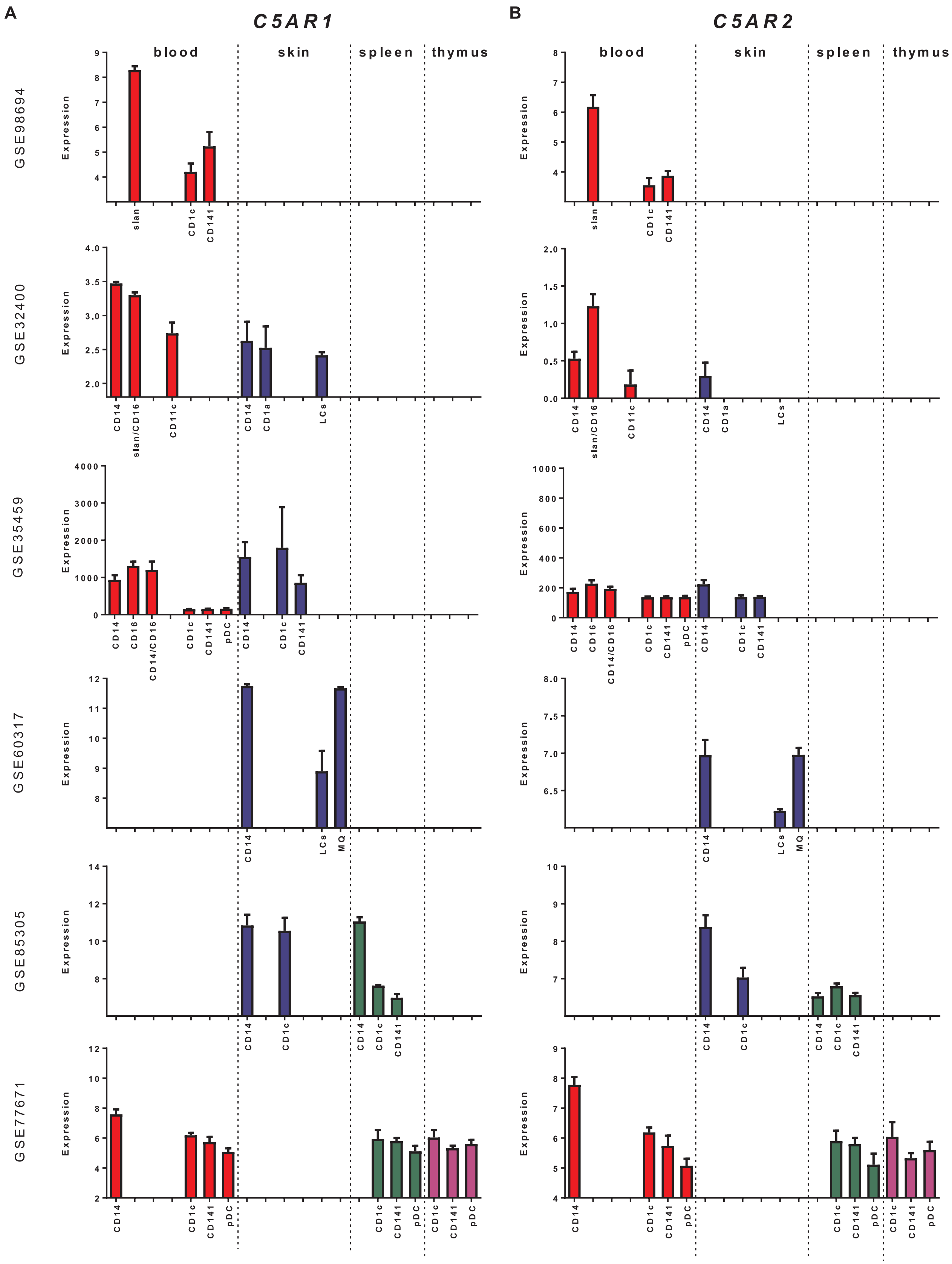

Fig. 1. *C5aR1* and *C5aR2* mRNA expression in various human APC subsets found in blood, skin, spleen and thymus. (A) *C5AR1* and (B) *C5AR2* mRNA expression was analyzed in various human APC subsets (indicated on x-axis) using the GEO2R online webtool (https://www.ncbi.nlm.nih.gov/geo/geo2r/) and publicly available online datasets accessible at NCBI Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) [82] In GEO2R, default settings were used. The following GEO series accession numbers from GEO were used: GSE98694, GSE32400, GSE35459, GSE60317, GSE85305, GSE77671 [53,58–61]. Expression levels of each dataset are plotted in individual graphs.

slanDC inhibits TLR-induced pro-inflammatory cytokine production via ERK/p38-induced CREB1 phosphorylation and subsequent IL-10 induction [29], and in addition induces a more regulatory phenotype in human moDCs [69]. Other groups have also reported that the inhibitory effect of C5a on IL-12 production and the stimulatory effect of C5a on IL-10 production [70] by monocyte-derived APCs is dependent on ERK1/2 signaling [11,14,55] and this was demonstrated to be dependent on C5aR1-C5aR2 heterodimerisation [55].

The inhibitory effect of C5a on TLR4-mediated IL-12, TNF-α and IL-23 production by human moDCs was almost completely abolished by blocking C5aR1 with a receptor antagonist (W-54011) [28]. This receptor antagonist used to determine involvement of C5aR1 has been reported to be specific for C5aR1 [71,72]. It can be envisioned, however, that blockage of C5aR1 may also interfere with functioning of the C5aR1-C5aR2 heterodimer complex and as such interference with C5aR1 may also indirectly affect C5aR2 signaling.

Combination of all currently available data thus suggest that the inhibitory effect of C5a in human moDC may well occur via downstream signaling of C5aR1-C5aR2 heterodimers. It should be kept in mind, however, that heterodimerisation of C5aR1 and C5aR2 has until now only been investigated in MQs, and thus needs to be confirmed in other APC subsets. The recently discovered functionally selective C5aR2 ligands will contribute to future studies on the role of C5aR1 and C5aR2 in various APC subsets and are needed to shed more light on the importance of C5aR1-C5aR2 heterodimerisation [70].

5. C5aR2 expression correlates with the differential effect of C5a on different APC subsets

Based on C5aR1 and C5aR2 expression profiles and the possible involvement of C5aR1-C5aR2 heterodimerization during C5a-mediated inhibition of TLR-induced pro-inflammatory cytokine production, we speculate that specific APC subsets are more sensitive to the inhibitory effect of C5a compared to others. MQ subsets, lung resident CD11b$^+$ DC and moDC, for example, are more likely to be subject to the regulatory effect of C5a because of their high expression levels for both C5aR1 and C5aR2, allowing the C5aR1-C5aR2 heterodimerisation needed for downstream propagation of the inhibitory signal [51,52]. This is in concordance with the observed inhibitory effect of C5aR and TLR crosstalk on pro-inflammatory cytokine production by human moDCs [28,29] and peritoneal MQs [11–16]. In line with this, BMDCs lack C5aR2 expression and are much less susceptible to C5a-mediated inhibition. This might clarify why findings regarding the effect of C5aR and TLR crosstalk on cytokine production in human moDC and slanDC [28,29] are not in line with observations in mouse DCs (see Tables 1, 2) [9,10].

In conclusion, we propose that the inhibitory capacity of C5a on TLR-induced pro-inflammatory cytokine production depends on C5aR1 and C5aR2 co-expression, needed for C5aR1-C5aR2 heterodimerisation. This explains why C5a does not inhibit pro-inflammatory cytokine production in BMDCs as BMDCs only express C5aR1 and not C5aR2. Future analyses are needed to fully elucidate if the inhibitory effect of C5a on TLR-induced APC activation is indeed restricted to monocyte-derived APCs and indeed depends on the combined presence of C5aR1 and C5aR2 on the APCs.

As mentioned before, the inhibitory effect of C5a on cytokine production by human moDC and mouse peritoneal MQ depends on the presence of maturation triggers, as C5a promotes pro-inflammatory cytokine production in the absence of a TLR stimulus [22,26,28]. This adds another layer of complexity on top of involvement of two C5a receptors who may propagate downstream effects of C5a. Since TLRs signal via similar signal transduction pathways as described downstream of C5aR1 and/or C5aR2, these signaling routes will likely integrate and the end result will be dependent on all the signals induced, via TLR, C5aR1 and/or C5aR1-C5aR2 heterodimerisation. In human moDC we observed that C5a induced ERK1/2 and p38 phosphorylation both in absence and presence of additional stimuli, such as LPS [29]. Strikingly, C5a-induced ERK1/2 and p38 activation did not lead to IL-10 induction without TLR stimulation, most probably due to lack of prolonged CREB1 phosphorylation in absence of TLR stimulation [29]. ERK1/2 and p38 activation upon C5a stimulation in absence of TLR dependent CREB1-induced IL-10-mediated negative feedback signaling, therefore, likely leads to induction of several pro-inflammatory cytokines in human moDCs.

5.1. Consequences

Monocyte-derived APCs are of great importance upon infection and in several inflammatory diseases. Inflammatory DCs, including slanDCs, moDCs and moMQ, strongly contribute to local immune activation [38,44]. In addition, slanDCs and moDCs are elevated in many autoimmune diseases, such as psoriasis, SLE, MS [38,44,73–78] and induce T cell immune responses [38,44,79]. Since cytokine production by APCs dictates T cell polarization, changes in the production profile of APC cytokines can strongly affect adaptive immune responses. The inhibitory effect of C5a on APC cytokine production during stimulation can, therefore, affect the modulating capacity of APCs on T cells. This fits with the observation that IFN-γ production by Th1 and cytotoxic T cells is reduced upon stimulation with C5a-primed and TLR-activated human moDCs [29] and mouse peritoneal MQs [11]. Although Th1 and Th17 immune responses are required to induce efficient pathogen clearance, immune regulation of these most pro-inflammatory Th subsets is crucial to prevent overwhelming and uncontrolled immune activation, tissue damage and prevention of immunopathology [80,81].

If indeed, as we propose, the balance/(co-)expression of the two different C5a receptors is fundamental to either inducing an pro- or anti-inflammatory effect on APC activation, C5aR1 and C5aR2 expression is most likely tightly regulated. In addition, differences in the expression patterns among various APC subsets or during APC activation status will probably serve specific functions. It can be speculated that C5aR2 expression may, for example, increase during the retraction phase of an infection, or that C5aR2 expression is restricted to only the most inflammatory DC subsets to prevent uncontrolled activation. Because of the dichotomous effect of C5a on APC function, we hypothesis that dysregulation of C5aR1 and C5aR2 expression or signaling can either lead to severe problems in pathogen clearance or can contribute to uncontrolled immune activation (which may for example be the case in autoimmunity). Since the field just starts to reveal the effect of C5aR1 and C5aR2 in APC biology, the extent of C5a induced APC regulation definitely deserves further investigation.

Declarations of interest

None.

Conflict of interest

The authors declare that they have no conflict of interest.

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