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Complement receptor immunoglobulin: a control point in infection and immunity, inflammation and cancer

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Summary

The B7 family-related protein, V-set and Ig domain (VSIG4) / Z39Ig / complement receptor immunoglobulin (CRIg), is a new player in the regulation of immunity to infection and inflammation. The unique features of this receptor as compared with classical complement receptors, CR3 and CR4, have heralded the emergence of new concepts in the regulation of innate and adaptive immunity. Its selective expression in tissue macrophages and dendritic cells has been considered of importance in host defence and in maintaining tolerance against self-antigens. Although a major receptor for phagocytosis of complement opsonised bacteria, its array of emerging functions which incorporates the immune suppressive and anti-inflammatory action of the receptor have now been realised. Accumulating evidence from mouse experimental models indicates a potential role for CRIg in protection against bacterial infection and inflammatory diseases, such as rheumatoid arthritis, type 1 diabetes and systemic lupus erythematosus, and also in promotion of tumour growth. CRIg expression can be considered as a control point in these diseases, through which inflammatory mediators, including cytokines, act. The ability of CRIg to suppress cytotoxic T cell proliferation and function may underlie its promotion of cancer growth. Thus, the unique properties of this receptor open up new avenues for understanding of the pathways that regulate inflammation during infection, autoimmunity and cancer with the potential for new drug targets to be identified. While some complement receptors may be differently expressed in mice and humans, as well as displaying different properties, mouse CRIg has a structure and function similar to the human receptor, suggesting that extrapolation to human diseases is appropriate. Furthermore, there is emerging evidence in human conditions that CRIg may be a valuable biomarker in infection and immunity, inflammatory conditions and cancer prognosis.

Key words: *CRIg/VSIG4/Z39Ig; macrophages; dendritic cells; infection and immunity; cytokines; alternative complement pathway, complement receptors; inflammatory diseases; CRIg-Fc fusion protein; cancer*

Abbreviations

APC	antigen presenting cell
CR	complement receptor
CRIg	complement receptor immunoglobulin
HBV	hepatitis B virus
IL	interleukin
MAP	mitogen activated protein
PI3	phosphatidylinositol 3
PKC	protein kinase C
TNF	tumour necrosis factor
VSIG4	B7 family-related protein V-set and Ig domain
Z39Ig	Protein with immunoglobulin domains derived from expressed sequence tag #Z39624

Introduction

Complement plays an important role in the opsonisation of circulating pathogens, facilitating the phagocytosis and removal of these pathogens by phagocytes. Fragments of complement are recognised by four complement C3 fragment receptors, CR1 (CD35), CR2 (CD21), CR3 (CD11b/CD18), and CR4 (CD11c/CD18), with an additional receptor, complement receptor immunoglobulin (CRIg), being added to this list in 2006 (fig. 1; [1, 2]). The major developments surrounding CRIg are summarised in table 1. Although CRIg (originally named Z39Ig) was first described in 2000 [3], its biological properties were not evident until van Lookeren Campagne and colleagues published their extensive work on its prime role as a complement receptor promoting phagocytosis of bacteria *in vivo* and *in vitro* [4]. Working with mice, this group not only continued to provide supportive evidence of its importance in defence against infection and promoting phagocytosis but also discovered its property of uniquely interacting with components of the alternative complement pathway and inhibiting its activation [2, 5]. This led to the development of a CRIg-Fc fusion protein, which demonstrated anti-inflammatory activity in several murine models of inflammatory diseases [6–10]. In a parallel publication, Vogt et al. [6] demonstrated the immunosuppressive activity of CRIg by using a VSIG4(CRIg)-Fc fusion protein *in vitro* and also when injected into mice. Interestingly, human dendritic cells ex-

pressing CRIg were found to suppress T cell proliferation, expression of activation markers (CD25 and CD69) and production of helper T cell (Th1) cytokines [11]. This suggests that CRIg$^+$ dendritic cells may promote tolerance and immunosuppression. This may possibly explain recent findings that CRIg$^+$ human macrophages may have a role in regulating malignancy [12–15].

Inflammatory mediators exert their effects on macrophages by regulating CRIg expression. Accordingly, cytokines have been shown to alter CRIg expression on human macrophages [16, 17]. Work along this line should be expanded to gain a better understanding of how inflammatory mediator networks operate in infection and immunity, chronic inflammatory diseases and cancer. Since CRIg and the classical complement receptors, CR3 and CR4, which also bind complement opsonised bacteria, are expressed concomitantly on macrophages, the relative commonalities and differences when engaged need to be appreciated. Finally, the mechanisms of CRIg-mediated protection against infection and inflammatory conditions as well as presumed susceptibility to cancer are becoming research topics of intense interest.

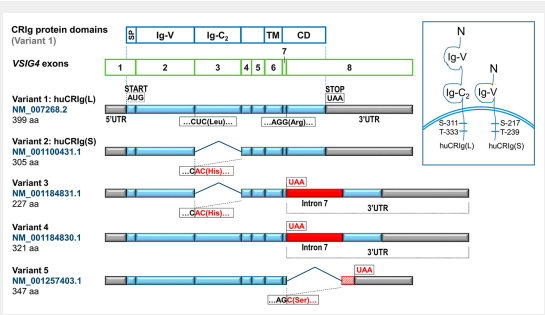

Figure 1

Schematic representation of the CRIg protein domains aligned with the five *VSIG4* splice variant transcript structures. The transcripts structures are derived from the NCBI Reference Sequence Database (RefSeq) using the following mRNA accession numbers: NM_007268.2, NM_001100431.1, NM_001184831.1, NM_001184830.1, and NM_001257403.1, as noted in the figure. The structure of the longest CRIg isoform (variant 1, huCRIg(L)) is used as the reference, with the protein domains denoted as: SP, signal peptide; Ig-V, immunoglobulin domain V-type; Ig-C_2, immunoglobulin domain V-type; TM, transmembrane region, and CD, cytoplasmic domain. The relative *VSIG4* exon structure reveals that SP, Ig-V, Ig-C_2, and TM are encoded specifically by exons 1, 2, 3, and 6, respectively, whereas CD is encoded by exons 7 and 8. The short form (variant 2, huCRIg(S)) differs from the long form by the exclusion of exon 3, the Ig-C_2 domain, and a change at codon 138 from CUC (Leucine) to CAC (Histidine). In this isoform, the overall size of the CRIg protein is reduced from 399 to 305 amino acids (aa). Variant 3 features this identical alteration, in addition to the inclusion of intron 7 which creates a stop codon (UAA) adjacent the last codon of exon 7, causing the majority of the CD to be missing (227 aa). Variant 4 features the same loss of the CD as variant 3, but retains all other domains (321 aa). Variant 5 retains the same domains as variants 1 and 4 (exon 1 to 7 inclusive), but is unique from the other four variants in that a portion of exon 8 is excluded, which creates a change at codon 321 (exon 7-8 junction) from AGG (Arginine) to AGC (Serine), followed by a 'frameshift' that results in a stop codon generated 26 aa downstream (347 aa). Insert: shows a schematic representation of the long and short forms of CRIg, expressed in macrophages. The extracellular portion of CRIg contains an Ig-V and/or Ig-C_2 domain(s). Putative phosphorylation sites for cAMP/cGMP-dependent protein kinase (S-311 or S-217) and protein kinase C (T-333 or T-239) have been proposed to be present in the cytoplasmic domain of CRIg.

CRIg structure

CRIg, a member of the transmembrane protein of the type 1 immunoglobulin (Ig) superfamily, is encoded by the *VSIG4* gene located in the pericentromeric region of the human X chromosome. The gene, first documented as "Z39Ig" by Langnaese et al. [3], contains eight exons and has a length of 18.3 kb. In humans, the product, CRIg, referred to as the long form (huCRIg(L) [4], contains both a constant (C2-type) and a variable (V-type) immunoglobulin domain (fig. 2). In addition, a short form, huCRIg(S), was also identified. The huCRIg(S) contains only the V-type immunoglobulin domain, with no C2-type [4]. The two different forms of CRIg arise from alternative splicing of the *VSIG4* gene, which also has the potential to give rise to a total of five different variants (fig. 2). Only one form of CRIg, containing a single IgV-type domain, is expressed in murine macrophages and the data show that the V-type domain of the CRIg protein is essential for its ability to bind complement components and to promote phagocytosis, whereas the significance of the C2-type domain remains uncertain. The intracellular portion of huCRIg(L) harbours two potential phosphorylation sites, a cAMP/cGMP-dependent protein kinase phosphorylation site at S-311, and a protein kinase C phosphorylation site at T-333 [3]. Both S and T

Table 1: Key developments in the field of complement receptor immunoglobulin (CRIg).

Development	References
Identification of Z39Ig – immunoglobulin superfamily member, gene localisation in the pericentriomere region of human X chromosome.	[3]
Z39Ig/CRIg expressed predominantly in fixed tissue macrophages.	[4]
CRIg promotes clearance of bacteria and viruses in experimental models; a key role for CRIg+ Kupffer cells.	[4, 19]
CRIg is a complement receptor which promotes phagocytosis of bacteria by macrophages. Unique properties of the receptor described.	[4, 20, 21]
CRIg uniquely interacts with components of the alternative complement activation pathway; is an inhibitor of the alternative pathway.	[2, 5]
VSIG4 induces T cell immunosuppression; CRIg+ dendritic cells and tolerogenic responses; negatively regulates T cell-dependent immunoglobulin isotype switching in the mouse and human immune system.	[6, 11, 24]
Development of CRIg-Fc fusion protein; protects against experimental arthritis, systemic lupus erythematosus, type 1 diabetes and other diseases.	[6–10, 27]
Adoptive transfer of CRIg+ macrophages protects against immune-mediated liver injury in a mouse model.	[29]
CRIg expression in tumour- associated macrophage; poor prognosis; promotes tumour growth (mouse and human immune system).	[12–15]
Regulation of CRIg expression in human macrophages by inflammatory mediators (including cytokines) and anti-inflammatory agents.	[16, 17, 33]
CRIg expression in macrophages in human tissues, large intestine, synovial tissue, liver.	[12–15, 26, 28, 30, 33, 36]
CRIg as a biomarker in human conditions: preeclampsia, chronic hepatitis B virus infection, heart failure, cancer	[12–15, 33–36]

residues are present on the intracellular domain of huCRIg(S) at residues 217 and 239 (fig. 1). The significance of these sites is currently unknown but could provide a means to regulate CRIg function. Surprisingly, the reported sizes of the protein (50kDa and 45kDa for the long and short forms, respectively) [4] do not agree with the estimated protein sizes. This suggests that some post-translational modifications, such as glycosylation, have occurred [18].

CRIg promotes phagocytosis and antimicrobial action of macrophages

Although there has been limited publication of data showing that CRIg plays a role in protection against infection, the results are quite convincing. Using CRIg$^{-/-}$ mice, Helmy et al. [4] showed that CRIg was important in the clearance of the intracellular bacterium *Listeria monocytogenes* by liver resident/fixed macrophages (Kupffer cells), and preventing dissemination of the bacteria to other organs. This protection provided by CRIg was evident by a reduction in numbers of bacteria and an increase in mouse survival. Similar results were obtained when the extracellular pathogen *Staphylococcus aureus* was examined, leading to the conclusion that CRIg is required for the rapid clearance of both intracellular and extracellular C3-opsonised bacteria. More recently, the anti-infective actions of CRIg have been demonstrated with adenoviruses in mice [19]. The work showed that CRIg-mediated viral clearance by liver Kupffer cells was significantly reduced in CRIg$^{-/-}$ mice compared with wild type mice.

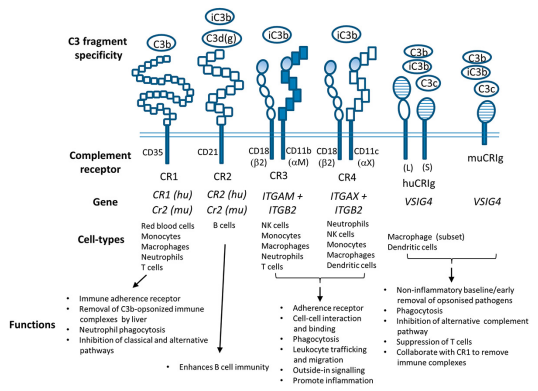

Figure 2

Characteristics, function and expression of complement receptors
The structural domains of the five known types of complement receptors are depicted, together with their specificity of C3 fragments, the genes encoding them, their distribution amongst the different leucocyte types and their known functions. CR1, CR2 and CRIg are single transmembrane proteins with extracellular portions, transmembrane domains and cytoplasmic tails whereas CR3 and CR4 are transmembrane heterodimers of a common β2 integrin (CD18) chain and an α integrin chain, αM (CD11b) or αX (CD11c). Murine CR1 and CR2 are derived from the same gene by alternative splicing whereas the human counterparts are encoded by 2 different genes. CR1 contains thirty short consensus repeats (SCR) and CR2 has fifteen SCR. CD18 contains four repeats and a Von Willebrand factor type A domain (lightly shaded oblong shape). The α integrins contain, within their extracellular portions, seven FG-GAP repeats (rectangles) and a Von Willebrand factor type A domain. Two human CRIg isoforms, huCRIg(L) for the long form and huCRIg(S) for short, have been described. Both isoforms contain an N-terminal ligand binding domain that belongs to the IgV-type of immunoglobulin domains (horizontal stripes). The long form of CRIg also contains a membrane proximal domain that is an IgC-type immunoglobulin domain. The function of this domain is unclear. The murine form, similar to CRIg(S), contains only the IgV-type of immunoglobulin domain but the cytoplasmic tail is shorter than that of huCRIg(S). The IgV domains are believed to be responsible for binding C3 fragments.

CRIg functions as a complement receptor on macrophages, promoting phagocytosis by binding to C3b and iC3b-coated particles [4]. CRIg with bound C3b is internalised into Kupffer cells and becomes localised in a pool of constitutively recycling membranes. At the initial stages of phagosome formation, CRIg was actively recruited from recycling endosomes to the sites of C3b-coated particle ingestion. Then, prior to the fusion of the phagosome and lysosome to avoid degradation, CRIg is likely recycled from the phagosome to the endosome for use in further phagocytic events, ensuring a readily available source of CRIg on the cell surface and thereby enabling a faster rate of phagocytosis when bacteria are encountered [1].

CRIg differs from CR3 not only in its expression (selectively in macrophages and dendritic cells) but also in the mechanisms of phagocytosis and induction of immune responses. Whereas CRIg binds to both C3b and iC3b, CR3 binds only iC3b ([1, 4], table 2). In murine peritoneal macrophages, Gorgani et al. [20] reported that, whereas efficient binding of opsonised particles to CR3 required divalent cations (Mg^{2+} and Ca^{2+}), binding to CRIg did not require these ions. CRIg-mediated binding also did not require integrin activation. Gorgani et al. [20] further investigated the relative contribution of CRIg and CR3 to complement-mediated phagocytosis, finding that CRIg enhanced phagocytosis only in resident macrophages, while CR3 acted as the dominant complement receptor in infiltrating activated macrophages at sites of inflammation. It was demonstrated that CRIg expression is capable of increasing the rate of phagocytosis in resident peritoneal macrophages, and is absent from inflammatory macrophages, suggesting that CRIg has a function in immune clearance and tissue homeostasis, and that initiating CRIg-mediated phagocytosis avoids the inflammatory cytokine cascade that is associated with phagocytosis via CR3 [4, 16]. Consistent with this idea, CRIg has been shown to play a role in clearing pathogens during early infection such as in the liver sinusoidal lumen through which invading pathogens have to pass [4]. Furthermore, this receptor may participate in the baseline removal of C3-opsonised apoptotic cells and cell debris without involving CR3 [1, 2], the engagement of which leads to a systemic inflammatory response. CRIg may not work alone in this function. CR1 is known to recognise C3b without internalisation. In this manner, CR1 would remove immune complexes from the circulation, thus preventing pathological deposition. The role for CRIg may be to mediate removal of potentially pathological agents through internalisation. Thus, CR1 and CRIg may have important roles in maintenance of homeostasis, providing baseline clearance of pathogens [1, 2].

Apart from promoting phagocytosis of opsonised bacteria, CRIg may also affect their killing. It has been reported in the human macrophage cell line THP1 and the J774 mouse macrophage cell line that CRIg binds chloride intracellular channel 3 (CLIC3), an intracellular chloride channel protein

that is required for the clearance of *Listeria monocytogenes* [21]. Thus, CRIg was shown to co-localise with CLIC3 on the membranes of *Listeria monocytogenes*-containing vacuoles in the mouse macrophage cell line. Using an anti-CRIg antibody that enhanced macrophage-mediated killing of intracellular *Listeria monocytogenes*, it was demonstrated that this killing-enhancing effect of anti-CRIg antibody required CLIC3 as the effect was abolished in macrophages from $CLIC3^{-/-}$ mice. The mechanism probably involved a CRIg-mediated increase in Cl^- concentration and a decrease in pH in the vacuoles. This contrasts with CR3 in the killing of phagocytosed microorganisms such as *Salmonella* serovar Typhimurium by human phagocytes in that this receptor is solely needed for the phagocytic step whereas the killing mechanism depends on a Toll-like receptor 4-mediated activation of NADPH oxidase [22].

Since the majority of the above data have been generated from mouse macrophages and experimental mouse models, care needs to be exercised when translating the findings to human diseases. There are major differences in complement receptors between mouse and humans; for example, CR1 has a very limited expression profile in the mouse. In terms of CRIg, mouse macrophages express one form that also contains the IgV domain ([4]; fig 1 and 2, table 2). Studies with human macrophages and phagocytosis have been limited to monocyte-derived macrophages in culture which express CRIg. The levels of this receptor in these macrophages correlated with the degree of phagocytosis of complement-opsonised *Candida albicans* [17]. The comparison between mouse and human CRIg expressing macrophages are shown in table 2.

Immunosuppressive and anti-inflammatory functions

CRIg is able to bind the alternative pathway complement components C3b and iC3b, which functions to bind foreign particles for phagocytosis. A study by Wiesmann et al. [5] investigated whether the binding of CRIg to C3b inhibits the inflammatory convertase activation of the alternative complement pathway. They successfully solved the crystal structure of CRIg bound to C3b, which indicated that unlike most C3b binding molecules, CRIg predominantly binds to the β-chain (rather than the α-chain). It was demonstrated that CRIg inhibited both the C3 and C5 convertases of the alternative but not the classical pathway. This inhibition is related to the ability of CRIg to bind C3b, whose function in both convertases is to recruit C3 and C5, thereby enabling their cleavage by factor Bb, the catalytic subunit of the convertases. Further studies with C5 revealed that when CRIg was bound to C3b, it prevented C5 from interacting with C3b. As a consequence, C5 could not be cleaved by the C5 convertase (C3bBb3b) to C5a and C5b [2]. Selectivity for alternative pathway convertases can be explained on the basis that CRIg does not bind C4b and hence cannot compete effectively with the classical pathway C5 convertase (C4b2a3b) for C5 binding [2].

Katschke et al. [7] demonstrated, through the generation of a soluble CRIg fusion protein (CRIg-Fc), that it was possible to reverse inflammation and bone loss in two different experimental models of arthritis. By inhibiting the alternative complement pathway with the fusion protein, the study demonstrated that the alternative pathway of complement is essential for disease induction and progression. This fusion protein was further investigated in a recent study in which it was administered to lupus-prone MRL/lpr mice [8]. The group reported decreased skin and kidney inflammation, and decreased proteinuria and pyuria in mice administered CRIg-Fc fusion protein. The protein also protected against other conditions known to involve complement. Chen et al. [9] demonstrated protection against experimental ischaemia/reperfusion injury in a mouse model. Administration of the CRIg-Fc protein prevented local intestinal and remote lung damage, which was associated with decreased complement deposition. In experimental autoimmune uveoretinitis, Chen et al. [10] showed that retinal inflammation was suppressed by treatment with the CRIg-Fc protein, which was also associated with reduced deposition of C3b and factor B in the tissues.

Vogt et al. [6] further investigated the immune function of CRIg. It is known that T cell activation by antigen presenting cells (APCs) is both positively and negatively regulated by the B7 family of proteins that are found on the surface of the APC [23]. Through screening, it was found that a mouse copy DNA sequence that appeared to be derived from messenger RNA encoding the CRIg protein, when translated was 20% identical to the amino acid sequences of known B7 family members. It was therefore hypothesised that CRIg, being related to the B7 protein family, would show some degree of regulation over T cells. They showed CRIg to be a strong negative regulator of murine and human T cell proliferation and interleukin-2 (IL-2) production, though the mechanisms of this regulation remain uncertain. It was further found that CRIg was only expressed on the surface of resting tissue macro-

Table 2: Comparison between mouse and human complement receptor immunoglobulin (CRIg) on macrophage phagocytosis.

Function	Experimental approach	Human (L)	Human (S)	Murine	References
Recognition of C3 fragments	Formation of rosettes following incubation of CRIg+ cells with complement-opsonised sheep erythrocytes	Yes	Not determined	Yes	[4]
Binding to C3b and iC3b	Binding of huCRIg (L)-Fc, huCRIg(S)-Fc or muCRIg-Fc to C3b and iC3b	Yes	Yes	Yes	[4]
Phagocytosis	Phagocytosis of complement-opsonised particles	(Yes; but isoform not determined)		Yes	[16, 17, 20]
Clearance of *Listeria monocytogenes* and *Staphylococcus aureus*	Assessment of bacterial load in the blood, spleen and lung of mice	Not determined	Not determined	Yes	[4]
Inhibition of the alternative complement pathway	Cleavage of C3 and C5 by their respective convertases	Yes (but isoform not stated)		Yes	[5, 7]

phages, and not on the surface of macrophages activated by lipopolysaccharide. This fact indicates that CRIg may have an important role in maintaining T cell unresponsiveness in healthy tissues. The regulatory role of CRIg on antibody production has also been demonstrated [24]. The work conducted in mice showed that CRIg on macrophages negatively regulates T cell-dependent immunoglobulin isotype switching through an action on T cell activation and differentiation.

Expression of CRIg in human dendritic cells was first reported by Ahn et al. [25]. While the immunosuppressive effects of CRIg-Fc fusion protein have been demonstrated by its direct interaction with T cells, Xu et al. [11] transfected human dendritic cells with CRIg such that they constitutively expressed CRIg and showed that these dendritic cells inhibited proliferation of allogenic T-cells, and decreased expression of activation markers and pro-inflammatory cytokines production from these cells. These suggest that CRIg expression on dendritic cells has anti-inflammatory outcomes; plays a role in tissue homeostasis and host defence, and suggest a potential function in suppressing effector T cells.

It has been reported with respect to inflammatory diseases that CRIg expression and levels of $CRIg^+$ macrophages relate to the intensity of the inflammatory reaction. For example, Tanaka et al. [26] showed that $CRIg^+$ macrophages were present in the large intestine of mice and this expression decreased during inflammatory colitis. Fu et al. [27], using the nonobese diabetic mouse model of type 1 diabetes, found that $CRIg^+$ macrophages were associated with diabetes resistance. Mice given injections of CRIg-Fc fusion protein had lower incidence of diabetes. CRIg expression has been found in macrophages infiltrating tissue in other inflammatory conditions, including in atherosclerosis where receptor expression was associated with foamy macrophages in human carotid atherosclerotic plaques [28]. The role of $CRIg^+$ macrophages in protection against inflammation is best seen from the results of Jung et al. [29], who demonstrated the protective role of these macrophages in a model of immune-mediated liver injury. Mice lacking CRIg showed increased liver pathology and poor survival rates, associated with increased antigen-induced responses by liver T and natural killer T cells. Interestingly the effect of lack of CRIg in these mice could be overcome by adoptive transfer of $CRIg^+$ Kupffer cells.

Since at least one of the human CRIg receptors, the S form, is structurally similar to the murine CRIg in that they contain only the IgV-type domain (fig. 1 and fig. 2), we can tentatively conclude that these results from experimental models of human diseases are relevant to the human immune system and inflammatory diseases, but this obviously requires examination. The presence of $CRIg^+$ macrophages in synovial tissue has been reported in rheumatoid arthritis [28, 30] and in experimental arthritis in mice [31]. In one study the CRIg+ macrophages in the synovial tissue were defined as $CRIg^+CR4^+$ and $CRIg^+CR4^-$. Interestingly, the former predominated in rheumatoid arthritis compared with osteoarthritis. Although data were not presented, CR3 was expressed in a similar manner to CR4 [30]. It is tempting to speculate that the $CRIg^+CR4^-$ subpopulation may be playing a protective role in this disease and that manipulating the number of this subpopulation may be potential new avenues to treat rheumatoid arthritis [29].

$CRIg^+$ macrophages and cancer

Recently, it has been shown that macrophages infiltrating lung tissue in patients with non-small-cell lung cancer express high level of CRIg [12]. The authors of this article highlighted that CRIg downregulated $CD4^+$ and $CD8^+$ T-cell proliferation and cytokine production. This work was further extended into a mouse model of Lewis lung carcinoma in which the effect of CRIg deficiency on tumour growth was examined. The result showed that $CRIg^{-/-}$ mice had significantly smaller tumours than wild type mice [12]. The role of CRIg in cancer pathogenesis is likely to be of relevance across different cancers. Sturtz et al. [13] conducted a gene microarray study in breast cancer patients and showed that tumour-adjacent tissue had >5 fold increase in CRIg expression compared with distant tissue. Investigation extended to CRIg expression in glioma using tissue microarray. The result of this study showed that CRIg expression in glioma patients is higher than control. Indeed, CRIg upregulation correlated with poor prognosis in this type of cancer [14]. Similarly, by using gene microarray from lymphoma patients, CRIg was found to be one of the most upregulated genes in T cell / histiocyte-rich large B-cell lymphoma [15]. Cancer has been shown to be associated with decreased production of interferon-γ by T cells in the tumour environment [32]. Previous studies have shown that this cytokine causes a decrease in CRIg expression in human macrophages, *in vitro* and *in vivo* [17, 33], and is conducive to the increased CRIg expression in tumour-associated macrophages.

The outcome of immune responses following the engagement of the T cell antigen receptor to the peptide of the antigen expressed on the major histocompatibility complex of the APC is dependent on the costimulatory and coinhibitory signals between the CD28 receptor family on the T cells and B7 family on the APC. These costimulatory and coinhibitory signals may be exploited by tumours for immune evasion. CRIg, a member of the B7 family which is a coinhibitory molecule, being increased in tumour associated macrophages is likely to prevent T-cell mediated tumour destruction [34].

Regulation of CRIg expression in macrophages

Since experimental disease models have shown that CRIg promotes anti-infective and anti-inflammatory events, there is a need to understand whether inflammatory mediators regulate CRIg expression on macrophages. The major evidence for inflammatory networks regulating CRIg expression and associated phagocytosis comes from the work of Gorgani et al. [17]. They demonstrated that the inflammatory mediator and cell activator, arachidonate, caused a marked decrease in CRIg expression in human macrophages, both at the mRNA and cell surface protein expression level [17]. The action of cytokines on this expression in human monocyte-derived macrophages is interesting. The results showed that tumour necrosis factor (TNF),

interferon-γ, IL-4 and transforming growth factor-β1 decreased CRIg expression but the immunosuppressive cytokine IL-10 caused a marked increase in expression. These changes in CRIg expression correlated with the amount of phagocytosis of *Candida albicans* [17]. Further studies in human monocyte-derived macrophages also demonstrated that IL-1β and IL-6 caused a decrease in expression, but were not as potent as TNF [16]. Interestingly, Guo et al. [33] found that interferon-γ not only decreased expression of CRIg on human macrophages *in vitro* but also played a role in decreasing expression in liver macrophages of patients with chronic hepatitis B virus (HBV) infection. A reduced CRIg expression was associated with an increase in plasma HBV load and increased serum alanine aminotransferase levels. This finding supports the infection protective actions of CRIg as well as the anti-inflammatory characteristics.

The findings reported by Gorgani et al. [17] and Ma et al. [16] collectively suggest that mediators of inflammation, including cytokines, may control CRIg expression at two levels: firstly by regulating the development of monocytes into CRIg positive macrophages and secondly on mature macrophages *per se*. The results suggest that exogenously and endogenously generated mediators not only regulate tissue/resident macrophage function by modulating CRIg expression but also act on infiltrating monocytes to control their development into $CRIg^+$ macrophages. This is supported by the findings of Vogt et al. [6] and Gorgani et al. [20] in mice.

While arachidonate is a powerful down-regulator of CRIg expression in human macrophages, the steroidal anti-inflammatory agent, dexamethasone, caused a marked up-regulation of CRIg expression [16, 17]. Evidence suggested that these agents regulate CRIg expression via protein kinase Cα (PKCα) [16, 17]. The arachidonate action on CRIg expression was found to be independent of the cyclooxygenase and lipoxygenase pathways, and did not involve the mitogen-activated protein kinases p38 and ERK1/ERK2, as well as independent of PI3 kinase but dependent on PKC activation [17]. The increase induced by dexamethasone can also be accounted for by an action on PKCα, namely an inhibitory effect [16, 17]. It is interesting that an anti-inflammatory agent has the ability to up-regulate a macrophage receptor which has both phagocytic and anti-inflammatory functions. This suggests that some of the anti-inflammatory properties of dexamethasone may be, in part, mediated by causing changes in CRIg expression. Ma et al. [16] made the further observation that TNF production by human macrophages may autoregulate the full expression of CRIg. Thus the addition of anti-TNF neutralising antibodies increased CRIg expression in cultured human macrophages. Since $CRIg^+$ macrophages are found in synovial tissue of rheumatoid arthritis patients, it is tempting to speculate that the anti-TNF therapy may be protective via this mechanism.

CRIg as a potential disease biomarker

Once we understand better the relationship between CRIg expression and disease progression in inflammatory disorders, it is possible that CRIg expression may be a helpful biomarker to ascertain diagnosis or disease progression and outcomes. The work of Tanaka et al. [30] suggests that there may be benefits for gauging inflammation in rheumatoid arthritis and this is supported by the findings in experimental mouse arthritis [31]. The most convincing example to date comes from the work on preeclampsia, a leading cause of neonatal and maternal morbidity and death [35]. Although there are biomarkers which distinguish between normal pregnancy and preeclampsia, these markers do not distinguish between the non-severe versus the severe form. Using a microarray approach, the upregulated *VSIG4* gene was found to be a marker for the severe form [35]. Others have found that CRIg was differentially expressed between right ventricular and left ventricular dysfunction in human heart failure and suggested this as a biomarker [36]. Furthermore it is evident that CRIg expression in tumour-associated macrophages can be a potential biomarker of prognostic value in cancer patients [12–15]. In chronic HBV infection the levels may be useful to gauge viral load and liver damage [33].

Concluding remarks

The exciting properties of CRIg, first revealed in animal models just over a decade ago, appear not to have escalated into a search for its role in health and disease, which is often associated with findings of this type. The information available, however, places the role of the receptor, from potentially being a major player in protection against infection and chronic inflammatory disease to the other end of the spectrum of increasing susceptibility to cancer. Clearly there is justification to pursue more actively work that will culminate in a greater understanding of (i) the regulatory inflammatory mediator network which controls the expression of the receptor on both macrophages and dendritic cells, (ii) the consequences of complement opsonised bacteria engaging CRIg versus CR3/CR4 on macrophages, (iii) the translation of the experimental models findings to the clinical diseases. The therapeutic potential in chronic inflammatory diseases has already been realised through the generation of CRIg-Fc fusion protein but the many effects of this protein may warrant other approaches of altering the actual expression of CRIg on macrophages at inflammatory sites. Finally, it remains now for "experiments of nature" to teach us what role CRIg and CRIg+ macrophages / dendritic cells play in human diseases, by identifying genetic mutations in the *VSIG4* gene and clinical presentation associated with CRIg deficiency.

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Figures (large format)

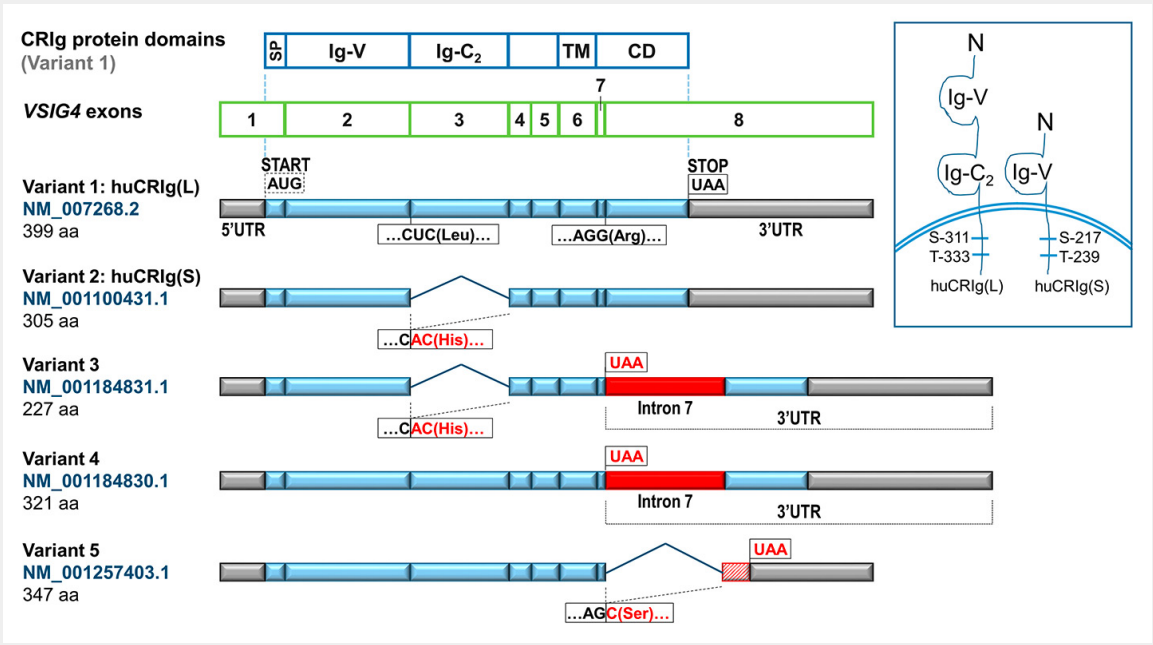

Figure 1

Schematic representation of the CRIg protein domains aligned with the five *VSIG4* splice variant transcript structures. The transcripts structures are derived from the NCBI Reference Sequence Database (RefSeq) using the following mRNA accession numbers: NM_007268.2, NM_001100431.1, NM_001184831.1, NM_001184830.1, and NM_001257403.1, as noted in the figure. The structure of the longest CRIg isoform (variant 1, huCRIg(L)) is used as the reference, with the protein domains denoted as: SP, signal peptide; Ig-V, immunoglobulin domain V-type; Ig-C_2, immunoglobulin domain V-type; TM, transmembrane region, and CD, cytoplasmic domain. The relative *VSIG4* exon structure reveals that SP, Ig-V, Ig-C_2, and TM are encoded specifically by exons 1, 2, 3, and 6, respectively, whereas CD is encoded by exons 7 and 8. The short form (variant 2, huCRIg(S)) differs from the long form by the exclusion of exon 3, the Ig-C_2 domain, and a change at codon 138 from CUC (Leucine) to CAC (Histidine). In this isoform, the overall size of the CRIg protein is reduced from 399 to 305 amino acids (aa). Variant 3 features this identical alteration, in addition to the inclusion of intron 7 which creates a stop codon (UAA) adjacent the last codon of exon 7, causing the majority of the CD to be missing (227 aa). Variant 4 features the same loss of the CD as variant 3, but retains all other domains (321 aa). Variant 5 retains the same domains as variants 1 and 4 (exon 1 to 7 inclusive), but is unique from the other four variants in that a portion of exon 8 is excluded, which creates a change at codon 321 (exon 7-8 junction) from AGG (Arginine) to AGC (Serine), followed by a 'frameshift' that results in a stop codon generated 26 aa downstream (347 aa). Insert: shows a schematic representation of the long and short forms of CRIg, expressed in macrophages. The extracellular portion of CRIg contains an Ig-V and/or Ig-C_2 domain(s). Putative phosphorylation sites for cAMP/cGMP-dependent protein kinase (S-311 or S-217) and protein kinase C (T-333 or T-239) have been proposed to be present in the cytoplasmic domain of CRIg.

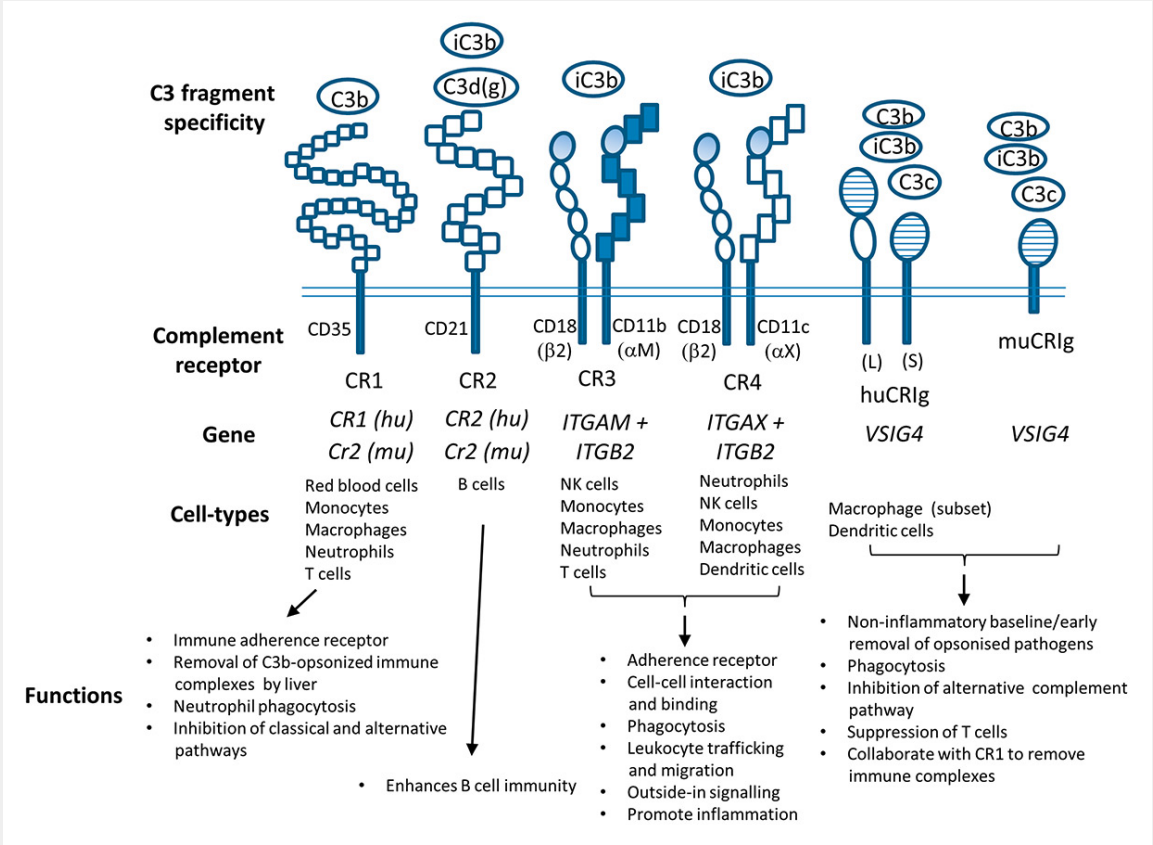

Figure 2

Characteristics, function and expression of complement receptors The structural domains of the five known types of complement receptors are depicted, together with their specificity of C3 fragments, the genes encoding them, their distribution amongst the different leucocyte types and their known functions. CR1, CR2 and CRIg are single transmembrane proteins with extracellular portions, transmembrane domains and cytoplasmic tails whereas CR3 and CR4 are transmembrane heterodimers of a common β2 integrin (CD18) chain and an α integrin chain, αM (CD11b) or αX (CD11c). Murine CR1 and CR2 are derived from the same gene by alternative splicing whereas the human counterparts are encoded by 2 different genes. CR1 contains thirty short consensus repeats (SCR) and CR2 has fifteen SCR. CD18 contains four repeats and a Von Willebrand factor type A domain (lightly shaded oblong shape). The α integrins contain, within their extracellular portions, seven FG-GAP repeats (rectangles) and a Von Willebrand factor type A domain. Two human CRIg isoforms, huCRIg(L) for the long form and huCRIg(S) for short, have been described. Both isoforms contain an N-terminal ligand binding domain that belongs to the IgV-type of immunoglobulin domains (horizontal stripes). The long form of CRIg also contains a membrane proximal domain that is an IgC-type immunoglobulin domain. The function of this domain is unclear. The murine form, similar to CRIg(S), contains only the IgV-type of immunoglobulin domain but the cytoplasmic tail is shorter than that of huCRIg(S). The IgV domains are believed to be responsible for binding C3 fragments.