TITLE: AIM/CD5L: a key protein in the control of immune homeostasis and inflammatory disease

AUTHORS: Lucía Sanjurjo*, Gemma Aran*, Nerea Roher †, Annabel F. Valledor‡, Maria-Rosa Sarrias*,§

*Innate Immunity Group, Health Sciences Research Institute Germans Trias i Pujol (IGTP), †Evolutive Immunology Group, Institute of Biotechnology and Biomedicine, Universitat Autònoma de Barcelona, ‡Nuclear Receptor Group, Department of Physiology and Immunology, School of Biology, University of Barcelona, § CIBERehd. Barcelona, Spain.

SUMMARY SENTENCE:

Review of the involvement of CD5L protein in the modulation of inflammatory responses and its putative use as a biomarker of disease.

ADDRESS FOR CORRESPONDENCE:

Maria-Rosa Sarrias, PhD

Health Sciences Research Institute Germans Trias i Pujol (IGTP), Ctra Can Ruti, Camí de les Escoles s/n, Edifici de Recerca, Planta 1

08916 Badalona, Spain. TF: 34 93-4978693. Fax: 34-934978654.

Email: mrsarrias@igtp.cat

KEY WORDS:

Spα/ Api-6/ CT-2/ SRCR domain/ evolution/ macrophage/CD36/IgM/LXR/RXR/ apoptosis/tuberculosis/atherosclerosis/obesity/hepatocellular carcinoma.

CHARACTER COUNT:

Total: 40635

Number of figures: 2

Number of color figures: 1

Number of references:

Number of words in the Abstract: 129

Number of words in the summary sentence: 22

Abbreviations:

2D-DIGE, twodimensional difference gel electrophoresis; 2DE, two-dimensional electrophoresis; 2DLC, two-dimensional liquid chromatography; 2D-PAGE, two-dimensional polyacrylamide gel electrophoresis; AD, atopic dermatitis; AIM, apoptosis inhibitor expressed by macrophages; AMs, alveolar macrophages; Api-6, Apoptosis inhibitor-6; ATII, alveolar type II; BALF, bronchoalveolar lavage fluid; BCG, bacillus Calmette Guérin; CD5L, CD5-like molecule; CFU, colony forming units; Ch25h, cholesterol-25-hydroxylase; CLI, critical limb ischemia; COPD, chronic obstructive pulmonary disease; DMBT1, deleted in malignant brain tumors; ELISA, enzyme-linked immunosorbent assay; FASN, fatty acid synthase; FSP27, fat-specific protein 27 ; GM-CSF, granulocyte macrophage colony-stimulating factor; HCC, hepatocarcinoma; HCV, hepatitis C virus; HMDM, human monocyte-derived macrophages; ICAM-1, intercellular adhesion molecule 1; Ig, immunoglobulin; IFN-γ, interferon-gamma; iTRAC, isobaric tag for relative and absolute quantitation; KD, Kawasaki disease; LAL, lysosomal acid lipase; LDL, low density lipoprotein; LDLR, LDL receptor; LFA-1, lymphocyte function-associated antigen; LPS, lipopolysaccharide; LXR, liver X receptor; LM, *Listeria monocytogenes* ; M-CSF, macrophage colony-stimulating factor;

MAC-1, macrophage 1 antigen; MARCO, macrophage receptor with collagenous structure; MS, mass spectrometry; MTB, Mycobacterium tuberculosis; NAFLD, non-alcoholic fatty liver; NK, natural killer; oxLDL, oxidized low density lipoproteins; PMA, phorbol myristate acetate; PPARγ, peroxisome proliferator-activated receptor; RCA, regulators of complement activation; RXR, retinoid X receptor; Spα, soluble protein alpha; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SRA, scavenger receptor A; SRCR, scavenger receptor cysteine-rich; SREBP, sterol regulatory element binding protein; TGF-β, transforming growth factor beta; TB, tuberculosis; TLR, toll like receptor; TS, Turner syndrome; WB, Western blot; WT, wild-type.

ABSTRACT

CD5L, a soluble protein belonging to the scavenger receptor cysteine-rich superfamily, is expressed mostly by macrophages in both lymphoid and inflamed tissues. The expression of this protein is transcriptionally controlled by liver X receptors, members of the nuclear receptor family that play major roles in lipid homeostasis. Research undertaken over the last decade has uncovered critical roles of CD5L as a pattern recognition receptor of bacterial and fungal components and in the control of key mechanisms in inflammatory responses, with involvement in processes such as infection, atherosclerosis, and cancer. In this review, we summarize the current knowledge of CD5L, its roles at the intersection between lipid homeostasis and immune response, and its potential use as a diagnostic biomarker in a variety of diseases, such as tuberculosis and liver cirrhosis.

INTRODUCTION

The immune system serves to protect the host from pathogenic and sterile insults. In both types of aggression a variety of cellular and protein systems contribute to a coordinated response aimed at resolving the infection and/or recovering homeostasis. CD5L (CD5-like molecule) is an emerging key component among the repertoire of immune effectors. It was identified in 1997 as a macrophage secreted protein, hence its original name, soluble protein alpha (Spα) [1]. Given its anti-apoptotic role on leukocytes, it was later termed AIM (Apoptosis Inhibitor expressed by Macrophages) and Api-6 (Apoptosis inhibitor-6) [8]. Research in the last decade has shown that this protein plays a myriad of additional functions, from the modulation of leukocyte migration and inflammatory responses to the control of lipid metabolism. In this review, we will refer to this molecule as CD5L in order to comply with the HUGO Gene Nomenclature. We will provide an overview of the current knowledge of CD5L, covering basic aspects like its cloning and evolution, tissue and cellular expression, as well as its role in leukocyte biology and pathology. Furthermore, current data supporting the capacity of CD5L to serve as a diagnostic marker in several diseases of inflammatory origin will be discussed.

CD5L: BASIC ASPECTS

Cloning

In 1997, Gebe et al. screened a cDNA library comprising human spleen mRNA and discovered that the two longest isolated clones—of 1804 and 2152 bp—encode for a 347-amino acid polypeptide [1]. The coding sequence had the features of a secreted

protein and so it was named soluble protein alpha (Spα) (Figure 1). Analysis of the primary sequence of human CD5L revealed 19 hydrophobic amino acids at its amino terminal end that act as a secretory signal sequence. N-terminal sequencing of two distinct recombinant forms of the protein, namely CD5L-immunoglobulin (Ig) fusion protein produced by COS cells [1] and a CD5L form synthesized by HEK cells [2] respectively, confirmed that these 19 amino acids are absent in the mature protein. This secretory signal sequence is followed by three cysteine-rich domains, each approximately 100 amino acids in length, followed by an in-frame stop codon (Figure 1). Sequence comparison with several other proteins revealed that the three cysteine-rich domains show significant homology to the Scavenger Receptor Cysteine-Rich (SRCR) domain.

Evolutionary insights

The SRCR domain consists of 90 to 110 residues containing 6–8 cysteines with a well conserved disulfide bond pattern [3, 4]. SRCR domains are present in more than 30 different secreted and/or membrane-anchored proteins. Examples of proteins containing SRCR domains are scavenger receptor A (SRA)-I/II [5], macrophage receptor with collagenous structure (MARCO) [6], CD163 [7], and deleted in malignant brain tumors (DMBT1) [8], among others. Many of these proteins are found on cells associated with the immune system and some of them have been implicated in the development and regulation of innate and adaptive immune responses. Interestingly, sequences containing SRCR domains are highly conserved and have been identified in representatives of a variety of animal phyla, from Ciona, sea urchin or *C. elegans* to amphioxus, lamprey, teleost or mammals [3, 4]. The genes that encode SRCR-domain containing proteins are easy to predict from genome sequences but, conversely, they are

difficult to classify in a particular family of proteins. Moreover, it is still not clear whether the SRCR domain originated from a single ancestral gene very early in the evolution or arose independently several times [5, 6].

Besides the presence of three SRCR domains and an N-terminal peptide, CD5L has an additional important feature: it lacks a transmembrane domain. With these three premises we searched for CD5L orthologous genes along the evolutionary tree and found CD5L orthologues in several mammalian species (Table 1). More specifically, we found a high degree of conservation between the primate sequences (identities ranging from 74 to 99%), having dN/dS ratios below one, which indicated evolutionary constrain and also between the placental mammalian sequences (identities ranging from 47 to 99%). Interestingly, we detected that cow CD5L, despite having 51% identity and a predicted signal peptide, shows an extra SRCR domain (methods for these alignments are detailed in supplementary methods). We also identified predicted orthologues in other vertebrates such as birds, reptiles and fish. The avian and reptilian sequences have identities ranging from 8 to 36% with hCD5L and only one avian (Turkey) and one reptilian (Turtle) orthologues meet the 3 domain criteria but only the Turkey homologue has a predicted signal peptide. Interestingly, we detected annotated fish CD5L orthologues (e.g Medaka, *O. latipes* or Stickleback *G. aculeatus*), but they showed an expansion in the number of SRCR domains (from 5 to 16 SRCR domains), which did not match with our three main criteria for CD5L identification. Even the putative homologous of CD5L in sea lamprey (*P. marynus*) showing the highest identity did not meet the main criteria of its mammalian counterparts. Similarly, we could not find CD5L orthologues in *D. melanogaster*, *C. intestinalis*, *C. savigni*, *C. elegans*, *S. purpuratus*, *B. floridae* or *B. belcheri*. Overall, these preliminary analyses may suggest

that CD5L could be an innovation of the mammalian lineage. Alternatively, other vertebrates may have homologous CD5L proteins with variations in the structural characteristics as we have observed in cow and some avian, reptilian or fish sequences. Additional functional data will be necessary to clarify this issue.

Expression in cells and tissue

Regarding tissue expression, Northern Blot analysis identified three distinct RNAs hybridizing to hCD5L in bone marrow, spleen, lymph node, thymus, and fetal liver, but not in non-lymphoid tissues (~2.4, 2.1, and 1.8 Kb in length) [1]. The authors found that the sequence of the three hCD5L mRNA transcripts differed in their 3´ regions, which were of different lengths and differed in the number of AUUUA elements. They suggested that these AU-rich elements participate in regulating hCD5L mRNA stability [1]. Interestingly, subsequent cloning and Northern blot analysis of murine CD5L (mCD5L) revealed a unique band of 1.9 Kb strongly expressed in the spleen and liver and weakly in the lung [7, 8]. In these initial studies, mCD5L mRNA was also detected by RT PCR in the thymus and by in situ mRNA hybridization in the thymus, spleen, and liver tissues. In addition, bacillus Calmette Guérin (BCG)-induced granulomas, which harbor large numbers of infiltrating macrophages, were found in the liver [8]. It was proposed that the lack of variation in the presence of AU-rich elements explained the single mRNA encoding for mCD5L. In this regard, it was suggested that while the tissue distribution of CD5L mRNA transcripts is similar in human and mouse, the abundance of these transcripts may be differentially regulated in the two species [7].

At the protein level, human and mouse CD5L share a high level of sequence identity (68%) and their predicted sizes on the basis of amino acid sequences are similar

(~37 kDa). However, SDS-PAGE and Western blot analysis show that the molecular weights of the human and mouse proteins differ. This observation is attributable to different post-translational modifications (Figure 1). In humans, two forms were defined at 38 and 40 kDa, resulting from distinct sialic acid content [2]. Accordingly, the primary sequence of hCD5L contains a potential region of O-linked glycosylation in a Pro-Ser-Thr-rich polypeptide (PST) separating SRCR domains 1 and 2 [2]. In the mouse, the larger molecular weight (55 kDa) of the protein can be explained by other post-translational modifications [1, 9]. In this regard, while hCD5L contains no N-linked glycans, the mCD5L sequence presents three putative N-glycosylation sites, two of which were verified to bind to N-glycans [9].

It has recurrently been hypothesized that differences in glycosylation patterns between human and mouse proteins result in distinct functional activities. Accordingly, in the present review, we will distinguish between data on mCD5L and hCD5L or refer to both as CD5L. Regarding the relevance of glycosylation in mCD5L function, the mutation of two N-glycosylation sites in the protein was found to affect its secretion and enhance its lipolytic activity in adipocytes (see below) [9]. Further studies will be required to determine whether this differential glycosylation also influences other functional aspects of CD5L biology.

Regulation of expression

Various studies using experimental models and/or human sample analysis have revealed two cellular sources of CD5L, namely epithelial cells in the lung and, to a higher extent, tissue macrophages. Macrophages are the main source of CD5L in the organism, and CD5L expression is upregulated under inflammatory conditions of infectious origin such as endotoxin-induced fulminant hepatitis [10], heat-killed

Corynebacterium parvum injection [11], *Listeria monocytogenes* infection [12], as well as in the course of cardiovascular and metabolic pathologies, such as in atherosclerotic lesions [13] and in the adipose tissue of obese mice [14]. However, in contrast to tissue macrophages, *in vitro* cultured macrophages do not apparently express CD5L unless previously activated with specific stimuli. In this regard, in early studies, mCD5L mRNA expression was lost in freshly isolated thioglycollate-activated peritoneal macrophages after 16 hours of culture in plastic dishes, and its expression could not be re-induced by phorbol myristate acetate (PMA), lipopolysaccharide (LPS), or interferon-gamma (IFN-γ) [8, 12]. These data suggested that other factors within the tissue are required for mCD5L gene expression. However, our later results indicated that cellular hCD5L mRNA and protein levels are increased in two *in vitro* settings, namely in *Mycobacterium tuberculosis* infection of THP1 macrophages [15] and in cultured human monocyte-derived macrophages (HMDM) by maturation with macrophage colony-stimulating factor (M-CSF) or with granulocyte macrophage colony-stimulating factor (GM-CSF) [16]. These data reinforced the notion that CD5L expression is tightly regulated in cells and tissues.

CD5L expression is positively controlled by liver X receptor (LXR) [12, 17], a transcription factor that belongs to the nuclear receptor family and that plays key roles in lipid homeostasis [18]. Two LXR isoforms have been defined, namely LXRα and LXRβ, both activated by oxysterols and specific intermediates in the cholesterol biosynthetic pathway (revised in [19]). LXRα is expressed in tissues with a high metabolic activity, including liver, adipose and macrophages, whereas LXRβ is ubiquitously expressed [19]. Of the two isoforms, LXRα is selectively involved in the regulation of CD5L expression [12]. To positively regulate gene expression, LXRs form heterodimers with another member of the nuclear receptor family, the retinoid X

receptor (RXR). CD5L expression is induced in macrophages by natural LXR ligands, including 25-hydroxycholesterol and oxidized low density lipoproteins (oxLDL), and by synthetic LXR agonists (TO901317 and GW3965) [12, 16, 17, 20], with synergistic induction of CD5L expression resulting from the combined activation by LXR and RXR agonists [12, 17]. Initial studies identified a potential LXR response element at position 5 Kb upstream of the CD5L transcriptional start site [12]. In addition, CD5L is a target gene for sterol regulatory element binding protein (SREBP)-1a, a transcription factor that positively regulates lipogenic genes [21]. A functional SREBP-responsive element corresponding to an E-box element was identified at position -507 in the CD5L promoter [21]. More recently, it has been shown that the transcription factor MafB is required for the induction of CD5L by agonist–activated LXR/RXR through a MafB response element at position -54 in the CD5L promoter [22]. MafB expression is indeed upregulated upon LXR activation through direct and indirect mechanisms, depending on the cellular context [21-23]. These observations would suggest that CD5L expression is coordinately regulated by a complex transcriptional network including LXR/RXR, MafB, and SREBP-1 transcription factors.

Epithelial cells have been described to be an additional cellular source of CD5L. This finding came from massive expression analysis of the genes implicated in tumorigenesis and emphysema in the lung in association with pulmonary inflammation that occurs in lysosomal acid lipase (LAL) knockout (*lal* -/-) mouse. In that study, overexpression of the mRNA encoding CD5L was detected in lung tissue from LAL-deficient mice in comparison to wild-type (WT) animals. Interestingly, subcellular fragmentation revealed that the CD5L mRNA derived from alveolar type II (ATII) cells and not cells purified from the bronchoalveolar lavage (containing 95% macrophages) [24]. This work set the foundations for the generation of a transgenic mouse model

overexpressing mCD5L in ATII cells, which resulted in an increased incidence of lung adenocarcinoma [25] (see more details below).

Cell surface receptors for CD5L

Recent evidence obtained *in vitro* and *in vivo* support the involvement of scavenger receptor CD36 as a *bona fide* cell surface receptor for CD5L [14]. In those studies, non-expressing adipocytes internalized a recombinant form of mCD5L (rmCD5L)—a process that was drastically decreased in the presence of CD36-neutralizing antibodies. Moreover, cellular uptake of systemically administered rmCD5L was markedly lower in CD36-deficient mice compared to WT mice [14]. Internalized rmCD5L colocalized with early endosomes, but not with late or recycling endosomes, thereby suggesting that CD5L could be transported into the cytosol during endosome maturation. In addition, rmCD5L was also internalized by macrophages through CD36, indicating that this surface molecule may serve as a cellular receptor for CD5L endocytosis in several cell types [14].

CD36 is an 88-kDa transmembrane glycoprotein expressed in a wide variety of cell types, such as microvascular endothelial cells, "professional" phagocytes (including macrophages, dendritic cells, and microglia), retinal pigment epithelial cells, erythroid precursors, hepatocytes, adipocytes, cardiac and skeletal myocytes, and specialized epithelial cells of the breast, kidney, and gut [26]. Accumulating evidence shows that CD36 recognizes many types of ligands, including thrombospondin [27], *Plasmodium falciparum* [28], bacterial cell wall components [29], phosphatidyl serine and oxidized phosphatidylserine on the surface of apoptotic cells [30], and additional endogenous ligands such as oxLDL [31], among others. The multivariate ligand recognition of CD36 allows it to exert several functions, depending on the cell type. Importantly, the

capacity of CD36 to internalize modified lipoproteins (e.g. oxLDL), which facilitates cholesterol accumulation in macrophages, links the activity of this receptor to the initiation and perpetuation of atherosclerosis. Also, CD36 is intimately involved in the regulation of fatty acid uptake across the plasma membrane and the subsequent metabolism of this substrate [32]. Thus, it has been proposed that CD36 expression and function influences susceptibility to certain metabolic diseases, such as obesity, insulin resistance, and fatty liver disease [33, 34]. In phagocytes, CD36 is also involved in phagocytosis and the development of an inflammatory response upon pathogen aggression [29, 35, 36]. By analogy with membrane protein CD14, it has been suggested that CD36 functions as an accessory protein to present bacterial and modified host proteins to some toll like receptors (TLRs) [37-41]. Whether the interaction of CD5L with CD36 modulates the binding of CD36 ligands and/or any of its activities remains to be elucidated. Likewise, the biochemical nature of the CD36-CD5L interaction is still unknown.

In addition, given the observation that thymocytes and natural killer (NK)-T cells—in which CD5L is also active [8, 11]—do not express CD36, distinct cell types may have alternative receptors for CD5L. In this regard, recent findings have highlighted the interaction of CD5L with the molecules CD55, Crry, CD59, and factor H [42]. The interaction of CD5L with CD55, Crry, CD59, and factor H was demonstrated by co-immunoprecipitation assays in cellular lysates from transfected HEK293T cells [42]. These are membrane-bound (CD55, Crry and CD59) and soluble (factor H) regulators of complement activation (RCA). The complement system is an essential constituent of host innate immunity that involves around 50 players, including pattern recognition molecules, protein components, proteases, and cell surface receptors. It constitutes a central mechanism of immune surveillance that fights against

pathogens and also against altered homeostasis of healthy and damaged host cells. To prevent self-reactivity, RCA protects host cells from complement attack [43]. CD5L binding to the RCA blocked RCA activity and allowed immune recognition of cancer cells (see below) [42]. These findings open a wide perspective on the functional relationship between CD5L and the complement system.

Presence in blood

In blood, CD5L circulates in high concentrations (~10 μg/mL) [2, 44] in association with IgM [2, 7], and the plasma levels of both proteins are positively correlated [45-47]. The IgM-CD5L interaction was initially discovered upon CD5L detection in IgM but not in IgG or IgA fractions of human serum [48]. The interaction was later demonstrated in direct binding studies using recombinant forms of CD5L (rCD5L) and FCS-free monoclonal hybridoma-produced IgM antibodies [2, 46]. Further interaction studies with rCD5L showed that each of the three SRCR domains of CD5L binds to the Fc region of IgM. Although there is no direct interaction between CD5L and the J-chain, the latter appears to be required for the binding of CD5L to IgM. Therefore, CD5L may bind circulating but not cell surface IgM [46].

Further analysis showed that circulating mCD5L is stabilized in serum as a result of its interaction with IgM, a process that protects mCD5L from renal excretion [46]. This notion was reinforced with a model of intravenous injection of a synthetic Fc portion of IgM heavy chain into mice lacking circulating IgM. Synthetic IgM-Fc associated with endogenous mCD5L, protecting mCD5L from renal excretion and preserving the levels of circulating mCD5L [44].

ROLES IN LEUKOCYTE FUNCTION

CD5L has been implicated in the modulation of many important aspects of leukocyte function. These are explained below and summarized in Figure 2.

Leukocyte apoptosis

Apoptosis is a programmed form of cell death that is considered a key component of various physiological processes. In fact, inappropriate apoptosis occurs in many human pathological conditions, including atherosclerosis, autoimmune disorders, and cancer [49].

Murine CD5L was initially named Apoptosis Inhibitor of Macrophages (AIM), in accordance with its anti-apoptotic effects *in vivo* and *in vitro.* In CD5L-deficient mice, before thymic selection, CD4/CD8 double-positive (DP) thymocytes were more susceptible to apoptosis induced by dexamethasone and irradiation. *In vitro,* rmCD5L significantly inhibited cell death of double positive (DP) thymocytes and CD95/Fas-crosslinking-mediated apoptosis of the monocyte-derived cell line J774A.1 [8]. Later, apoptotic function of mCD5L was corroborated in CD5L-deficient mice, which showed a reduction of T and NKT cells in liver granulomas, compared to WT mice, when challenged with heat-killed *Corynebacterium parvum* [11]. In addition, administration *in vitro* of rmCD5L significantly inhibited apoptosis of liver NKT and T cells obtained from mice injected with *C. parvum* [11]. All together, these observations suggested that mCD5L had the capacity to rescue these cell types from programmed cell death.

In line with the previous findings, mCD5L also contributes to protecting macrophages against apoptosis induced by various pathogens, namely *Bacillus anthracis*, *Escherichia coli*, *Salmonella typhimurium*, and *Listeria monocytogenes* [12,

17, 20]. Moreover, both in humans and mice, CD5L produced by macrophages was identified as a factor that protects these phagocytic cells from the apoptotic effects of diverse agents such as anisomycin [17], cycloheximide [16], cigarette smoke extract [50], and oxidized lipids [13, 16]—the latter facilitating the progression of atherosclerotic disease (see below).

Given all these observations, both mouse and human CD5L forms can be defined as apoptosis inhibitors that support the survival of macrophages and other cell types when challenged by various apoptosis-inducing insults of infectious origin and chemical compounds.

Autophagy and inflammation

Autophagy is a highly conserved cellular degradation process found in eukaryotes ranging from yeast to mammals, and it serves to recycle obsolete damaged or superfluous cell components into basic biomolecules. In this regard, autophagy drives a flow of biomolecules in a continuous degradation-regeneration cycle [51]. During the last decade, autophagic dysfunction has been associated with a broad variety of human pathologies, from cancer to infectious and metabolic diseases. Thus, the modulation of autophagy may represent a pharmacological target for drug development and therapeutic intervention for various human disorders [52, 53].

Autophagy controls inflammation through regulatory interactions with innate immune signaling pathways, through the removal of endogenous inflammasome activators, and through effects on the secretion of immune mediators [54]. CD5L has been shown to influence the monocyte inflammatory response. On the one hand, hCD5L inhibits monocyte TNF [55, 56] and IL-1β production, while enhancing IL-10 secretion [57] upon TLR2 and TLR4 stimulation. We have recently been revealed that

the macrophage anti-inflammatory pattern induced by CD5L is mediated through enhanced autophagy mechanisms in this cell type [57]. Examination of autophagy markers in both THP1 macrophages and PB monocytes reinforced this notion. In this regard, hCD5L increased cellular LC3-II content, LC3 puncta, as well as LC3-LysoTracker Red colocalization. Furthermore, electron microscopy analysis showed increased presence of cytoplasmic autophagosomes in THP1 macrophages overexpressing hCD5L. Silencing experiments indicated that the receptor CD36 was required for hCD5L-induced autophagy, thereby revealing a novel function for the CD36-CD5L axis in the induction of macrophage autophagy and in the control of cellular homeostasis. These observations suggest that the modulation of CD5L-CD36 activity may offer therapeutic options for severe inflammatory conditions associated with deregulated autophagy.

B-cell proliferation

hCD5L was initially described as a novel secreted protein produced in lymphoid tissues that may regulate monocyte activation, function, and/or survival [1]. This suggestion was based on the results of cell binding studies using an hCD5L-immunoglobulin (hCD5L-mIg) fusion protein, which showed that hCD5L binds to peripheral blood monocytes but not to T or B cells. This finding is in apparent contrast with the evidence provided by Yusa et al., who showed that, when in combination with TGF-β, hCD5L inhibits LPS-induced proliferation in B lymphocytes [58]. These authors proposed that CD5L exerts distinct functions depending on the target cell types and/or its combined effects with other cytokines [58].

PATHOPHYSIOLOGICAL IMPLICATIONS

The involvement of CD5L in modulating the activity of macrophages and other cell types may have consequences on the outcome of serious pathologies. In this regard, CD5L has been implicated in several diseases that are highly relevant for human health, mostly of inflammatory origin, ranging from infection and obesity through to cancer.

Antimicrobial responses

Infectious diseases are a major cause of morbidity and mortality worldwide. The mammalian innate immune system is a remarkable complex of cellular and biochemical processes that enable efficient detection and elimination of pathogens that threaten host viability. However, although the generation of a potent immune response is crucial for the containment and eradication of microbial infection, excessive or inappropriate inflammation may be harmful to the host and may result in immunopathology or autoimmunity [59, 60]. Like other members of the SRCR superfamily, such as MARCO [61], DMBT1/SAG/gp340 [62], CD6 [63], CD163 [64], and CD5 [65], CD5L is able to bind bacterial components. Both human and mouse CD5L proteins bind to and aggregate Gram-negative and Gram-positive bacteria [55, 56] and to saprophytic and pathogenic fungi [55]. Moreover, hCD5L has been found to act as a pattern recognition receptor for LPS and lipoteichoic acid (LTA), and competition-binding studies revealed that the binding of hCD5L to LPS and LTA is mediated by two independent sites [56]. In addition to its pathogen-binding properties, CD5L exerts antimicrobial activities. In this regard, initial evidence showed that mCD5L increases macrophage phagocytosis of latex beads [66]. Later on, *in vitro* studies by our group indicated that hCD5L enhances the mycobactericidal activity of macrophages, thus actively participating in the

macrophage response against *M. tuberculosis*. CD5L expression peaked in the early phase of infection, thereby inducing the synthesis of vitamin D-dependent antimicrobial peptides and subsequent autophagy mechanisms that led to mycobacterial killing. These data thus demonstrated that hCD5L plays a role in the host response against *M. tuberculosis* [15].

Moreover, mice that do not express functional LXRs displayed increased susceptibility to infection with the intracellular bacteria *L. monocytogenes*, mainly because of altered macrophage function, accelerated apoptosis, and defective bacterial clearance [12]. Loss of regulation of CD5L expression upon *L. monocytogenes* administration greatly contributed to the increased macrophage apoptosis and higher susceptibility to infection in the LXR-deficient mice. In another experimental model of *L. monocytogenes* infection, transient overexpression of cholesterol-25-hydroxylase (Ch25h), the enzyme that synthesizes the LXR natural ligand 25-hydroxycholesterol, promoted the survival of *L. monocytogenes*-infected cells through mCD5L induction but increased susceptibility of the host to infection [20]. In this scenario, infected mice showed higher bacterial loads in liver and spleen, which correlated with increased bacterial content in macrophages infected *in vitro*. These results thus apparently contradicted the findings described previously in LXR-deficient mice. The authors suggested that these discrepancies reflect different effects of constitutive *versus* transient changes in macrophage apoptosis, supported by the notion that increased survival of *L. monocytogenes*-infected macrophages by transient Ch25h overexpression at the time of infection intensifies the disease. Also, 25-hydroxycholesterol influences immune response independently of LXRs, which may help to explain the differences between these two models [67]. Interestingly, mCD5L inhibited *L. monocytogenes*-induced macrophage death in part by blocking caspase-1 cleavage. The authors

proposed that these events could be part of a strategy evolved by the pathogen to maintain a protected cellular environment for its replication and to prevent immune activation by pyroptotic death of macrophages. Overall, these studies revealed new intersection points between metabolic and inflammatory pathways in which CD5L plays a central role and also highlighted the delicate balance between cell survival and cell death required for the host to resist infection.

Inflammation

A model of LPS-induced hepatitis in mice was used to assess the involvement of mCD5L in the progression of inflammation *in vivo*. Mice overexpressing mCD5L were immunized by heat-inactivated *Propionibacterium acnes* and challenged with intravenous injection of LPS. In these studies, the anti-apoptotic action of mCD5L was linked to increased numbers of liver infiltrating macrophages and hence increased inflammation [66]. The authors also showed that mCD5L promotes macrophage phagocytosis and thus hypothesized that CD5L-dependent support of macrophage survival and phagocytic activity results in efficient clearance of dead cells and infectious or toxic reagents in hepatitis.

Atherosclerosis

Atherosclerosis is an inflammatory pathology characterized by an accumulation of fat deposits and cellular debris within the arterial wall. Two major factors contributing to the pathophysiology of atherosclerosis are hyperlipidemia and inflammation. Low-density lipoprotein (LDL) is a major extracellular carrier of cholesterol and, as such, it plays key physiologic roles, distributing cholesterol to peripheral tissues through the circulatory system. However, under conditions of

hyperlipidemia, specific components of LDL become oxidized (oxLDL) or otherwise modified, and these modifications substantially alter the function of these components. Modified LDL particles cause alterations in the endothelium and are chemotactic for monocytes, facilitating monocyte migration and subsequent differentiation into macrophages. Also, they are avidly taken up by macrophages via scavenger receptors to generate lipid-rich foam cells. The accumulation of these cells and subsequent pro-inflammatory reactions in the artery wall lead to the development of atherosclerotic lesions, which may obstruct the arterial lumen and/or eventually rupture and thrombose, causing myocardial infarction or stroke [68, 69].

In humans and mice, CD5L is highly expressed in lipid-laden macrophages at atherosclerotic lesions. In this regard, the induction of CD5L expression supports macrophage survival within the artery wall and is thus associated with atherogenesis [13]. Indeed, in mice with a double deficiency in CD5L and LDL receptor (LDLR), the development of atherosclerotic lesions induced by a high fat, high cholesterol diet was markedly reduced in comparison to LDLR-deficient mice [13]. Accordingly, a recent report showed that MafB, which directly regulates CD5L expression, also participates in the acceleration of atherosclerosis by inhibiting foam-cell apoptosis [22].

In addition to its anti-apoptotic effects, CD5L participates in other key aspects of atherogenesis. We have recently demonstrated that hCD5L increases macrophage foam cell formation. Together with the finding that rhCD5L binds to oxLDL, we hypothesized that CD5L serves as a soluble protein that transfers oxLDL to CD36 and showed that hCD5L does promote CD36-mediated oxLDL uptake [16]. Furthermore, hCD5L may contribute to macrophage-endothelial cell adhesion to endothelial intercellular adhesion molecule 1 (ICAM-1) by enhancing the expression of the

integrins lymphocyte function-associated antigen (LFA-1) and macrophage 1 antigen (MAC-1) [16].

Chronic kidney disease

CD5L may also have an additional role related to vascular damage, this time localized in the small arteries and arterioles in the kidney, causing nephrosclerosis. This is one of the main pathologies underlying chronic kidney disease, and it may lead to ischemic changes in the glomeruli and interstitium, consequently compromising renal function. In a rat model of nephrosclerosis, immunohistochemistry analysis showed CD5L strongly expressed in macrophages that infiltrated the diseased kidney [70]. Furthermore, treatment with drugs that inhibited kidney damage and lowered macrophage infiltration reduced CD5L expression in renal tissue, thus suggesting the that CD5L expression is critical for the progression of nephrosclerosis [70].

Obesity-associated inflammatory diseases

Obesity is closely associated with insulin resistance—a condition that triggers and/or accelerates multiple metabolic disorders including type 2 diabetes, cardiovascular diseases, and fatty liver dysfunction. Insulin resistance is caused, in part, by chronic, low-grade inflammation in adipose tissue of the obese [71]. This subclinical state of inflammation is dependent mainly on the innate immune system. Activation of TLRs expressed on adipocytes by fatty acids leads to the production of inflammatory adipokines and the recruitment of classically activated inflammatory macrophages (M1 macrophages) into the adipose tissue of obese subjects, enhancing the chronic subacute inflammatory stage [72, 73].

In studies focused on analyzing the putative role of CD5L in inflammation and obesity, mCD5L was shown to induce lipolysis in adipose tissue after its internalization into adipocytes through CD36 [74]. Once in the cytosol, mCD5L binds to fatty acid synthase (FASN), a metabolic enzyme that is highly expressed in adipose tissue and that catalyzes the synthesis of saturated fatty acids, such as palmitate, from acetyl-CoA and malonyl-CoA precursors. Through the interaction between mCD5L and FASN, the former remarkably reduced the enzymatic activity of the latter, thereby decreasing the amount of saturated fatty acids in adipocytes [14]. This response ablated transcriptional activity of peroxisome proliferator-activated receptor (PPARγ), a master transcription factor for the differentiation of adipocytes, leading to diminished gene expression of lipid-droplet coating proteins, including fat-specific protein 27 (FSP27) and Perilipin, which are indispensable for triacylglycerol storage in adipocytes [74]. These events resulted in decreased lipid droplet size, lower numbers of mature adipocytes, and decreased weight and fat mass induced by high-fat diet in mice—findings that are physiological relevant for the prevention of obesity [14, 74]. However, this CD5L-dependent lipolytic response also induced an efflux of free fatty acids from adipose cells, which stimulated chemokine production in surrounding adipocytes through TLR4 activation, concomitant with an infiltration of inflammatory macrophages [75]. Supporting these observations, the progression of obesity-associated inflammation was prevented both locally and systemically in obese CD5L-deficient mice as a result of the abolished infiltration of inflammatory macrophages. Similarly, whole-body glucose intolerance and insulin resistance were ameliorated in obese CD5L null mice. Thus, the absence of mCD5L apparently prevented insulin resistance in obese mice [75]. Consequently, the regulation of hCD5L levels has been proposed as a potential

therapeutic strategy to treat inflammatory diseases associated with obesity, such as the metabolic syndrome [76].

Winer et al. suggested that obesity in humans often increases the serum levels of multiple auto-antibodies, thus causing autoimmune diseases. In accordance, pathogenic IgG antibodies, including a unique profile of auto-antibodies, have been found in obese humans and mice [77]. In this context, it was recently demonstrated that mCD5L modulates the homeostasis of IgM in obese mice fed a high-fat diet, subsequently contributing to auto-antibody production [46]. In that study, the mCD5L-IgM association inhibited IgM binding and internalization by follicular dendritic cells through the Fcα/μ receptor. The authors proposed that this response prolongs the presence of the IgM immunocomplexes on the surface of splenic follicular dendritic cells and may increase IgM-dependent antigen presentation to germinal center B cells, thereby enhancing the development of long-lived plasma cells that produce high-affinity IgG auto-antibodies [46]. Whether CD5L is also associated with the pathogenesis of other autoimmune diseases remains to be seen.

.

Chronic obstructive pulmonary disease

Macrophages are key cellular mediators of immune defense and inflammation in the lung. Among these cells, alveolar macrophages (AMs) are the most abundant population [78]. Although they are essential for pulmonary host defense, they are also involved in enhanced inflammation in chronic obstructive pulmonary disease (COPD) [78]. COPD is characterized by airflow limitation that is not fully reversible and it is a major cause of chronic morbidity and mortality worldwide [79]. AM resistance to apoptosis has been implicated in the pathogenesis of this disease. In this regard, the numbers of AMs, including those positive for CD5L, were found to be significantly

increased in the lungs of a mouse model of COPD [50]. CD5L expression was demonstrated at both mRNA and protein levels in AMs isolated from the bronchoalveolar lavage. *In vitro*, conditioned medium containing CD5L protected U937 macrophage cells from the apoptotic effects of cigarette smoke extract. These studies support the notion that CD5L participates in resistance to apoptosis in COPD-associated AMs.

Cancer

Several reports have pointed to the contribution of CD5L to two highly prevalent malignancies, namely lung adenocarcinoma and hepatocellular carcinoma. Interestingly, by modulating immune responses, CD5L may have opposite effects on the outcome of these conditions, thereby promoting lung and inhibiting liver cancer.

Lung adenocarcinoma

In a transgenic mouse model of specific overexpression of CD5L in myeloid cells, myeloid cell apoptosis was inhibited [80]. These mice also displayed systemically increased myeloid cell proliferation. Interestingly, CD5L overexpression led to lung inflammation and the formation of bronchoalveolar adenocarcinoma, thus lowering animal survival significantly. At the molecular level, it was observed that oncogenic signaling pathways (i.e. increased phospho-Stat3-, phospho-Erk1/2-, and phospho-p38-positive cells) were activated in blood and lung macrophages, dendritic cells, and neutrophils of these mice [80]. Similar effects were observed upon CD5L overexpression in alveolar type II epithelial cells *in vivo* [25]. These mice also presented malignant transformation of the lung, as a result of decreased epithelial cell apoptosis and enhanced pro-inflammatory cytokines/chemokines amounts in lung and serum [25].

Hepatocellular carcinoma in steatosis

A recent study has shown that circulating mCD5L prevented hepatocellular carcinoma (HCC) that arose in a steatotic liver in obese mice [42]. Under these conditions, mCD5L accumulated on the surface of tumor hepatocytes. There, CD5L it interacted with the negative RCA molecules CD55, CD59 and Crry, leading to RCA inactivation and subsequent C3 activation and/or membrane attack complex deposition. The final outcome was induced necrotic death of tumor hepatocytes, which could then be removed by bystander Kuppfer cells. Accordingly, CD5L-deficient mice were highly susceptible to steatosis-associated HCC development. When fed a high-fat diet for one year, all CD5L-deficient mice developed multiple liver tumors, which were confirmed HCC upon histological examination. In contrast, mice with normal mCD5L expression did not develop these tumors after the same dietary challenge [42]. The specific mechanism of RCA inactivation and whether CD5L has the capacity to eliminate other type of tumor cells remain unknown. Interestingly, the authors of that study also observed that CD5L interference with RCA activity was specific for tumor cells, as normal hepatocytes internalized mCD5L through CD36, with concomitant modulation of intracellular lipid metabolism, as described for adipocytes [42]. These results support the use of CD5L as a therapy to specifically target and destroy liver cancer cells through activation of the complement system.

CD5L AS A DISEASE BIOMARKER

Biomarkers have gained significant clinical value in medical practice and have recently been introduced into clinical patient management for the diagnosis, treatment stratification, and prognosis of diverse pathologies. As mentioned, hCD5L is detected in serum in relatively high amounts (μg/mL range), and a large-scale analysis in a healthy

population revealed higher levels in women than in men (mean 6.06±2.1 μg/ml in women, 4.99±1.8 μg/ml in men) [47]. Interestingly, hCD5L peaked in women in their 20s and decreased with age (mean 6.75 ± 2.05 μg/ml at 20 years old, 4.91±1.57 at 70 years old) [47]. Moreover, plasma levels of CD5L are altered in several conditions that arise in an inflammatory context. In this regard, we have seen that mCD5L plasma levels increase up to 10-fold in mice infected with *M. tuberculosis* three weeks after infection, coinciding with peak colony forming units (CFU) numbers in spleen and lung [15]. Likewise, septic shock induction with LPS and zymosan modulates mCD5L plasma levels in mice, resulting in increased levels 24 hours post injection [55].

Several proteomic studies using human plasma/serum, and synovial and bronchoalveolar lavage fluid have highlighted hCD5L protein as a putative biomarker for a number of inflammatory conditions (Table 2). The list of conditions is steadily increasing, ranging from atopic dermatitis [81] to Kawasaki disease [82] and osteoarthritis [83]. Interestingly, most of the studies focused on the value of hCD5L as a biomarker of liver disease [84].

In the liver, chronic inflammation causes high morbidity and mortality worldwide. Hepatitis virus infection, alcohol abuse, and non-alcoholic fatty liver (NAFLD) are the main etiologies associated with this disease. In this context, continuous inflammation as a result of liver damage leads to hepatic fibrosis, which frequently brings about cirrhosis and ultimately HCC [62]. The determination of a plasma biomarker of liver fibrosis or HCC would be of great relevance for the clinical management of these patients. In this context, proteomic analysis based on 2D gel electrophoresis identified enhanced levels of hCD5L in the sera of individuals with liver cirrhosis related to hepatitis C virus (HCV) infection as compared to healthy control

serum, and this protein was proposed as a potential biomarker for assessing liver fibrosis [85]. A later study confirmed that serum levels of hCD5L are indicators of advanced liver fibrosis in HCV [86]. Also, on the basis of its proposed role in immune system regulation, hCD5L was thought to be most likely associated with viral infection rather than cirrhosis [85]. In the context of HCV infection, a study on 19 HCV-positive patients that included 7 with cirrhosis and 5 with HCC suggested that CD5L might be a useful biomarker for early diagnosis of HCC in HCV cirrhotic patients [87]. A more recent report showed that certain combinations of hCD5L indexes normalized to liver marker score distinguished HCC patients from non-HCC patients (mostly HCV) and thus could be applicable for HCC diagnosis [47]. However, a proteomic approach applied in different stages of NAFLD identified and further validated hCD5L as an upregulated serum biomarker for cirrhotic NAFLD, but discarded hCD5L as a surveillance tool for HCC in these patients [88]. These studies suggest that, blood levels of hCD5L may serve as a biomarker of liver cirrhosis in HCV and NAFLD, and HCC only in HCV. Future studies are required to confirm the potential of hCD5L as a biomarker of liver damage/HCC and of other inflammatory diseases. Moreover, significant alterations of hCD5L levels point to a functional implication of this protein in these pathologies-a question that deserves further investigation.

CONCLUDING REMARKS

Research during the last decade has provided a wealth of information on CD5L that places this molecule at the crossroads between immunity and metabolism. However, important questions like the mechanism/s induced by CD5L to inhibit cellular apoptosis remain unanswered. Through its multiple activities, mostly in leukocytes but

also in adipocytes and epithelial cells, CD5L may affect the modulation of diseases of high prevalence, such as atherosclerosis, obesity, and liver cancer. Given our increasing understanding of CD5L function, strategies involving anti/pro-CD5L compounds could provide the basis for novel therapies in these pathological settings. CD5L has an added value in its potential use as a biomarker of disease. The presence of this protein in human fluids facilitates its use as a diagnostic and prognostic biomarker of various aspects of pathology. Future studies will reveal the therapeutic and biomarker utility of this versatile molecule.

ACKNOWLEDGMENTS

This work was supported by grants from the *Fundació la Marató de TV3* (MTV3 12-922), and also from the Spanish Ministry of Health, ISCIII, and FEDER funds (FIS IP13/1906) to MRS. MRS and NR are supported by Miguel Servet (CPII14/00021) and Ramón y Cajal (RYC-2010-06210) fellowships, respectively.

REFERENCES

1. Gebe, J.A., Kiener, P.A., Ring, H.Z., Li, X., Francke, U., Aruffo, A. (1997) Molecular cloning, mapping to human chromosome 1 q21-q23, and cell binding characteristics of Spalpha, a new member of the scavenger receptor cysteine-rich (SRCR) family of proteins. *J Biol Chem* **272**, 6151-8.
2. Sarrias, M.R., Padilla, O., Monreal, Y., Carrascal, M., Abian, J., Vives, J., Yelamos, J., Lozano, F. (2004) Biochemical characterization of recombinant and circulating human Spalpha. *Tissue Antigens* **63**, 335-44.
3. Sarrias, M.R., Gronlund, J., Padilla, O., Madsen, J., Holmskov, U., Lozano, F. (2004) The Scavenger Receptor Cysteine-Rich (SRCR) domain: an ancient and highly conserved protein module of the innate immune system. *Crit Rev Immunol. 2004;24(1):1-37.*
4. Buckley, K.M., Rast, J.P. (2015) Diversity of animal immune receptors and the origins of recognition complexity in the deuterostomes. *Dev Comp Immunol* **49**, 179-89.
5. Bowdish, D.M., Gordon, S. (2009) Conserved domains of the class A scavenger receptors: evolution and function. *Immunol Rev* **227**, 19-31.
6. Dzik, J.M. (2010) The ancestry and cumulative evolution of immune reactions. *Acta Biochim Pol* **57**, 443-66.
7. Gebe, J.A., Llewellyn, M., Hoggatt, H., Aruffo, A. (2000) Molecular cloning, genomic organization and cell-binding characteristics of mouse Spalpha. *Immunology* **99**, 78-86.
8. Miyazaki, T., Hirokami, Y., Matsuhashi, N., Takatsuka, H., Naito, M. (1999) Increased susceptibility of thymocytes to apoptosis in mice lacking AIM, a novel murine macrophage-derived soluble factor belonging to the scavenger receptor cysteine-rich domain superfamily. *J Exp Med* **189**, 413-22.

9. Mori, M., Kimura, H., Iwamura, Y., Arai, S., Miyazaki, T. (2012) Modification of N-glycosylation modulates the secretion and lipolytic function of apoptosis inhibitor of macrophage (AIM). *FEBS Lett. 2012 Oct 19;586(20):3569-74.*

10. Haruta, I., Hashimoto, E., Kobayashi, M., Shiratori, K. (2007) Systemic multifocal epithelial inflammations associated with PBC-like bile duct damage in chronic colitis harboring TCR alpha -/- x AIM -/- mice: does lipoteichoic acid affect the pathogenesis of epithelial inflammation followed by fibrosis? *Ann N Y Acad Sci* **1107**, 300-7.

11. Kuwata, K., Watanabe, H., Jiang, S.Y., Yamamoto, T., Tomiyama-Miyaji, C., Abo, T., Miyazaki, T., Naito, M. (2003) AIM inhibits apoptosis of T cells and NKT cells in Corynebacterium-induced granuloma formation in mice. *Am J Pathol* **162**, 837-47.

12. Joseph, S.B., Bradley, M.N., Castrillo, A., Bruhn, K.W., Mak, P.A., Pei, L., Hogenesch, J., O'Connell R, M., Cheng, G., Saez, E., Miller, J.F., Tontonoz, P. (2004) LXR-dependent gene expression is important for macrophage survival and the innate immune response. *Cell* **119**, 299-309.

13. Arai, S., Shelton, J.M., Chen, M., Bradley, M.N., Castrillo, A., Bookout, A.L., Mak, P.A., Edwards, P.A., Mangelsdorf, D.J., Tontonoz, P., Miyazaki, T. (2005) A role for the apoptosis inhibitory factor AIM/Spalpha/Api6 in atherosclerosis development. *Cell Metab* **1**, 201-13.

14. Kurokawa, J., Arai, S., Nakashima, K., Nagano, H., Nishijima, A., Miyata, K., Ose, R., Mori, M., Kubota, N., Kadowaki, T., Oike, Y., Koga, H., Febbraio, M., Iwanaga, T., Miyazaki, T. (2010) Macrophage-Derived AIM Is Endocytosed into Adipocytes and Decreases Lipid Droplets via Inhibition of Fatty Acid Synthase Activity. *Cell Metabolism* **11**, 479-492.

15. Sanjurjo, L., Amezaga, N., Vilaplana, C., Caceres, N., Marzo, E., Valeri, M., Cardona, P.J., Sarrias, M.R. (2014) The scavenger protein apoptosis inhibitor of macrophages (AIM) potentiates the antimicrobial response against Mycobacterium tuberculosis by enhancing autophagy. *PLoS One* **8**, e79670.

16. Amezaga, N., Sanjurjo, L., Julve, J., Aran, G., Perez-Cabezas, B., Bastos-Amador, P., Armengol, C., Vilella, R., Escola-Gil, J.C., Blanco-Vaca, F., Borras, F.E., Valledor, A.F., Sarrias, M.R. (2014) Human scavenger protein AIM increases foam cell formation and CD36-mediated oxLDL uptake. *J Leukoc Biol* **95**, 509-20.

17. Valledor, A.F., Hsu, L.C., Ogawa, S., Sawka-Verhelle, D., Karin, M., Glass, C.K. (2004) Activation of liver X receptors and retinoid X receptors prevents bacterial-induced macrophage apoptosis. *Proc Natl Acad Sci U S A* **101**, 17813-8.

18. Hong, C., Tontonoz, P. (2014) Liver X receptors in lipid metabolism: opportunities for drug discovery. *Nat Rev Drug Discov* **13**, 433-44.

19. Pascual-Garcia, M., Valledor, A.F. (2012) Biological roles of liver X receptors in immune cells. *Arch Immunol Ther Exp (Warsz)* **60**, 235-49.

20. Zou T, G.O., Berland R, Boyartchuk VL. (2011) Listeria monocytogenes Infection Induces Prosurvival Metabolic Signaling in Macrophages. *Infect Immun* **79**, 1526-35. Epub 2011 Jan 24.

21. Im, S.S., Osborne, T.F. (2012) Protection from bacterial-toxin-induced apoptosis in macrophages requires the lipogenic transcription factor sterol regulatory element binding protein 1a. *Mol Cell Biol* **32**, 2196-202.

22. Hamada, M., Nakamura, M., Tran, M.T.N., Moriguchi, T., Hong, C., Ohsumi, T., Dinh, T.T.H., Kusakabe, M., Hattori, M., Katsumata, T., Arai, S.,

Nakashima, K., Kudo, T., Kuroda, E., Wu, C.-H., Kao, P.-H., Sakai, M., Shimano, H., Miyazaki, T., Tontonoz, P., Takahashi, S. (2014) MafB promotes atherosclerosis by inhibiting foam-cell apoptosis. *Nat Commun* **5**.

23. Menendez-Gutierrez, M.P., Roszer, T., Fuentes, L., Nunez, V., Escolano, A., Redondo, J.M., De Clerck, N., Metzger, D., Valledor, A.F., Ricote, M. (2015) Retinoid X receptors orchestrate osteoclast differentiation and postnatal bone remodeling. *J Clin Invest* **125**, 809-23.

24. Lian, X., Yan, C., Qin, Y., Knox, L., Li, T., Du, H. (2005) Neutral lipids and peroxisome proliferator-activated receptor-{gamma} control pulmonary gene expression and inflammation-triggered pathogenesis in lysosomal acid lipase knockout mice. *Am J Pathol* **167**, 813-21.

25. Li, Y., Qu, P., Wu, L., Li, B., Du, H., Yan, C. (2011) Api6/AIM/SpÎ±/CD5L Overexpression in Alveolar Type II Epithelial Cells Induces Spontaneous Lung Adenocarcinoma. *Cancer Research*.

26. Silverstein, R.L., Febbraio, M. (2009) CD36, a scavenger receptor involved in immunity, metabolism, angiogenesis, and behavior. *Sci Signal* **2**, re3.

27. Asch, A.S., Barnwell, J., Silverstein, R.L., Nachman, R.L. (1987) Isolation of the thrombospondin membrane receptor. *J Clin Invest* **79**, 1054-61.

28. Ockenhouse, C.F., Tandon, N.N., Magowan, C., Jamieson, G.A., Chulay, J.D. (1989) Identification of a platelet membrane glycoprotein as a falciparum malaria sequestration receptor. *Science* **243**, 1469-71.

29. Hoebe, K., Georgel, P., Rutschmann, S., Du, X., Mudd, S., Crozat, K., Sovath, S., Shamel, L., Hartung, T., Zahringer, U., Beutler, B. (2005) CD36 is a sensor of diacylglycerides. *Nature* **433**, 523-7.

30. Mikolajczyk, T.P., Skrzeczynska-Moncznik, J.E., Zarebski, M.A., Marewicz, E.A., Wisniewska, A.M., Dzieba, M., Dobrucki, J.W., Pryjma, J.R. (2009) Interaction of human peripheral blood monocytes with apoptotic polymorphonuclear cells. *Immunology* **128**, 103-13.

31. Endemann, G., Stanton, L.W., Madden, K.S., Bryant, C.M., White, R.T., Protter, A.A. (1993) CD36 is a receptor for oxidized low density lipoprotein. *J Biol Chem* **268**, 11811-6.

32. Ibrahimi, A., Abumrad, N.A. (2002) Role of CD36 in membrane transport of long-chain fatty acids. *Curr Opin Clin Nutr Metab Care* **5**, 139-45.

33. Coburn, C.T., Hajri, T., Ibrahimi, A., Abumrad, N.A. (2001) Role of CD36 in membrane transport and utilization of long-chain fatty acids by different tissues. *J Mol Neurosci* **16**, 117-21; discussion 151-7.

34. Kennedy, D.J., Kuchibhotla, S., Westfall, K.M., Silverstein, R.L., Morton, R.E., Febbraio, M. (2010) A CD36-dependent pathway enhances macrophage and adipose tissue inflammation and impairs insulin signalling. *Cardiovasc Res* **89**, 604-13.

35. Stuart, L.M., Deng, J., Silver, J.M., Takahashi, K., Tseng, A.A., Hennessy, E.J., Ezekowitz, R.A., Moore, K.J. (2005) Response to Staphylococcus aureus requires CD36-mediated phagocytosis triggered by the COOH-terminal cytoplasmic domain. *J Cell Biol* **170**, 477-85.

36. Means, T.K., Mylonakis, E., Tampakakis, E., Colvin, R.A., Seung, E., Puckett, L., Tai, M.F., Stewart, C.R., Pukkila-Worley, R., Hickman, S.E., Moore, K.J., Calderwood, S.B., Hacohen, N., Luster, A.D., El Khoury, J. (2009) Evolutionarily conserved recognition and innate immunity to fungal pathogens by the scavenger receptors SCARF1 and CD36. *J Exp Med* **206**, 637-53.

37. Jimenez-Dalmaroni, M.J., Xiao, N., Corper, A.L., Verdino, P., Ainge, G.D., Larsen, D.S., Painter, G.F., Rudd, P.M., Dwek, R.A., Hoebe, K., Beutler, B., Wilson, I.A. (2009) Soluble CD36 ectodomain binds negatively charged diacylglycerol ligands and acts as a co-receptor for TLR2. *PLoS One* **4**, e7411.

38. Triantafilou, M., Gamper, F.G., Lepper, P.M., Mouratis, M.A., Schumann, C., Harokopakis, E., Schifferle, R.E., Hajishengallis, G., Triantafilou, K. (2007) Lipopolysaccharides from atherosclerosis-associated bacteria antagonize TLR4, induce formation of TLR2/1/CD36 complexes in lipid rafts and trigger TLR2-induced inflammatory responses in human vascular endothelial cells. *Cell Microbiol* **9**, 2030-9.

39. Triantafilou, M., Gamper, F.G., Haston, R.M., Mouratis, M.A., Morath, S., Hartung, T., Triantafilou, K. (2006) Membrane sorting of toll-like receptor (TLR)-2/6 and TLR2/1 heterodimers at the cell surface determines heterotypic associations with CD36 and intracellular targeting. *J Biol Chem* **281**, 31002-11.

40. Abe, T., Shimamura, M., Jackman, K., Kurinami, H., Anrather, J., Zhou, P., Iadecola, C. (2010) Key role of CD36 in Toll-like receptor 2 signaling in cerebral ischemia. *Stroke* **41**, 898-904.

41. Stewart, C.R., Stuart, L.M., Wilkinson, K., van Gils, J.M., Deng, J., Halle, A., Rayner, K.J., Boyer, L., Zhong, R., Frazier, W.A., Lacy-Hulbert, A., El Khoury, J., Golenbock, D.T., Moore, K.J. (2009) CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. *Nat Immunol* **11**, 155-61.

42. Maehara, N., Arai, S., Mori, M., Iwamura, Y., Kurokawa, J., Kai, T., Kusunoki, S., Taniguchi, K., Ikeda, K., Ohara, O., Yamamura, K.-i., Miyazaki, T. (2014)

Circulating AIM Prevents Hepatocellular Carcinoma through Complement Activation. *Cell Reports* **9**, 61-74.

43. Ricklin, D., Lambris, J.D. (2013) Complement in Immune and Inflammatory Disorders: Pathophysiological Mechanisms. *The Journal of Immunology* **190**, 3831-3838.

44. Kai, T., Yamazaki, T., Arai, S., Miyazaki, T. (2014) Stabilization and Augmentation of Circulating AIM in Mice by Synthesized IgM-Fc. *PLoS One* **9**, e97037.

45. Levin, Y., Wang, L., Schwarz, E., Koethe, D., Leweke, F.M., Bahn, S. (2010) Global proteomic profiling reveals altered proteomic signature in schizophrenia serum. *Mol Psychiatry* **15**, 1088-1100.

46. Arai, S., Maehara, N., Iwamura, Y., Honda, S.-i., Nakashima, K., Kai, T., Ogishi, M., Morita, K., Kurokawa, J., Mori, M., Motoi, Y., Miyake, K., Matsuhashi, N., Yamamura, K.-i., Ohara, O., Shibuya, A., Wakeland, EdwardÂ K., Li, Q.-Z., Miyazaki, T. (2013) Obesity-Associated Autoantibody Production Requires AIM to Retain the Immunoglobulin M Immune Complex on Follicular Dendritic Cells. *Cell Reports* **3**, 1187-1198.

47. Yamazaki, T., Mori, M., Arai, S., Tateishi, R., Abe, M., Ban, M., Nishijima, A., Maeda, M., Asano, T., Kai, T., Izumino, K., Takahashi, J., Aoyama, K., Harada, S., Takebayashi, T., Gunji, T., Ohnishi, S., Seto, S., Yoshida, Y., Hiasa, Y., Koike, K., Yamamura, K.-i., Inoue, K.-i., Miyazaki, T. (2014) Circulating AIM as an Indicator of Liver Damage and Hepatocellular Carcinoma in Humans. *PLoS One* **9**, e109123.

48. Tissot, J.D., Sanchez, J.C., Vuadens, F., Scherl, A., Schifferli, J.A., Hochstrasser, D.F., Schneider, P., Duchosal, M.A. (2002) IgM are associated to Sp alpha (CD5 antigen-like). *Electrophoresis* **23**, 1203-6.

49. Elmore, S. (2007) Apoptosis: a review of programmed cell death. *Toxicol Pathol* **35**, 495-516.

50. Kojima, J., Araya, J., Hara, H., Ito, S., Takasaka, N., Kobayashi, K., Fujii, S., Tsurushige, C., Numata, T., Ishikawa, T., Shimizu, K., Kawaishi, M., Saito, K., Kamiya, N., Hirano, J., Odaka, M., Morikawa, T., Hano, H., Arai, S., Miyazaki, T., Kaneko, Y., Nakayama, K., Kuwano, K. (2013) Apoptosis inhibitor of macrophage (AIM) expression in alveolar macrophages in COPD. *Respiratory Research* **14**, 30-30.

51. Feng, Y., He, D., Yao, Z., Klionsky, D.J. (2013) The machinery of macroautophagy. *Cell Res* **24**, 24-41.

52. Levine, B., Kroemer, G. (2008) Autophagy in the pathogenesis of disease. *Cell* **132**, 27-42.

53. Lucin, K.M., Wyss-Coray, T. (2013) Targeting autophagy for disease therapy. *Nat Biotechnol* **31**, 322-3.

54. Deretic, V., Saitoh, T., Akira, S. (2013) Autophagy in infection, inflammation and immunity. *Nat Rev Immunol* **13**, 722-37.

55. Martinez, V.G., Escoda-Ferran, C., Tadeu Simoes, I., Arai, S., Orta Mascaro, M., Carreras, E., Martinez-Florensa, M., Yelamos, J., Miyazaki, T., Lozano, F. (2014) The macrophage soluble receptor AIM/Api6/CD5L displays a broad pathogen recognition spectrum and is involved in early response to microbial aggression. *Cell Mol Immunol* **11**, 343-54.

56. Sarrias, M.R., Rosello, S., Sanchez-Barbero, F., Sierra, J.M., Vila, J., Yelamos, J., Vives, J., Casals, C., Lozano, F. (2005) A role for human Sp alpha as a pattern recognition receptor. *J Biol Chem* **280**, 35391-8.

57. Sanjurjo, L., Amézaga, N., Aran, G., Naranjo-Gómez, M., Arias, L., Armengol, C., Borràs, E.F., Sarrias, M.R. (2015) The human CD5L/AIM-CD36 axis: a novel autophagy inducer in macrophages that modulates inflammatory responses. *Autophagy*.

58. Yusa, S., Ohnishi, S., Onodera, T., Miyazaki, T. (1999) AIM, a murine apoptosis inhibitory factor, induces strong and sustained growth inhibition of B lymphocytes in combination with TGF-beta1. *Eur J Immunol* **29**, 1086-93.

59. Casadevall, A., Pirofski, L.-a. (2003) Antibody-mediated regulation of cellular immunity and the inflammatory response. *Trends in Immunology* **24**, 474-478.

60. Mantovani, A., Allavena, P., Sica, A., Balkwill, F. (2008) Cancer-related inflammation. *Nature* **454**, 436-44.

61. Brannstrom, A., Sankala, M., Tryggvason, K., Pikkarainen, T. (2002) Arginine residues in domain V have a central role for bacteria-binding activity of macrophage scavenger receptor MARCO. *Biochem Biophys Res Commun* **290**, 1462-9.

62. Bikker, F.J., Ligtenberg, A.J., End, C., Renner, M., Blaich, S., Lyer, S., Wittig, R., van't Hof, W., Veerman, E.C., Nazmi, K., de Blieck-Hogervorst, J.M., Kioschis, P., Nieuw Amerongen, A.V., Poustka, A., Mollenhauer, J. (2004) Bacteria binding by DMBT1/SAG/gp-340 is confined to the VEVLXXXXW motif in its scavenger receptor cysteine-rich domains. *J Biol Chem* **279**, 47699-703.

63. Sarrias, M.R., Farnos, M., Mota, R., Sanchez-Barbero, F., Ibanez, A., Gimferrer, I., Vera, J., Fenutria, R., Casals, C., Yelamos, J., Lozano, F. (2007) CD6 binds to pathogen-associated molecular patterns and protects from LPS-induced septic shock. *Proc Natl Acad Sci U S A* **104**, 11724-9.

64. Fabriek, B.O., van Bruggen, R., Deng, D.M., Ligtenberg, A.J., Nazmi, K., Schornagel, K., Vloet, R.P., Dijkstra, C.D., van den Berg, T.K. (2009) The macrophage scavenger receptor CD163 functions as an innate immune sensor for bacteria. *Blood* **113**, 887-92.

65. Vera, J., Fenutria, R., Canadas, O., Figueras, M., Mota, R., Sarrias, M.R., Williams, D.L., Casals, C., Yelamos, J., Lozano, F. (2009) The CD5 ectodomain interacts with conserved fungal cell wall components and protects from zymosan-induced septic shock-like syndrome. *Proc Natl Acad Sci U S A* **106**, 1506-11.

66. Haruta, I., Kato, Y., Hashimoto, E., Minjares, C., Kennedy, S., Uto, H., Yamauchi, K., Kobayashi, M., Yusa, S., Muller, U., Hayashi, N., Miyazaki, T. (2001) Association of AIM, a novel apoptosis inhibitory factor, with hepatitis via supporting macrophage survival and enhancing phagocytotic function of macrophages. *J Biol Chem* **276**, 22910-4.

67. Blanc, M., Hsieh, W.Y., Robertson, K.A., Kropp, K.A., Forster, T., Shui, G., Lacaze, P., Watterson, S., Griffiths, S.J., Spann, N.J., Meljon, A., Talbot, S., Krishnan, K., Covey, D.F., Wenk, M.R., Craigon, M., Ruzsics, Z., Haas, J., Angulo, A., Griffiths, W.J., Glass, C.K., Wang, Y., Ghazal, P. (2013) The transcription factor STAT-1 couples macrophage synthesis of 25-hydroxycholesterol to the interferon antiviral response. *Immunity* **38**, 106-18.

68. Ross, R. (1999) Atherosclerosis--an inflammatory disease. *N Engl J Med* **340**, 115-26.

69. Libby, P. (2002) Inflammation in atherosclerosis. *Nature* **420**, 868-74.

70. Uramatsu, T., Nishino, T., Obata, Y., Sato, Y., Furusu, A., Koji, T., Miyazaki, T., Kohno, S. (2013) Involvement of Apoptosis Inhibitor of Macrophages in a Rat Hypertension Model with Nephrosclerosis: Possible Mechanisms of Action of Olmesartan and Azelnidipine. *Biological and Pharmaceutical Bulletin* **36**, 1271-1277.

71. Shoelson, S.E., Lee, J., Goldfine, A.B. (2006) Inflammation and insulin resistance. *J Clin Invest* **116**, 1793-801.

72. Xu, H., Barnes, G.T., Yang, Q., Tan, G., Yang, D., Chou, C.J., Sole, J., Nichols, A., Ross, J.S., Tartaglia, L.A., Chen, H. (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* **112**, 1821-30.

73. Weisberg, S.P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R.L., Ferrante, A.W., Jr. (2003) Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* **112**, 1796-808.

74. Iwamura, Y., Mori, M., Nakashima, K., Mikami, T., Murayama, K., Arai, S., Miyazaki, T. (2012) Apoptosis inhibitor of macrophage (AIM) diminishes lipid droplet-coating proteins leading to lipolysis in adipocytes. *Biochemical and Biophysical Research Communications* **422**, 476-481.

75. Kurokawa, J., Nagano, H., Ohara, O., Kubota, N., Kadowaki, T., Arai, S., Miyazaki, T. (2011) Apoptosis inhibitor of macrophage (AIM) is required for obesity-associated recruitment of inflammatory macrophages into adipose tissue.

Proceedings of the National Academy of Sciences of the United States of America **108**, 12072-12077.

76. Miyazaki, T., Kurokawa, J., Arai, S. (2011) AIMing at metabolic syndrome. -Towards the development of novel therapies for metabolic diseases via apoptosis inhibitor of macrophage (AIM). *Circ J.***75(**11):2522-31.

77. Winer, D.A., Winer, S., Shen, L., Wadia, P.P., Yantha, J., Paltser, G., Tsui, H., Wu, P., Davidson, M.G., Alonso, M.N., Leong, H.X., Glassford, A., Caimol, M., Kenkel, J.A., Tedder, T.F., McLaughlin, T., Miklos, D.B., Dosch, H.M., Engleman, E.G. (2011) B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. *Nat Med* **17**, 610-7.

78. Vlahos, R., Bozinovski, S. (2014) Role of alveolar macrophages in chronic obstructive pulmonary disease. *Front Immunol.* **5**:435. doi: 10.3389/fimmu.2014.00435.

79. Pauwels, R.A., Rabe, K.F. (2004) Burden and clinical features of chronic obstructive pulmonary disease (COPD). *Lancet.* **364** (9434):613-20.

80. Qu, P., Du, H., Li, Y., Yan, C. (2009) Myeloid-Specific Expression of Api6/AIM/SpÎ± Induces Systemic Inflammation and Adenocarcinoma in the Lung. *The Journal of Immunology* **182**, 1648-1659.

81. Kim, W.K., Hwang, H.R., Kim do, H., Lee, P.Y., In, Y.J., Ryu, H.Y., Park, S.G., Bae, K.H., Lee, S.C. (2008) Glycoproteomic analysis of plasma from patients with atopic dermatitis: CD5L and ApoE as potential biomarkers. *Exp Mol Med* **40**, 677-85.

82. Yu, H.R., Kuo, H.C., Sheen, J.M., Wang, L., Lin, I.C., Wang, C.L., Yang, K.D. (2009) A unique plasma proteomic profiling with imbalanced fibrinogen cascade in patients with Kawasaki disease. *Pediatr Allergy Immunol* **20**, 699-707.

83. Balakrishnan, L., Bhattacharjee, M., Ahmad, S., Nirujogi, R.S., Renuse, S., Subbannayya, Y., Marimuthu, A., Srikanth, S.M., Raju, R., Dhillon, M., Kaur, N., Jois, R., Vasudev, V., Ramachandra, Y., Sahasrabuddhe, N.A., Prasad Ts, K., Mohan, S., Gowda, H., Shankar, S., Pandey, A. (2014) Differential proteomic analysis of synovial fluid from rheumatoid arthritis and osteoarthritis patients. *Clin Proteomics* **11**, 1-14.

84. Armengol, C., Bartoli, R., Sanjurjo, L., Serra, I., Amezaga, N., Sala, M., Sarrias, M.R. (2013) Role of scavenger receptors in the pathophysiology of chronic liver diseases. *Crit Rev Immunol* **33**, 57-96.

85. Gangadharan, B., Antrobus, R., Dwek, R.A., Zitzmann, N. (2007) Novel serum biomarker candidates for liver fibrosis in hepatitis C patients. *Clin Chem* **53**, 1792-9.

86. Mera, K., Uto, H., Mawatari, S., Ido, A., Yoshimine, Y., Nosaki, T., Oda, K., Tabu, K., Kumagai, K., Tamai, T., Moriuchi, A., Oketani, M., Shimada, Y., Hidaka, M., Eguchi, S., Tsubouchi, H. (2014) Serum levels of apoptosis inhibitor of macrophage are associated with hepatic fibrosis in patients with chronic hepatitis C. *BMC Gastroenterol* **14**, 27-37.

87. Sarvari, J., Mojtahedi, Z., Taghavi, S.A., Kuramitsu, Y., Shamsi Shahrabadi, M., Ghaderi, A., Nakamura, K. (2013) Differentially Expressed Proteins in Chronic Active Hepatitis, Cirrhosis, and HCC Related to HCV Infection in Comparison With HBV Infection: A proteomics study. *Hepat Mon* **13**, e8351.

88. Gray, J., Chattopadhyay, D., Beale, G.S., Patman, G.L., Miele, L., King, B.P., Stewart, S., Hudson, M., Day, C.P., Manas, D.M., Reeves, H.L. (2009) A proteomic strategy to identify novel serum biomarkers for liver cirrhosis and

hepatocellular cancer in individuals with fatty liver disease. *BMC Cancer* **9**, 271-82.

89. Kolialexi, A., Anagnostopoulos, A.K., Papantoniou, N., Vougas, K., Antsaklis, A., Fountoulakis, M., Mavrou, A., Tsangaris, G.T. (2010) Potential biomarkers for Turner in maternal plasma: possibility for noninvasive prenatal diagnosis. *J Proteome Res* **9**, 5164-70.

90. Hung, P.H., Chen, Y.W., Cheng, K.C., Chou, H.C., Lyu, P.C., Lu, Y.C., Lee, Y.R., Wu, C.T., Chan, H.L. (2011) Plasma proteomic analysis of the critical limb ischemia markers in diabetic patients with hemodialysis. *Mol Biosyst.* **7**(6):1990-8.

91. Wu, J., Kobayashi, M., Sousa, E.A., Liu, W., Cai, J., Goldman, S.J., Dorner, A.J., Projan, S.J., Kavuru, M.S., Qiu, Y., Thomassen, M.J. (2005) Differential Proteomic Analysis of Bronchoalveolar Lavage Fluid in Asthmatics following Segmental Antigen Challenge. *Mol Cell Proteomics* **4**, 1251-1264.

92. Xu, D.-D., Deng, D.-F., Li, X., Wei, L.-L., Li, Y.-Y., Yang, X.-Y., Yu, W., Wang, C., Jiang, T.-T., Li, Z.-J., Chen, Z.-L., Zhang, X., Liu, J.-Y., Ping, Z.-P., Qiu, Y.-Q., Li, J.-C. (2014) Discovery and identification of serum potential biomarkers for pulmonary tuberculosis using iTRAQ-coupled two-dimensional LC-MS/MS. *Proteomics* **14**, 322-331.

93. Balfoussia, E., Skenderi, K., Tsironi, M., Anagnostopoulos, A.K., Parthimos, N., Vougas, K., Papassotiriou, I., Tsangaris, G.T., Chrousos, G.P. (2013) A proteomic study of plasma protein changes under extreme physical stress. *J Proteomics* **98**, 1-14.

FIGURE LEGENDS

Figure 1 Schematic representation of human and mouse CD5L proteins. Schematic diagram of human and mouse CD5L. S.P, signal peptide, SRCR domains 1, 2 and 3, N-glycosylation (black circles) and O-glycosylation sites [2, 9] (white circles). The amino acid number is shown below each sequence, and the observed molecular weights for each protein, according to references [2, 7, 9], are indicated.

Figure 2. The many roles of CD5L in inflammation. Schematic drawing that summarizes the settings in which CD5L is involved, including its target cells and known interacting proteins. The main mechanisms modulated by CD5L are indicated. Abbreviations:Ag.: antigen; FASN, fatty acid synthase; LM, *Listeria monocytogenes*; MTB, Mycobacterium tuberculosis; oxLDL, oxidized low density lipoproteins; TLR: toll-like receptor; ?: unknown interacting protein; ↓ decreased; ↑ increased.