Membrane cofactor protein (MCP; CD46): deficiency states and pathogen connections

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Membrane cofactor protein (MCP; CD46), a ubiquitously expressed complement regulatory protein, serves as a cofactor for serine protease factor I to cleave and inactivate C3b and C4b deposited on host cells. However, CD46 also plays roles in human reproduction, autophagy, modulating T cell activation and effector functions and is a member of the newly identified intracellular complement system (complosome). CD46 also is a receptor for 11 pathogens ('pathogen magnet'). While CD46 deficiencies contribute to inflammatory disorders, its overexpression in cancers and role as a receptor for some adenoviruses has led to its targeting by oncolytic agents and adenoviral-based therapeutic vectors, including coronavirus disease of 2019 (COVID-19) vaccines. This review focuses on recent advances in identifying disease-causing CD46 variants and its pathogen connections.

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Introduction

As one of the most ancient components of innate immunity, the complement system traces its origins to more than a billion years ago as it evolved to protect against pathogens and to engage in cellular processes [1,2]. Interestingly, 'living fossils' such as coral, sea urchin, sponge and horseshoe crab have complement activating and regulatory components similar to present day humans.

The contemporary primate complement system consists of at least 60 proteins and activation products and serves as an effector arm for the adaptive immune response. It features three activation cascades (alternative, classical and lectin) and a common terminal cytolytic pathway (reviewed in Refs. [3–5]). Within five minutes, millions of complement activation fragments can covalently swarm onto bacterial or viral pathogens to a) elicit opsonization and lysis (i.e. membrane perturbation phenomena) and b) promote the inflammatory response (e.g. the anaphylatoxins).

Such a powerful surveillance and membrane-modifying system requires strict control in order to avoid excessive host damage. Membrane cofactor protein (MCP; CD46) is a type one transmembrane complement regulatory protein expressed by almost every cell type (with the noteworthy exception of erythrocytes) (reviewed in Refs. [6–8]). It binds the two key activation fragments, C3b and C4b, that covalently deposit on self-tissue. Subsequently, CD46 serves as a cofactor for serine protease factor I (FI)-mediated cleavage of these two fragments to prevent their further engagement by the activation pathways.

CD46 is rather unique among complement proteins in that most cells coexpress four isoforms that arise by alternative splicing (see Figure 1). The *MCP* gene is located in the Regulators of Complement Activation (RCA) gene cluster located on the long arm of chromosome one (reviewed in Ref. [7]).

CD46 is particularly potent against the alternative pathway (AP) [9], although BC isoforms provide enhanced protection (relative to C isoforms) against the classical pathway [10]. CD46 also has other key capabilities. First, CD46 impacts reproduction as it is expressed as a hypoglycosylated isoform (C isoform) on the inner acrosomal membrane of human spermatozoa where it participates in the interaction between spermatozoa and oocyte during fertilization (reviewed in Refs. [7,8]). Second, because of its overexpression on a variety of human tumors, CD46 is emerging as a key player in both malignant transformation and in cancer therapeutics (reviewed in Refs. [11–13]). Third, CD46 signaling via motifs in its tails may critically impact cell behavior. For example, CD46-mediated intracellular signaling: a) enhances macrophage activity and survival, including cytokine and nitric oxide production and antigen presentation [14,15]; b) regulates autophagy of epithelial cells during pathogen invasion [16] or oxidative stress [17]; and c) modulates T cell activation by providing costimulatory signals during TCR engagement [18,19[**]] and for optimal CD8^{+} T cell effector functions [20]. CD46's signaling capabilities have

Figure 1
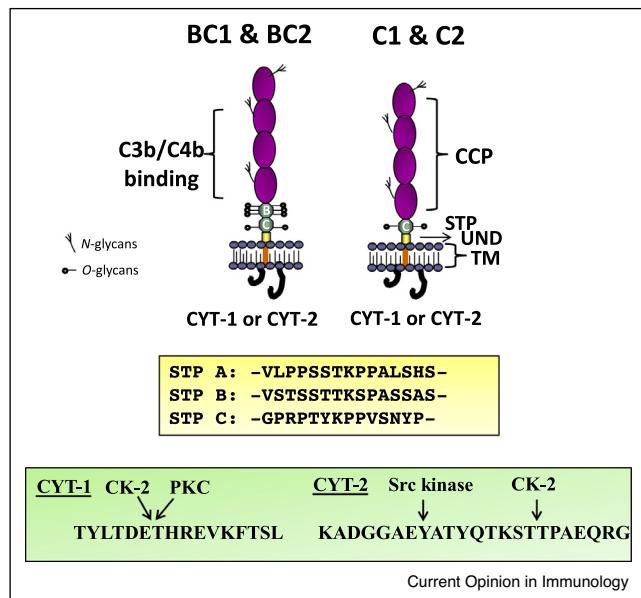
Schematic of CD46's protein structure. The amino-terminus consists of four contiguous complement control protein (CCP) modules. Each CCP bears ~60 amino acids consisting of four invariant cysteines (forming two disulfide bonds) and 10–18 highly conserved amino acids. CCPs 1, 2 and 4 bear *N*-glycans. Next is an alternatively spliced domain that is enriched in serines, threonines and prolines (STP region, site of *O*-glycosylation). While the *MCP* gene contains three STP exons (termed A, B and C), the commonly expressed isoforms contain B + C or C alone. This region is followed by a common, juxtamembraneous segment of 12 amino acids of undefined function. The carboxyl-terminus includes a transmembrane domain and one of two nonhomologous, alternatively spliced cytoplasmic tails; namely, CYT-1 with 16 amino acids or CYT-2 with 23 amino acids. Thus, isoforms are termed BC1 (343 amino acids), BC2 (350 amino acids), C1 (328 amino acids) or C2 (335 amino acids) to reflect splicing in the STP and cytoplasmic tail domains. The M_r varies: C isoforms range from ~51–58 kDa while BC isoforms range from ~59–68 kDa. UND, undefined domain; TM, transmembrane domain; CK-2 casein kinase 2; PKC, protein kinase C.

been best studied in CD4$^+$ T cells (reviewed in Refs. [21,22]). Indeed, a more detailed inspection of CD46 during T cell activation led to the discovery of an intracellular complement system, or complosome, which assists in immune defense via key interactions including modulating nutrient uptake and cellular metabolism [19••,23–25].

Of note, wild-type mice (and most other rodents) express *MCP* only on the inner acrosomal membrane of spermatozoa and in parts of the eye. Thus, murine models may rely on *MCP* transgenic animals. In rodents, the cellular complement regulator, Crry, replaces CD46 activity on most cells (reviewed in Refs. [7,8,26]) Crry is not expressed by other mammals, including primates.

This review focuses on recent advances in disease-causing CD46 variants and in its pathogen connections. To meet editorial guidelines, we often rely on reviews rather than original articles.

Deficiency states
Linkage analyses, genome-wide association studies and next generation sequencing have identified more than 80 disease-associated mutations in *MCP* ([27,28,29,30], reviewed Ref. [6] and as of 3/1/2021 unpublished tabulation by MK Liszewski, JP Atkinson and MK Herlin) (Figure 2). Most of these mutations have been linked to a rare thrombotic microangiopathy (TMA) called atypical hemolytic uremic syndrome (aHUS) ([31•] and see National Organization for Rare Disorders, https://rarediseases.org/rare-diseases/atypical-hemolytic-uremic-syndrome/). However, putative associations also have been suggested for other diseases including systemic lupus erythematosus [32], rheumatoid arthritis [33], asthma [17], multiple sclerosis [34,35], glomerulonephrites (reviewed in Ref. [6]), Alzheimer disease [36], bullous pemphigoid [37] and pregnancy-related disorders (see below and Ref. [38•]).

Hemolytic uremic syndrome
Typical features of HUS include the triad of microangiopathic hemolytic anemia, thrombocytopenia (i.e. low platelet count) and acute renal injury. Rare *MCP* mutations most commonly occur in the CCP domains and predispose to aHUS (also called complement-mediated HUS, C-HUS). Currently, they account for ~10−15% of aHUS cases. Penetrance is ~50%, suggesting the need for a secondary trigger ([39] and reviewed in Refs. [31•,40]). Most mutations are missense although nonsense and splice-site variants have been recognized (reviewed in Ref. [6]). In ~75% of aHUS cases, the mutant protein is not expressed on the cell membrane. Thus, a majority of mutations result in haploinsufficiency. However, a smaller portion of individuals are homozygous [39,41,42]. Additionally, a specific *MCP* SNP block in the promoter region, termed the *MCPggaac* risk haplotype, may be associated with decreased transcriptional activity. This has been linked to aHUS, but only if associated with a causative variant in another complement regulator or AP component ([43,44], and reviewed in Ref. [40]).

Intronic mutations also have been described, as in the case of CD46 splicing variant IVS2 + 2T > G (also known as c.286 + 2T > G, rs769742294). Two studies of this splicing variant pointed out that it can produce two different mRNA transcripts. In one case, the variant caused deletion of 155 base pairs at the 3′ of exon 2 (deleting 48 amino acids in CCP1) [42], while another study found it produced an mRNA causing a frame-shift mutation resulting in CD46 truncation in CCP2 (E97Kfs*33) [45]. The IVS2 + 2T > G variant was the most prevalent mutation (and a 'hot spot') in a cohort of

Figure 2
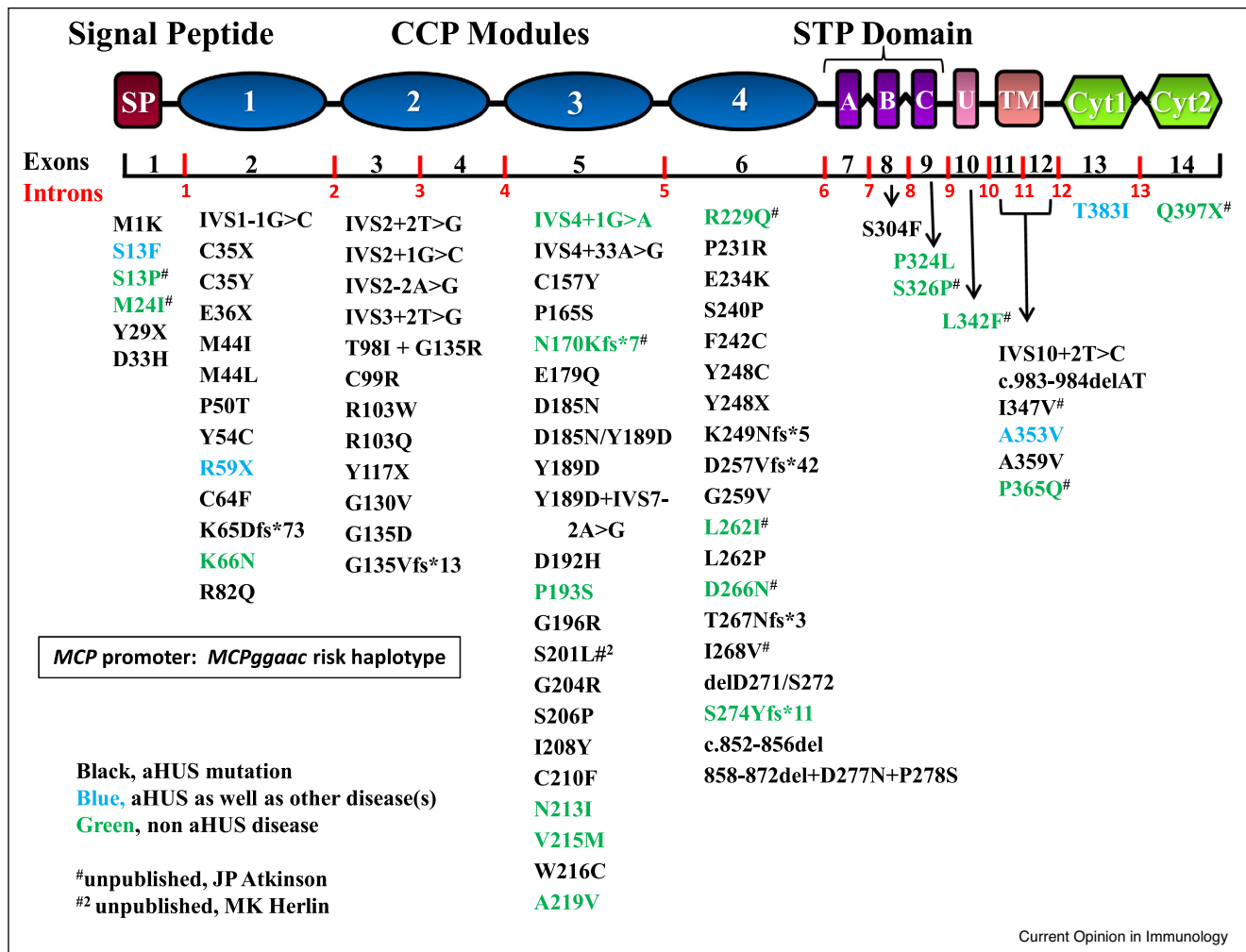
Disease-associated CD46 mutations. A schematic depicting CD46 protein, genomic organization, and disease-associated amino acid mutations. CD46 has a 34-amino acid signal peptide (SP). The gene consists of 14 exons (numbered in black) and 13 introns (numbered in red) for a minimum length of 43 kb. A majority of the mutations for aHUS and other diseases are located primarily in the four CCPs. Note also that a risk haplotype, *MCPggaac* (boxed), has been suggested to lie within the promoter region (see text). Black, aHUS mutations; blue, aHUS and other diseases; green, non-aHUS disease (see text). # indicates the mutation has not yet been published. Note that there is inconsistency in the literature for CD46 mutant numbering. Some published mutations do not count the SP or all STP exons. In this review, we follow the recommendations of the Human Genome Variation Society and include the SP and all exons. For the sake of uniformity, older published mutant numbers may have been updated. For original mutation citations, see Ref. [6] and as indicated in text.

aHUS-afflicted Indian children [46]. Further, it was also the most prevalent mutation (13/485) in an international aHUS cohort analyzed by Piras *et al*. [41]. These studies highlight the potential variable outcome of intronic mutations and the importance of their rigorous analyses.

The 'typical' or post-infection form of HUS represents ∼90% of cases. Patients, primarily children, develop diarrhea secondary to infection, most commonly by *Escherichia coli* serotype O157:H7, that produces a Shiga-like toxin (STEC). However, recent studies have determined that some cases originally diagnosed as

STEC-HUS were actually aHUS triggered by STEC infection in the setting of a complement deficiency. Two patients with a clinical history of STEC-HUS that progressed to end stage renal disease (ESRD) [47] had heterozygous complement gene rare variants, one for factor I and the other for the previously described *MCP* splice-site mutation (IVS2 + 2T > G) associated with aHUS. Additionally, a retrospective study assessed the frequency of complement gene rare variants in a French national cohort of children with STEC-HUS [48]. Next generation sequencing for six complement genes associated with aHUS identified rare variants in one or two

genes in Shiga-toxin positive patients, including one in CD46: N170Kfs*7. The authors concluded that genetic screening should be pursued in patients with post-diarrheal HUS who progress to end-stage renal disease.

Pregnancy-related and other disorders

CD46 mutated proteins have also been implicated in the pathophysiology of other disorders. For example, studies have evaluated the *MCP* gene in the pregnancy-related disorder: pre-eclampsia (PE), especially the HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome (reviewed by Burwick and Feinberg [38•] and Salmon *et al.* [49]). PE is a devastating multi-system disorder that occurs in 3–5% of pregnancies accounting for significant neonatal morbidity and mortality. HELLP is the most severe form of this disorder, accounting for 1% of all pregnancies. *MCP* mutations were identified in ∼8% of cases, although the range varied between 0–12% (summarized in Ref. [38•]). Similar to HUS, the precise etiology is unknown but likely relates (at least in part) to endothelial cell dysfunction secondary to excessive complement activation.

Since pregnancy in women with SLE and/or anti-phospholipid syndrome (APLS) is associated with PE or miscarriage, Salmon *et al.* sequenced *MCP* and other complement regulatory genes in a large cohort of patients [49]. They found that 18% of patients had heterozygous mutations (including in *MCP*), thus identifying the first genetic defects associated with PE in SLE or APLS. Additionally, rare CD46 variants have also been associated with miscarriage [50], systemic sclerosis, glomerulonephritis and thrombotic thrombocytopenic purpura (reviewed in Ref. [6]). Such studies have involved a small number of patients and require further investigation.

Value of genetic screening

What is clear from the above studies is that diseases featuring a failure to adequately control the complement system, in particular its powerful AP, can lead to tissue destruction, organ failure and death [51,52]. Determining which *MCP* mutations drive disease versus those that are simply rare variants is a current challenge. Additionally, since aHUS is successfully treated with eculizumab (a mAb to C5), decisions relative to treatment length may be assisted by genetic screening to identify if variants are known to be benign, pathogenic or, more commonly, catalogued as a 'variant of uncertain significance' (VUS) [53]. Alterations in complement proteins identified as a VUS may require functional analyses including quantification of CD46 on peripheral blood cells via flow cytometry as well as characterization and functional analyses of recombinantly produced protein [12].

Pathogen connections

Widespread expression, complement regulatory activities, immune-modulating signaling functions and

Figure 3
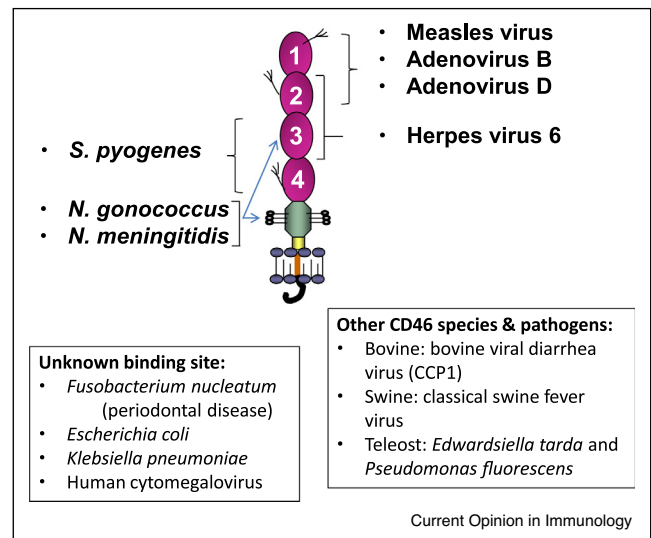
CD46 is a receptor for at least 11 pathogens. For seven, the attachment site has been identified (as indicated). A recent report established that 16/17 randomly selected adenovirus-D types use CD46 as cellular receptor [61••]. Bovine, swine and teleost CD46 serve as pathogenic viral receptors. Bovine CCP1 is the binding site of bovine viral diarrhea virus.

internalization mechanisms make CD46 an appealing candidate for exploitation by a diverse group of 11 pathogens (reviewed in Ref. [6]). CD46 has been called a 'pathogens' magnet' [54]. This group includes: five viruses — multiple species of adenoviruses (AdV) B and D, measles virus, herpesvirus 6A (reviewed in Ref. [6]), cytomegalovirus [55•]; and six bacteria — *Streptococcus pyogenes*, *Neisseria gonorrhea*, *Neisseria meningitides*, *E. coli*, *Klebsiella pneumoniae* [56] and *Fusobacterium nucleatum* (see Figures 3 and 4). Additionally, bovine CD46 (CCP1) serves as a receptor for the bovine viral diarrheal virus (reviewed in Refs. [6,57]), porcine CD46 serves as a receptor for the classical swine fever virus [58] and teleost CD46 may act as a receptor for *Edwardsiella tarda* and *Pseudomonas fluorescens* [59].

Pathogens target different CD46 domains for attachment and dissemination. Following engagement, CD46 can be shed or internalized via clathrin-coated pits or macropinocytosis. For example, the human herpesvirus 6 (HHV-6) envelope glycoprotein complex binds to CCPs 2 and 3. This is followed by internalization via clathrin-coated endocytosis and subsequent entry into the nucleus for viral nucleic acid replication (reviewed in Ref. [6]). Charvet *et al.* proposed a link between HHV-6A binding to CD46 and activation of a human endogenous retrovirus (HERV) element [34]. Specifically, they found that the binding of HHV-6A to CD46 CCPs 3 and 4 induced expression (via CYT-1) of a multiple-sclerosis-associated

Figure 4
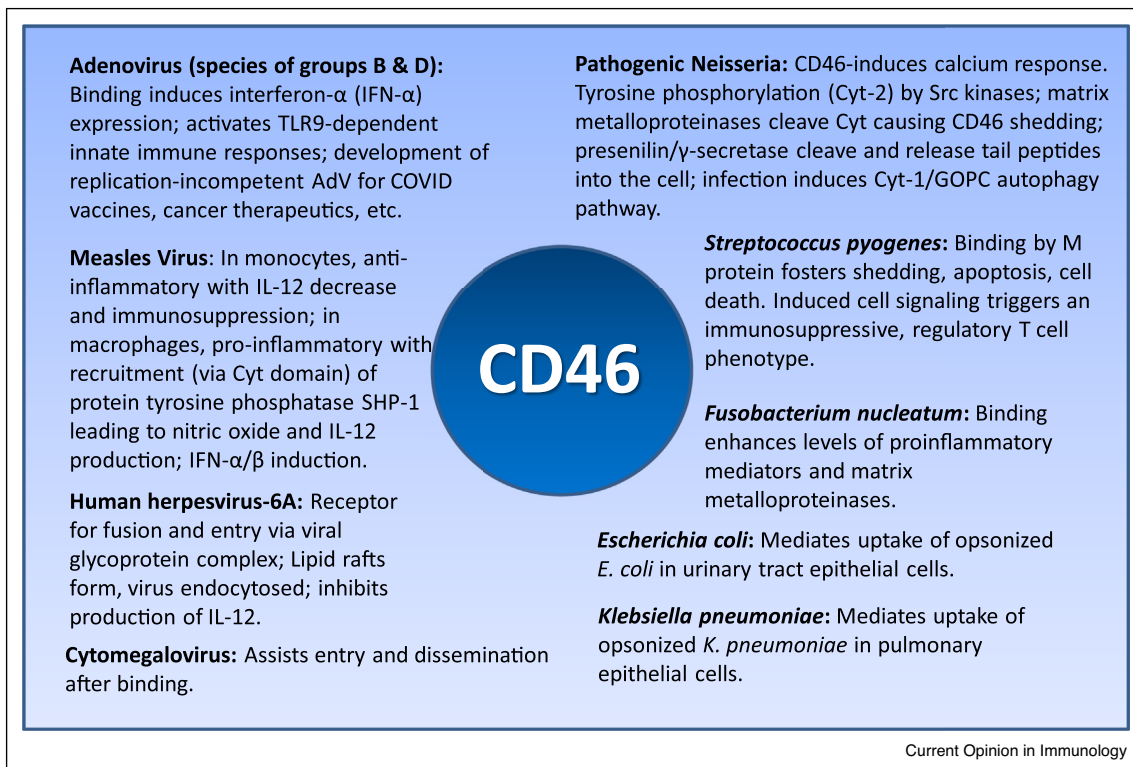
Connections and implications of pathogen binding to CD46. Abbreviations: TLR9, toll-like receptor 9; Cyt, the cytoplasmic domain of CD46 (alternative splicing produces a shorter tail of 16 amino acids, Cyt-1, or a longer tail of 23 distinct amino acids, Cyt-2); GOPC, Golgi-associated PDZ domain and coiled-coil motif containing protein.

retrovirus envelope protein, MSRV-Env [34]. The authors hypothesized that CD46 not only might serve as a transactivator of retroviral envelope genes, but also that this could impact the pathogenesis of inflammatory disorders such as multiple sclerosis.

A major virulence factor of *S. pyogenes*, M protein, binds CD46 via CCPs 3 and 4, a property that facilitates its adhesion and infectivity. This interaction leads to CD46 shedding, induction of apoptosis and cell death (reviewed in Ref. [6]). Further, M protein engagement of CD46 on T cells promotes an immunosuppressive/regulatory phenotype in T cells (reviewed in Ref. [6])

CD46 is also a receptor for *N. meningitides* (NM) and *Neisseria gonorrhoeae* (NG). The Type IV pilus of *Neisseria* mediates the initial attachment to epithelial cells by binding to CCP 3 as well as to the STP domain (reviewed in Refs. [6,8]). Infection by *Neisseria* stimulates the phosphorylation of CD46/CYT-2 by c-YES, a member of the Src family of protein tyrosine kinases. Further, Neisserial binding to CD46 triggers a cytoskeletal rearrangement and proteolytic cleavage of both CD46 tails. Additionally, during early infection NG binding to isoforms with CYT-

1 induces autophagy in epithelial cells by CD46 interaction with the scaffold protein, GOPC, and the autophagosome formation complex, Beclin1/VPS34 [60**]. However, later in infection, NG downregulates CD46/CYT-1 and disrupts lysosomes. This dual interference with the autophagy pathway promotes NG intracellular survival [60**]. Further, in a CD46 transgenic mouse model, NM engagement of CD46 accelerated the initiation of sepsis by modulating inflammation and survival of macrophages [15]. What is clear from these studies is that NM utilizes multiple strategies to overcome CD46-host mediated cytoprotection.

Measles virus (MV) and certain species of adenoviruses also target CD46. MV hemagglutinin as well as the AdV fiber knob protein in species of AdV types B and D attach to CD46 through CCPs 1 and 2. Intriguingly, the species of AdV-D bind CD46 through a noncanonical entry mechanism, the adenovirus hexon capsid protein [61**]. Indeed, 16 out of 17 randomly selected AdV-D types were shown to engage CD46 as a receptor in this manner [61**].

Studies investigating the role of MV-induced lymphopenia and systemic immunosuppression have provided key

information regarding CD46 responses in monocytes, dendritic cells and macrophages (reviewed in Ref. [8]). For example, binding by measles virus elicits internalization, alters intracellular processing and reduces antigen presentation (reviewed in Ref. [8]).

What is currently lacking in the field is a unified hypothesis that can identify whether CD46 will be either shed and/or internalized. Examples provided above illustrate the complexity of the issue. Likely, both processes are related. Further, the phenotypic expression differences of the four commonly coexpressed isoforms of CD46 probably complicate matters; that is, there is an expression polymorphism in the population in that 65% of individuals predominantly express the more heavily glycosylated BC isoforms, 29% express equivalent levels of BC + C, and 6% have C isoform predominance (reviewed in Ref. [62]). Also, our unpublished work suggests that BC isoforms shed more efficiently than C isoforms (Liszewski and Atkinson, unpublished). Further, the presence of two distinct CD46 cytoplasmic tails with independent signaling motifs may impact shedding or internalization. Thus, whether cell-specific, isoform-specific or condition-specific directed cell surface loss, much remains to be determined relative to the effect on CD46 engagement by its ligands and pathogens.

Pathogenic microbes also produce CD46-like proteins to subvert host defense (reviewed in Refs. [6,63]). For example, poxviruses express a protein that is ~35% homologous to CD46. Such complement regulatory inhibitors are called PICES (poxviral inhibitors of complement enzymes). Thus, proteins from variola (the causative agent of smallpox) and monkeypox are termed SPICE and MOPICE, respectively. The complement regulator from vaccinia, the vaccine strain, was named earlier as VCP (vaccinia complement protein, also called VICE). These inhibitors consist of three or four CCPs that structurally and functionally mimic CD46. They possess cofactor activity against C3b and C4b. However, they also have decay accelerating activity similar to fellow RCA regulator, decay accelerating factor (DAF; CD55). Further, these virulence proteins possess heparin-binding properties that allow them to attach to cell surface glycosaminoglycans in order to down-regulate complement activation.

CD46 and therapeutic applications
CD46 is emerging as a therapeutic oncologic target (reviewed in Ref. [11]). While CD46 expression level on peripheral blood mononuclear cells and granulocytes is ~10 000/cell, tumor-derived cells and cell lines range from 100 000–250 000/cell (reviewed in Refs. [13,64]). As a result of its overexpression in multiple cancers, a macropinocytosing CD46-antibody drug conjugate has been developed that is currently undergoing clinical trials for several oncologic applications [65]. The most remarkable

example of overexpression may be in relapsed multiple myeloma in which CD46 expression is increased up to 14-fold in patients who have the region on chromosome 1q carrying CD46 genomically amplified (reviewed in Ref. [11]). Additionally, species of AdV and MV (targeting CD46) are being exploited as engineered, modified vectors for wide-ranging therapeutic interventions. This includes a CD46-targeted AdV26-based vaccine against the spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) being developed by Janssen and as part of a vaccine 'Sputnik V' by the Gamaleya Research Institute (Ad5/Ad26) (reviewed in Ref. [66]). Additional therapeutic trials targeting CD46 include those for oncology [11,12], HIV, Ebola virus and Zika virus ([11] and reviewed in Refs. [61**,67,68]). Relative to MV, clinical trials are underway that employ an oncolytic MV encoding the thyroidal sodium iodide symporter (NIS) (facilitates viral gene expression and a tool for radiovirotherapy) [69,70]. Modified MV is also being developed as a vaccine against SARS-CoV-2 (reviewed in Ref. [71]). For example, the Pasteur Institute engineered MV to express spike protein (TMV-083) as a vaccine candidate. The same platform is being utilized against Chikungunya, Lassa, Zika and Middle East respiratory syndrome coronavirus. Further, CD46-targeted oncolytic adenoviral-based vectors are being developed as an alternative to AdV type C (e.g. AdV5) that binds to the coxsackie-adenovirus receptor, since the latter has low level expression [72].

Of considerable interest, Persson et al. suggest that vaccine vectors that are CD46-engaging (e.g. AdV-D) may more efficiently transduce antigen-presenting cells than AdV targeting receptors that are not expressed on such cells [61**]. Since 80% of adults have neutralizing antibodies against CAR-targeting AdV5, most frequently used in oncolytic viral therapy, Ono et al. developed a novel oncolytic adenovirus that recognizes CD46 (AdV35) and efficiently lysed tumor cells [73]. Additionally, a chimeric AdV therapy engineering two group B adenoviruses (Ad11/Ad3), called Enadenotucirev, is undergoing multiple trials for several types of cancer [69,74].

Further, a modified fiber knob protein of group B AdV35 is being used as a combination therapy with rituximab to treat patients with rituximab-refractory B-cell non-Hodgkin's lymphoma [75,76]. Pre-clinical studies in mice and non-human primates demonstrated that pre-treatment with Ad35K++, a high affinity, solubilized recombinant CD46-binding fiber knob, resulted in transient removal of CD46 from tumor cells. Because CD46 can be overexpressed by an order of magnitude on such cells (blocking complement-dependent cytotoxicity), the pre-treatment resulted in enhanced tumor killing by rituximab. These studies create the basis for the use of Ad35K++ as a combination therapy with rituximab in clinical trials to treat B-cell malignancies.

Conclusion

Since the discovery of CD46 as a membrane complement regulator more than 30 years ago, new knowledge has emerged not only about its structure and function as a complement regulator, but also its key interactions as a driver of cellular metabolism and component of the intracellular complement system [7]. Highlighted in this review were the disease-causing loss-of-function rare variants and the multiple connections of CD46 with a diverse group of pathogens. Importantly, new therapeutic applications targeting CD46 range from treatment of cancer to SARS-CoV-2 (COVID-19) vaccines. Undoubtedly, other surprises are yet in store as more knowledge is gained about this multi-functional protein.

Author contributions

MKL wrote and edited the manuscript; JPA conceived, outlined and edited the manuscript.

Conflict of interest statement

MKL has no competing interest; JPA reports serving as a consultant for Achillon Pharmaceuticals, Celldex Therapeutics, Clinical Pharmacy Services, Compliment Corporation, Gemini Therapeutics and Kypha; stock or equity options for AdMiRx, Inc, Compliment Corporation, Gemini Therapeutics, Kypha.

Acknowledgements

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- •• of outstanding interest

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