European Journal of Cell Biology 103 (2024) 151384

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.com/locate/ejcb

Controling the cytoskeleton during CEACAM3-mediated phagocytosis

Johannes W.P. Kuiper [a], Helena L. Gregg [a], Meike Schüber [a], Jule Klein [a], Christof R. Hauck [a,b,*]

[a] Lehrstuhl Zellbiologie, Fachbereich Biologie, Universität Konstanz, Germany
[b] Konstanz Research School Chemical Biology, Universität Konstanz, Germany

ARTICLE INFO

Keywords:
CEA-related cell adhesion molecule
Immunoreceptor tyrosine-based activation motif
Phagocytosis
Tyrosine phosphorylation
Rac
Pathogenic bacteria

ABSTRACT

Phagocytosis, an innate defense mechanism of multicellular animals, is initiated by specialized surface receptors. A phagocytic receptor expressed by human polymorphonuclear granulocytes, the major professional phagocytes in our body, is one of the fastest evolving human proteins implying a special role in human biology. This receptor, CEACAM3, is a member of the CarcinoEmbryonic Antigen-related Cell Adhesion Molecule (CEACAM) family and dedicated to the immediate recognition and rapid internalization of human-restricted pathogens. In this focused contribution, we will review the special adaptations of this protein, which co-evolves with different species of mucosa-colonizing bacteria. While the extracellular Immunoglobulin-variable (Ig_V)-like domain recognizes various bacterial adhesins, an Immunoreceptor Tyrosine-based Activation Motif (ITAM)-like sequence in the cytoplasmic tail of CEACAM3 constitutes the central signaling hub to trigger actin rearrangements needed for efficient phagocytosis. A major emphasis of this review will be placed on recent findings, which have revealed the multi-level control of this powerful phagocytic device. As tyrosine phosphorylation and small GTPase activity are central for CEACAM3-mediated phagocytosis, the counterregulation of CEACAM3 activity involves the receptor-type protein tyrosine phosphatase J (PTPRJ) as well as the Rac-GTP scavenging protein Cyri-B. Interference with such negative regulatory circuits has revealed that CEACAM3-mediated phagocytosis can be strongly enhanced. In principle, the knowledge gained by studying CEACAM3 can be applied to other phagocytic systems and opens the door to treatments, which boost the phagocytic capacity of professional phagocytes.

1. Introduction

Phagocytosis is an ancient process, by which multicellular organisms protect themselves against dangerous infection, but also use this process to remove disfunctional self components such as damaged cells or extracellular protein aggregates (Desjardins et al., 2005; Flannagan et al., 2012). The cells dedicated to this task, so-called professional phagocytes including macrophages, microglia, dendritic cells and polymorphonuclear granulocytes (PMNs), express membrane receptors, which initiate phagocytosis when engaged by their multivalent, particulate ligands (Swanson and Baer, 1995). Phagocytic receptors can operate in an opsonin-dependent or in an opsonin-independent manner. While opsonin-dependent receptors such as complement- or Fc-receptors require prior coating of particles with complement components or specific antibodies, opsonin-independent receptors, such as CEACAM3, Dectin-1, mannose receptor, or scavenger receptors directly recognize and bind microbial surfaces (Buntru et al., 2012; Rosales and Uribe-Querol, 2017; Underhill and Ozinsky, 2002). Despite these mechanistic distinctions on the extracellular ligand binding side, all these receptors connect to the intracellular cytoskeleton to accomplish the task of internalizing particles > 500 nm. Indeed, phagocytic receptors need to instruct the cytoskeleton to produce a local, temporary deformation of the plasma membrane, often in the form of lamellipodia, to wrap around and engulf the bound particle (Fig. 1) (Castellano et al., 2001; Diakonova et al., 2002).

The major transformations of the actin cytoskeleton and the resulting lamellipodia during phagocytosis are spectacular (Fig. 1). Human granulocytes, with an average diameter of ~10 μm, often form lamellipodia the size of the cell, which can completely enwrap particles with a length of several microns. These membrane structures are usually confined to the area of the cell, which is in direct contact with the particulate ligand. Lamellipodia are transient in nature and they appear and resolve within minutes (Aderem and Underhill, 1999; Greenberg, 1995). Therefore, it has been of interest to reveal the mechanisms that

Abbreviations: CEA, Carcinoembryonic Antigen; CEACAM, CEA-related cell adhesion molecule; Ig, immunoglobulin; ITAM, immunoreceptor tyrosine-based activation motif.

* Correspondence to: Lehrstuhl Zellbiologie, Fachbereich Biologie 621, Universität Konstanz, Universitätsstrasse 10, 78457 Konstanz, Germany.
E-mail address: christof.hauck@uni-konstanz.de (C.R. Hauck).

https://doi.org/10.1016/j.ejcb.2024.151384
Received 1 September 2023; Received in revised form 20 November 2023; Accepted 3 January 2024
Available online 5 January 2024

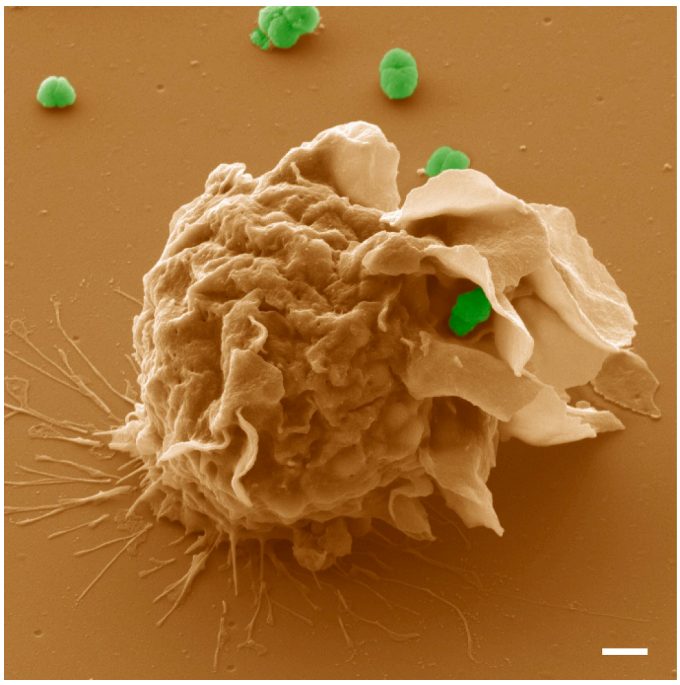

Fig. 1. CEACAM3-mediated, opsonin-independent phagocytosis by human granulocytes. Primary human granulocytes were incubated for 15 min with CEACAM3-binding *Neisseria gonorrhoeae* in the absence of opsonins. Upon CEACAM3-mediated contact, the granulocyte (orange) responds with massive, local membrane protrusions (lamellipodia), which allow the rapid engulfment of the bacteria (green). Shown is a scanning electron micrograph of the fixed sample, which has been pseudocolored for clarity. Scale bar represents 1 μm.

initiate the phagocytic process and to understand how the modulation of the underlying actin cytoskeleton is orchestrated in a spatial and temporal manner (May and Machesky, 2001; Niedergang and Chavrier, 2005; Nordenfelt and Tapper, 2011). In this review, we will use the example of the opsonin-independent receptor CEACAM3 to detail the principles of positive and negative regulatory signals emanating from this membrane protein. Thereby, we will highlight the multiple levels, where the phagocytic response is coordinated to balance maximum internalization of particles with minimum collateral damage to the tissue. Given the mechanistic parallels amongst phagocytic receptors, features revealed in the context of CEACAM3 will also be applicable to other receptor proteins engaged in phagocytosis.

2. Receptors of the CEACAM family present on the mucosal surface are prime targets for specialized microbial pathogens

CEACAM3 belongs to a subgroup of immunoglobulin (Ig)-superfamily cell adhesion molecules (IgCAMs), which are known as Carcinoembryonic antigen (CEA)-related cell adhesion molecules or for short CEACAMs (Beauchemin et al., 1999). To understand the peculiar specialization of CEACAM3 to serve as an opsonin-independent phagocytic receptor on human granulocytes, it is helpful to consider the functional context of the wider CEACAM family and their exploitation by pathogenic microorganisms (Tchoupa et al., 2014). Several members of this family are abundantly expressed by mucosal epithelial cells in the nasopharyngeal cavity, the lung, the intestine, and the genito-urinary tract (Kuespert et al., 2006; Thompson et al., 1991). In all these tissues, these membrane proteins are engaged in lateral cell-cell adhesion, but also localize to the apical surface of polarized epithelial cells, exposing their amino-terminal immunoglobulin (Ig)-domains to the external environment. This prominent surface exposure might be the reason, why CEACAMs appear to be a preferred target of bacterial pathogens colonizing the human mucosa (for review of this topic see (Mix et al., 2021; Tchoupa et al., 2014)). In particular, CEACAM1 and CEA (the product of the *CEACAM5* gene) are exploited for host cell attachment by specialized adhesins detected in a growing number of human-adapted bacterial pathogens such as *Neisseria gonorrhoeae, N. meningitidis, Haemophilus influenzae, Moraxella catarrhalis, Fusobacterium nucleatum, Helicobacter pylori*, *Streptococcus agalactiae*, *S. pyogenes*, and several pathovars of *Escherichia coli* (Virji et al., 1996; Hill and Virji, 2003; Berger et al., 2004; Tchoupa et al., 2015; Königer et al., 2016; Sheikh and Fleckenstein, 2023; Brewer et al., 2019; van Sorge et al., 2021; Catton et al., 2023)(Table 1).

Obviously, adhesin-mediated binding to the apically exposed CEACAMs allows these mucosa-colonizing bacteria to establish a firm connection with the epithelium. Indeed, heterologous expression of human CEA family members in epithelial tissues of mice has demonstrated that the presence of human CEACAMs facilitates colonization by *Neisseria gonorrhoeae*, *N. meningitidis*, or uropathogenic *E. coli* (Islam et al., 2018; Johswich et al., 2013; Muenzner et al., 2010; Muenzner et al., 2016). Moreover, such type of in vivo studies with CEA-transgenic mice have revealed a further consequence of intimate bacterial adhesion to epithelial CEACAM family members. In the stratified epithelium of the urogenital tract, CEACAM-mediated adhesion allows pathogens to suppress the detachment and exfoliation of the outermost cell layers (Muenzner et al., 2010; Muenzner et al., 2016; Muenzner et al., 2005). Thereby, the microorganisms reduce epithelial turnover, create a stable foothold on the mucosa for attachment and multiplication, and maximize their colonization success. Unexpectedly, the suppression of exfoliation is not a direct consequence of CEACAM-initiated signaling, but rather an indirect response to a soluble, cell-permeable bacterial metabolite, nitric oxide (NO), which acts as a short-range messenger and modulates integrin-based host cell adhesion (Muenzner and Hauck, 2020). This intricate microscale communication between pathogens and the host epithelium is enabled by bacterial nitrogen respiration under anaerobic conditions, but is also strictly dependent on the spatial proximity mediated by the intimate adhesion of the bacteria to host cell CEACAMs (Muenzner and Hauck, 2020).

Similar to the short range communication via NO, the close contact to the host cell provided by CEACAM binding can also be exploited for other cross-kingdom transfers. This is best examplified by the gastric pathogen *Helicobacter pylori*. These gram-negative bacteria express the surface protein HopQ, which can bind to CEACAM1, CEACAM5 and CEACAM6 on gastric epithelial cells (Javaheri et al., 2016; Königer et al., 2016). The mechanically dependable connection to the gastric surface provided by CEACAM-attachment allows *Helicobacter* to position one of its type-4-secretion systems (T4SSs), which are sophisticated outer membrane multiprotein complexes resembling nanoscale injection needles (Fischer et al., 2020). In this configuration, one of the T4SSs is able to translocate a major virulence factor of *H. pylori*, the CagA protein, directly into the cytosol of host epithelial cells (Backert et al., 2017). CagA then interacts with several host proteins, modulating multiple signaling pathways and reprogramming cellular functions (Tegtmeyer et al., 2017). Underscoring the important role of initial CEACAM-attachment for the proper function of this T4SS, a *Helicobacter* HopQ mutant is unable to inject CagA into host cells and CEACAM-deficient cells are refractory to T4SS-mediated CagA translocation (Behrens et al., 2020; Bonsor et al., 2018; Javaheri et al., 2016; Tegtmeyer et al., 2019).

CEACAM-binding pathogens might further benefit from engaging CEACAM1, which is also found on various leukocytes and has an immunosuppressive signaling function (Chen et al., 2012; Gray-Owen and Blumberg, 2006). Accordingly, CEACAM1 clustering can diminish B- and T-cell responses in the context of inflammatory settings (Lobo et al., 2009; Lu et al., 2012) and bacterial infection (Boulton and Gray-Owen, 2002; Galaski et al., 2021).

Given these multiple and mutually non-exclusive benefits diverse microorganisms gain from targeting epithelial CEACAMs, it appears as no surprise, that numerous bacteria independently evolved CEACAM-

Table 1
CEACAM-binding bacterial pathogens and their recognition by the granulocyte receptor CEACAM3. The table summarizes the results of CEACAM-binding assays with various strains of the indicated pathogenic microorganisms. Only studies, which in parallel tested binding of a particular bacterial strain or adhesin to epithelial CEACAMs (CEACAM1, CEACAM5) and granulocyte CEACAM3 were considered.

Pathogens	Adhesin	Investigated strains	Number of CEACAM-binding / CEACAM3-binding adhesins	Assay format*	References
Escherichia coli	Afa/Dr	IH11128	1/0	BCA	Berger et al. (2004)
		C1845	1/0	BCA	
		KS52	1/0	BCA	
		A30	1/0	BCA	
Fusobacterium nucleatum	CbpF	2B3	1/0	PP	Brewer et al. (2019)
		ATCC25586	1/0	PP	
		ATCC23726	1/0	BPD	Galaski et al. (2021)
Haemophilus influenzae	OMP P1	RD KW20	1/0	BPD, BCA	Tchoupa et al. (2015)
		Hib	1/0	BPD	
		NTHi strains	11/0	BPD	
		RD KW20	1/0	BPD	Adrian et al. (2019)
Haemophilus influenzae biovar *aegyptius*	OMP P1	ATCC11116	1/1	BPD	Tchoupa et al. (2015)
		ATCC11116	1/1	BPD	Adrian et al. (2019)
Helicobacter pylori	HopQ	P12	1/0	BPD	Königer et al., 2016
		26695	1/0	BPD	
		B8	1/0	BPD	
		SS1	1/0	BPD	
		X47	1/0	BPD	
		P12	1/1	PP, BCA	
		G27	1/1	BPD/PCB	Javaheri et al. (2016)
		NCTC11637	1/1	BCA	
		P12	1/1	PP	Bonsor et al. (2018)
		P12	1/1	BPD, PCB	Moonens et al. (2018)
		TX30	1/1	BPD, PCB	
Moraxella catarrhalis	UspA1	BBH18	1/1	BPD, BCA	Heinrich et al. (2016)
Neisseria gonorrhoeae	Opa_{CEA}	MS11	10/4	BCA	Bos et al. (1997)
		MS11	10/3	BCA	Gray-Owen et al. (1997)
		MS11	10/3	BPD	Roth et al. (2013)
		VP1	8/1	BPD	
		MS11	4/1	BCA	Sintsova et al. (2015)
Neisseria meningitidis	Opa_{CEA}	C311	3/0	BCA	Virji et al. (1996)
		C751	3/0	BCA	
		C1938	4/0	BCA	Muenzner et al. (2000)
		F6124	4/0	BCA	
		H44/76	4/0	BCA	de Jonge et al. (2003)
		MC58	4/0	BCA	Johswich et al. (2013)
Streptococcus agalactiae	β-Igl3	A909	1/0	BCA, PP	van Sorge et al. (2021)
Streptococcus pyogenes	R28	AL368	1/0	BCA, PP	Catton et al. (2023)
		emm28 strains	13/0	BPD	

* abbreviations for the used binding assay formats:
BCA = Adhesion of intact, adhesin-expressing bacteria to full-length CEACAM expressed on host cell
BPD = Binding of intact, adhesin-expressing bacteria to soluble CEACAM ectodomains; evaluated by flow cytometry, Western blotting or fluorescence microscopy
PCB = Binding of purified recombinant adhesin to full-length CEACAM expressed on host cell
PP = Binding of purified recombinant adhesin to soluble CEACAM ectodomains

binding adhesins. It is this context that singles out CEACAM3 as a special member of the CEACAM family, which can be best appreciated by looking at its evolutionary trajectory and by detailing the structure-function relationship of CEACAM3.

3. CEACAM3 in primate evolution

Genome comparison amongst higher primates has revealed that CEACAMs belong to the fastest evolving genes in humans (Gibbs et al., 2007; Adrian et al., 2019). Obviously, a pathogen-driven positive selection can explain the rapid fixation of missense mutations in CEACAM family members recognized by bacterial adhesins (Adrian et al., 2019; Baker et al., 2022), a process observed for other genes with functions in the host-pathogen interplay (Fumagalli et al., 2011). Interestingly, while a CEACAM1 homologue is found in all mammals, a CEACAM3-encoding gene has only been found in the genomes of higher primates, i.e. old world monkeys (Adrian et al., 2019). This striking observation suggests that CEACAM3 evolved as a novel family member during recent primate evolution around 35 million years ago (Adrian et al., 2019). With more and more primate genomes becoming available, it will be possible to delineate the origin of CEACAM3 in the future with higher precision (Kuderna et al., 2023). However, the comparison of human CEACAM3 with its ape homologues has already revealed that the amino acid changes predominantly map to the extracellular Ig_V-like domain of CEACAM3, while the exons encoding the transmembrane and the cytoplasmic portion of CEACAM3 are conserved (Fig. 2A) (Adrian et al., 2019). On the one hand, this finding suggests that CEACAM3′s intracellular portion conveys a conserved function into the cell (see also next sections). On the other hand, the situation found for the extracellular domain of CEACAM3 is rather unusual as most phagocytic receptors recognize given endogenous proteins (opsonins such as antibodies or complement factors) or conserved molecular structures found on various microbes. Therefore, their extracellular ligand binding domains are invariant. In contrast, the Ig_V-like domain of CEACAM3 seems to be under selective pressure to rapidly adapt to changing ligands. Indeed, the known CEACAM3-binding bacteria and their adhesins are structurally diverse, and even within a given microbial species the adhesins in unrelated isolates usually show high variation in their CEACAM-binding domains (Hill and Virji, 2003; Hill et al., 2012; Roth et al., 2013; Sintsova et al., 2015; Tchoupa et al., 2015). Clearly, these are hallmarks of an ongoing arms race between pathogens trying to escape CEACAM3 recognition (i.e. modulation of surface adhesin primary structure) and

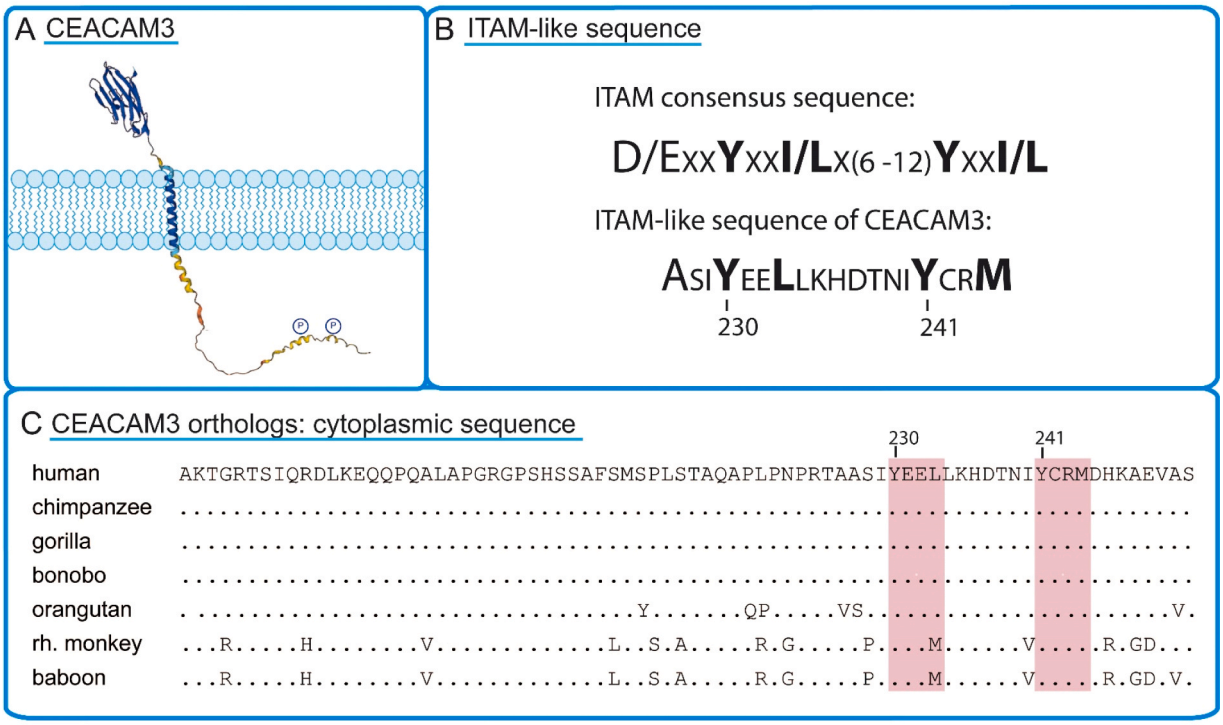

Fig. 2. Structural determinants of CEACAM3 function. A) Schematic rendering of CEACAM3 overall structure (based on Alphafold prediction). Colors indicate reliablity of structural prediction with blue: high certainty; yellow: low certainty. Segments representing the immunoglobulin variable-like (IgV)-like domain, the transmembrane domain, and the cytoplasmic domain have been stiched together in arbitrary angles for space reasons. B) Alignment of the immunoreceptor tyrosine-based activation motif (ITAM)-like sequence of CEACAM3 with the canonical ITAM primary structure. The critical tyrosine and neighboring residues are highlighted in bold. C) Amino acid sequence aligment of cytoplasmic domains of primate CEACAM3 orthologues. The critical tyrosine residues Y230 and Y241 (numbering according to human sequence) and their sequence context are highlighted with red color.

human CEACAM3, which is under positive selection to maintain the ability to bind novel microbial adhesin variants. Such an evolutionary process has been aptly named a Red-Queen scenario and is characteristic of predator-prey and host-pathogen relationships (van Valen, 1973). Two findings on the population level support the idea that CEACAM3 undergoes accelerated evolution to protect us from pathogen attack: i) genomic surveys reveal common CEACAM3 polymorphisms in geographically distinct human populations, which are able to recognize a wider spectrum of bacterial adhesins (Adrian et al., 2019); ii) most characterized bacterial adhesins preferentially bind epithelial CEACAMs (CEACAM1 and CEA), while avoiding recognition by CEACAM3 (Roth et al., 2013; Sintsova et al., 2015; Tchoupa et al., 2015)(Table 1). For example, the OMP P1 adhesins of various *H. influenzae* isolates selectively bind to CEACAM1 and CEA, and are not recognized by CEACAM3. So far, only OMP P1 expressed by a *H. influenzae* biovar *aegyptius* isolate binds to CEACAM1, CEA, and CEACAM3 (Tchoupa et al., 2015) (Table 1). However, a CEACAM3 polymorphism prevalent in Western Africa exhibits binding to all OMP P1 adhesins tested (Adrian et al., 2019). Together, the CEACAM3 gene appears as a recent primate invention to equip professional phagocytes with a germline-encoded receptor enabling rapid detection and phagocytosis of antigenically variable bacterial pathogens. Depending on local selective pressures (e.g. prevailing bacterial pathogens), particular human CEACAM3 variants might be advantageous. The increasing availability of genomic data will surely provide interesting novel insight into the spectrum of CEACAM3 polymorphisms in various human populations.

4. Structure-activity relationship of CEACAM3

As elaborated above, CEACAM3 has a distinct function among members of the CEACAM family, and the unique biology of this receptor is already highlighted by its recent emergence in a single mammalian lineage, the primates. A dedicated role of CEACAM3 in immune defense is also implied by its exclusive expression pattern in human granulocytes (Kuroki et al., 1991). In addition, the domain structure of CEACAM3 with a single extracellular immunoglobulin variable-like domain (Ig_V-domain) and the lack of immunoglobulin constant-like (Ig_C) domains signify that this receptor does not engage in homotypic interactions with other IgCAMs, as typically observed for epithelial CEACAMs (Fig. 2A). In contrast to such epithelial CEACAMs, CEACAM3 does not form homodimers supported by Ig_V-Ig_V as well as Ig_C-Ig_C interactions, but rather occurs in monomeric form (Bonsor et al., 2018). These structural features indicate that CEACAM3 is a CEACAM family member without cell-cell adhesive functions. Indeed, the only known ligands for CEACAM3 are various adhesins of bacterial pathogens, which bind, in direct protein-protein-interactions, to the non-glycosylated face of the CEACAM3 Ig_V-domain (Bonsor et al., 2018). The non-glycosylated β-sheet of the CEACAM3 Ig_V-domain is made up by the β-strands C, F, and G of the Ig-fold and is referred to as the CFG-face. Interestingly, common CEACAM3 polymorphisms, such as S43R, L44Q, or V49A, which occur at a frequency of $> 10\%$ in several human populations, all map to this CFG-face (Adrian et al., 2019). Together, these features are in line with the idea that the extracellular part of CEACAM3 is designed to recognize and bind a specific set of human pathogens. They also point to the cytoplasmic domain of CEACAM3, which should be involved in translating ligand binding into cellular responses.

The cytoplasmic domain of CEACAM3 comprises 75 amino acid residues and harbors as the most distinctive feature an immunoreceptor tyrosine-based activation motif (ITAM)-like sequence (Fig. 2B). ITAMs are found in various leukocyte receptors, including the T cell receptor, the B cell receptor, and Fc receptors (Reth, 1989). The canonical ITAM-sequence is characterized by precisely spaced tyrosine residues in a defined sequence context which corresponds to D/ExxYxxI/$Lx_{(6}$

$_{-12)}$YxxI/L (Abram and Lowell, 2007; Isakov, 1997; Reth, 1989). In CEACAM3, the tyrosine residues are located at positions Y230 and Y241 and possess ideal spacing (Fig. 2B). In contrast to the canonical sequence, these tyrosine residues are not preceded by an acidic (D/E) residue. Furthermore, Y241 is not accompanied by a leucine or isoleucine residue in carboxy-terminal direction, but rather by a methionine residue (Fig. 2B). This might have functional consequences for the binding of SH2-domain containing proteins upon phosphorylation of Y241 (see next section). Due to these deviations from the canonical sequence, the amino acid motif in CEACAM3 is referred to as ITAM-like. Interestingly, in some primates (e.g. rhesus monkey and baboon) both tyrosine residues of the ITAM-like sequence are accompanied by methionine instead of leucine/isoleucine (Adrian et al., 2019)(Fig. 2C). If such alterations affect CEACAM3 function in these primates has not been investigated.

Clearly, the ITAM-like sequence is critical for downstream signaling from human CEACAM3. Mutation of both tyrosine residues to non-phosphorylateable phenylalanine results in a strong reduction in CEACAM3-mediated phagocytosis (McCaw et al., 2003; Schmitter et al., 2004). Truncation of the complete cytoplasmic domain can further diminish CEACAM3-mediated bacterial uptake (Schmitter et al., 2004), suggesting that additional sequences contribute to downstream signaling. However, no other molecular determinants have been characterized in the cytoplasmic domain of CEACAM3 besides the ITAM-like sequence. Structure prediction by Alpha-fold points to a mostly unstructured C-terminal tail with short α-helical sections suggesting that

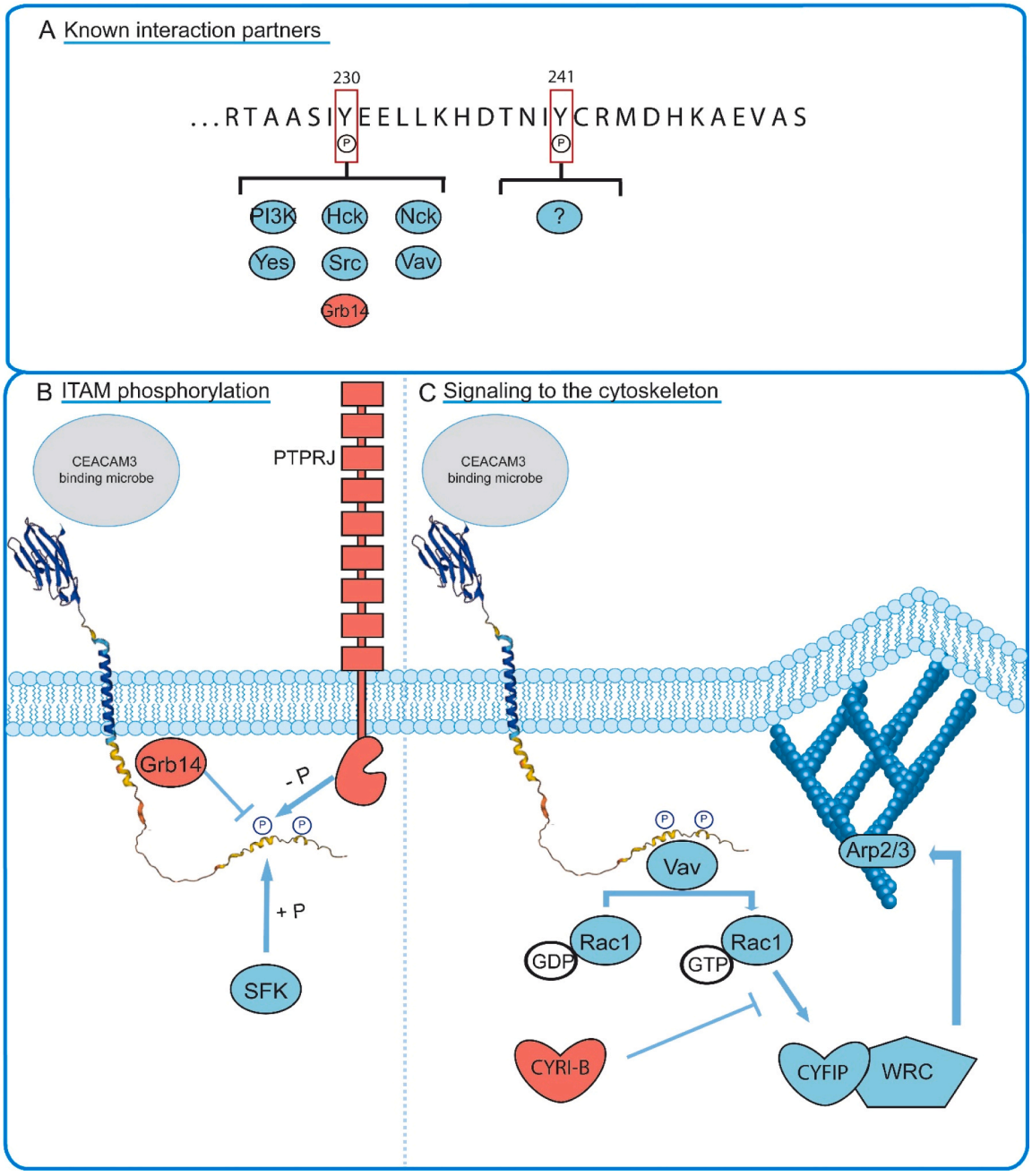

Fig. 3. Schematic overview of the negative regulation of CEACAM3. A) Summary of known binding partners of the CEACAM3 ITAM-like sequence. The critical phosphotyrosine residues are highlighted with a red frame. B) Proteins involved in phosphorylation and dephosphorylation of the CEACAM3 ITAM-like sequence. Phosphorylation via cytoplasmic Src family kinases (SFK) is indicated with '+P'. Dephosphorylation via the receptor-type protein tyrosine phosphatase J (PTPRJ) is indicated with '-P'. C) Positive and negative regulation of Rac-GTP-dependent cytoskeletal rearrangements during CEACAM3-mediated phagocytosis.

this part of the receptor provides ample space for protein-protein interactions with cytosolic proteins (Fig. 2A). In the next section, we will discuss, which proteins are known to interact with the cytoplasmic tail of CEACAM3 and how these interactions are regulated.

5. Regulation of CEACAM3 phosphorylation during phagocytosis

The opsonin-independent phagocytosis driven by CEACAM3 heavily depends on tyrosine phosphorylation. In particular, the phosphorylation status of the ITAM-like sequence, situated at Y230 and Y241 close to the carboxy-terminus of the protein, dictates the functionality of CEACAM3. Accordingly, the balance of protein kinase-mediated phosphorylation versus the phosphatase-mediated dephosphorylation determines if CEACAM3 engagement results in productive internalization (Fig. 3). Similar to other immuno-receptors harboring such tyrosine-based motifs, Src family kinases (SFKs) play an initial role in the phosphorylation of these tyrosines. Indeed, constitutively active Src kinases are able to use both residues as a substrate. Myristoyl-modified SFKs are ideally positioned at the inner leaflet of the plasma membrane to readily respond to CEACAM3 clustering by multivalent, adhesin-expressing bacterial pathogens. However, whether all SFKs expressed by granulocytes are involved in CEACAM3 phosphorylation is currently not known. A direct association of the Hck-SH2 domain with the phosphorylated ITAM-like motif of clustered CEACAM3 has been demonstrated by FRET measurements in intact cells (Buntru et al., 2009). Other granulocyte SFKs, such as Fgr and Lyn, have not been investigated in detail. Given the overlapping substrate specificity of these enzymes, it appears reasonable to assume that they all can act on CEACAM3 tyrosine residues. While the purified, recombinant SH2-domains of Hck, Yes, and Lck associate with tyrosine phosphorylated CEACAM3 in vitro, the Fyn SH2 domain fails to bind under these conditions (Kopp et al., 2012). This finding exemplifies that binding affinities between phosphorylated CEACAM3 and particular SFKs can vary, indicating that there might be preferential phosphorylation and binding of CEACAM3 by one or more SFK family members.

Interestingly and in contrast to other ITAM-containing immunoreceptors, the more distal located pY241 of the CEACAM3 ITAM-like sequence has so far not been found to engage in interactions with SH2 domains, while the more membrane proximal pY230 is a preferred binding site for various SH2-domain containing proteins (Fig. 3A). Besides SFKs, these pY230-binding proteins include the regulatory subunit of class I phosphoinositide 3-kinase (PI3K), the guanine nucleotide exchange factor Vav, and the adapter protein Nck (Fig. 3A). The binding of each of these SH2 domains in principle should exclude the docking of another SH2-domain to the same site in CEACAM3. However, the local clustering and tyrosine phosphorylation of CEACAM3, which is initiated by its multivalent bacterial ligands, would provide room for simultaneous association with several SH2-domain-possessing interaction partners, even though these binding partners share the binding preference for pY230 of CEACAM3.

The direct association of CEACAM3 pY230 with several important downstream signaling molecules might explain why a canonical ITAM-signaling constituent, the tyrosine kinase Syk, is not essential for CEACAM3-initiated phagocytosis. Syk is critical for FcγRI- and FcγRIII-initiated ITAM signaling leading to phagocytosis by macrophages and neutrophils (Crowley et al., 1997; Huang et al., 2006; Kiefer et al., 1998). In these contexts, Syk activity initiates multimolecular adaptor complexes by the association of phosphorylated Syk with its binding partners PI3K, Vav and SLP-76 (Mocsai et al., 2010). However, in the case of CEACAM3, PI3K and Vav can directly bind to pY230 of the receptor (Buntru et al., 2011; Schmitter et al., 2007), alleviating a strict requirement for Syk in promoting uptake of CEACAM3-bound particles (Sarantis and Gray-Owen, 2007).

The prominent role of CEACAM3 pY230 as a central hub for downstream signaling is also used to negatively impact CEACAM3-mediated phagocytosis. On the one hand, competitive binding by an SH2-domain containing adaptor protein called Grb14 can suppress downstream events initiated by CEACAM3 (Kopp et al., 2012). Similar to the above mentioned SH2-domain containing interaction partners of the phosphorylated CEACAM3 Y230, Grb14 selectively binds to this phospho-tyrosine residue (Kopp et al., 2012)(Fig. 3B). As the effect of Grb14 is due to its ability to compete with other proteins for the same binding site, the magnitude of the inhibitory effect of Grb14 depends on protein levels and their relative binding affinity for pY230. Clearly, the binding affinities and protein levels of each pY230 interaction partner could be regulated further by post-translational modifications such as ubiquitylation, phosphorylation, or acetylation. Such an additional level of control over pY230-mediated downstream events has not been explored to date.

In contrast to the stoichiometric control afforded by a competitive binding partner such as Grb14, CEACAM3 pY230 is also regulated enzymatically. Obviously, the removal of the phosphate residue at pY230 requires the activity of a phosphotyrosine-directed protein phosphatase. The enzyme behind this activity has been revealed recently via a focussed CRISPR/Cas9-based genetic screen in myeloid cells (Goob et al., 2022). Goob et al. have generated a CEACAM3-expressing HL60 cell line, which exhibits rapid and selective opsonin-independent uptake of bacteria expressing a CEACAM3-binding adhesin. At the same time, these human myeloid cells do not internalize non-CEACAM3-binding bacteria in the absence of opsonins (Adrian et al., 2019). Using lentiviral-encoded sgRNAs individually targeting each human receptor-type protein tyrosine phosphatase (RPTP), Goob et al. identified PTPRJ (CD148) as the single most important PTPR acting on the CEACAM3 ITAM-like sequence (Goob et al., 2022)(Fig. 3B). As predicted by the positive effect of CEACAM3 tyrosine phosphorylation, the overexpression of active PTPRJ, but not the expression of an inactive mutant version of this enzyme, severely blunted CEACAM3-mediated phagocytosis (Goob et al., 2022). In contrast, the genetic deletion of PTPRJ resulted in a prominent gain-of-function with elevated levels of CEACAM3 tyrosine phosphorylation and a dramatically enhanced phagocytic phenotype of the PTPRJ-knock-out HL60 cells towards CEACAM3-binding bacteria (Goob et al., 2022). It is interesting to point out, that PTPRJ has also been shown to impact Dectin-1-mediated, opsonin-independent phagocytosis (Goodridge et al., 2011). In this context, the lack of PTPRJ alone has no consequences on phagocytosis of β-glucan-covered particles (Goodridge et al., 2011). Only upon deletion of a second RPTP, deletion of the tyrosine phosphatase PTPRC (CD45), Dectin-1-mediated phagocytosis is affected. However, in contrast to the gain-of-function observed for CEACAM3-mediated phagocytosis in PTPRJ-ko cells, compound deficiency for PTPRJ and PTPRC decreases Dectin-1 mediated uptake of yeast particles. This seemingly contradictory result can be explained by the lack of Src family kinase activation in PTPRJ and PTPRC double-knock-out cells, as these RPTPs are required for the initial dephosphorylation of an inhibitory phospho-tyrosine residue in SFKs (Hermiston et al., 2009; Zhu et al., 2008). In PTPRJ-deficient myeloid cells, Src family kinases can become active via initial dephosphorylation by PTPRC, revealing the negative impact of PTPRJ on downstream events such as the SFK-dependent phosphorylation of the CEACAM3 ITAM-like sequence (Goob et al., 2022).

The adaptor protein Grb14 and the phosphatase PTPRJ illustrate the multimodal regulation of phosphorylation-dependent processes at the critical ITAM-like sequence, further underscoring the central role of this short amino acid motif in the cytoplasmic domain of CEACAM3. These examples also demonstrate that phagocytosis mediated by human professional phagocytes operates at a submaximal level and that there might be room for enhancing phagocytosis under specific circumstances to maximally control and potentially eradicate microbial intruders.

6. Controling CEACAM3-driven cytoskeletal re-arrangements

CEACAM3-mediated phagocytosis is accompanied by extensive

morphological changes with rapidly expanding lamellipodia, which engulf the bound particle (see Fig. 1). The expansion of these thin membrane lamellae is driven by actin polymerization and filament branching. Accordingly, blockade of actin dynamics by cytochalasin D or jasplakinolide, which destabilize or freeze f-actin, respectively, abolishes CEACAM3-mediated pahgocytosis (Muenzner et al., 2008). The branched network of short actin filaments found in lamellipodia depends on nucleation by the Arp2/3 complex (Rottner and Stradal, 2016). Several studies have detailed the molecular mechanisms connecting CEACAM3 engagement and its phosphorylation with the local recruitment and activation of Arp2/3 complex regulators (Fig. 3C). Indeed, phosphorylation of the Y230 residue within the ITAM-like motif of CEACAM3 provides a high affinity docking site for the SH2 domain of Nck adapter proteins (Pils et al., 2012). Both Nck1 and Nck2 can associate with pY230 and CEACAM3-transfected Nck1/Nck2-double knock-out fibroblasts, in contrast to wildtype fibroblasts, lack CEACAM3-mediated uptake of bacteria (Pils et al., 2012). Nck adapter proteins recruit the Wave regulatory complex (WRC) to the phagocytic cup, presumably via binding to the WRC component Nck-associated protein Nap1 (also known as Hem-2 or Kette in Drosophila). Recruitment of the WRC to the cell membrane induces lamellipodia formation in different cell types (Mehidi et al., 2021; Steffen et al., 2004). The central WRC component, the Wave protein, employs its C-terminal WH2-Central-Acidic (WCA) domain to dock to and activate the Arp2/3 complex, which then initiates the formation of branched f-actin (Bieling and Rottner, 2023; Rottner et al., 2021). In the context of CEACAM3-mediated phagocytosis, the formation of lamellipodia is completely suppressed upon overexpression of a truncated Wave protein lacking the WCA domain, demonstrating the essential role of the WRC in this process (Pils et al., 2012). WRC activation requires the interaction of Rac-GTP with another WRC subunit, the protein Cyfip1 (also called Sra1) (Fig. 3C). This interaction allosterically releases the WCA domain of Wave to allow binding of the Arp2/3 complex (Rottner et al., 2021). Rac-GTP loading is accomplished by Rac guanine nucleotide exchange factors (GEFs) and in the context of CEACAM3, the Rac GEF Vav plays a critical role (Schmitter et al., 2007). Similar to the adapter proteins Nck1 and Nck2, Vav associates via its SH2 domain with phosphorylated Y230 of the CEACAM3 ITAM-like sequence (Schmitter et al., 2007). Thereby, CEACAM3 phosphorylation assures the coincidence of two essential components needed for Arp2/3 complex activation: CEACAM3 pY230 brings together the Rac GEF Vav (for GTP-loading of the WRC activator Rac) with the Rac-GTP-binding protein Cyfip1 as a component of the WRC. This coincidence seems to provide an optimal local environment for maximal Arp2/3 driven f-actin nucleation, as CEACAM3-mediated phagocytosis occurs within minutes after bacterial contact (McCaw et al., 2004; Schmitter et al., 2004; Schmitter et al., 2007). Interestingly, this direct association of CEACAM3 with a Rac GEF might also be the reason, why phosphatidylinositol-(3,4,5)-trisphosphate (PIP3), the product of PI3 kinase activity, is not needed to accomplish CEACAM3-mediated phagocytosis (Buntru et al., 2011). This is in contrast to other phagocytic receptors, such as the Fcγ receptor, where PIP3 seems to be involved in recruiting and activating PIP3-binding GEFs to assure completion of phagocytosis (Araki et al., 1996; Booth, 2006).

Interestingly, the regulation of the WRC by GTP-loaded Rac provides another layer of control over CEACAM3-mediated phagocytosis. In a recent genome-wide CRISPR/Cas9-knock-out screen in myeloid cells, several potential negative regulators of CEACAM3-mediated uptake have been identified (Kuiper et al., 2023). The screen was based on CEACAM3-expressing human myeloid HL60 cells, which should, upon CRISPR/Cas9-mediated disruption of genes encoding potential negative regulator(s), exhibit increased phagocytosis of CEACAM3-binding gonococci. Indeed, disruption of several genes led to the predicted gain-of-function (Kuiper et al., 2023). One of the candidate genes, Cyri-B is homologous to the Rac-binding WRC subunit Cyfip1 and suppresses WRC activation by sequestering GTP-bound Rac (Shang et al., 2018; Yelland et al., 2021; Yuki et al., 2019)(Fig. 3C). Strikingly, targeted disruption of the CYRI-B gene more than doubled CEACAM3-mediated phagocytosis and this was accompanied by elevated Rac-GTP levels and overactivation of Rac-GTP effectors such as the kinase PAK (Shang et al., 2018). At the same time, the CYRI-B-deficient cells exhibited a comparable increase in Fcγ receptor-mediated phagocytosis of IgG-opsonized bacteria (Kuiper et al., 2023). This finding suggests that Cyri-B is a generic competitor of Rac-GTP binding effector proteins and that CEACAM3 as well as FcγR likewise depend on Rac-GTP for efficient phagocytosis. Restoring expression of wildtype Cyri-B in the Cyri-B knock-out cells diminished phagocytosis back to levels seen in wildtype cells, while a mutant version of Cyri-B, which lacks Rac-GTP binding, did not reduce the elevated levels of bacterial uptake (Kuiper et al., 2023).

Again, these results demonstrate that levels of phagocytosis seen in wildtype cells are far below the maximal phagocytosis rate, which phagocytes could accomplish, and that endogenous factors curb the maximum phagocytic response. Although it seems counterintuitive, uncontrolled activation of phagocytosis could also have negative consequences. Actin tread milling for instance requires a constant supply of ATP. Consequently, it has been shown in platelets and neurons that up to 50% of the cellular ATP is used to sustain actin cytoskeleton dynamics (Bernstein and Bamburg, 2003; Daniel et al., 1986). Phagocytosis could provoke a spike in actin-driven energy demands as it is accompanied by extensive actin remodeling. This idea is supported by the finding that ablation of brain-type creatine kinase - an enzyme that facilitates metabolic demands by connecting sites of ATP production with sites of ATP consumption - dramatically impedes phagocytosis (Kuiper et al., 2008). Therefore, one could speculate that phagocytosis needs to be restrained, so that cellular ATP pools are not exhausted, which could have negative effects on viability and/or energy-demanding downstream functions of phagocytes such as microbial killing by reactive oxygen species. However, the striking gain-of-function in CEACAM3-mediated phagocytosis observed upon deletion of PTPRJ or Cyri-B indicate that cellular energy supplies are sufficient to sustain even a 100% increase in phagocytosis. Therefore, the negative regulation of actin-driven phagocytosis could also be a physiological adaptation that is unrelated to metabolic cost. For example, phagocytosis in granulocytes is accompanied by the release of bactericidal and potentially harmful granule content, such as proteases, peroxidases, and reactive oxygen species (Mittal et al., 2014; Pechous, 2017; Winterbourn et al., 2016). Accordingly, limiting phagocytosis to a lower-than-maximum level might also be a means to keep collateral damage to host cells and tissues at bay. The knowledge about endogenous factors in myeloid cells, which constrain phagocytosis, might be useful for future approaches to maximize phagocytosis. It can be easily envisioned that the ability to boost phagocytosis in a temporally and spatially defined way, might offer clinical benefits in certain settings, such as acute or chronic bacterial infections. Therefore, future work might be directed towards identifying additional negative regulators of phagocytosis, characterizing their role in various phagocytic pathways, and finding means to modulate their activity. The continuing study of CEACAM3 as a highly efficient phagocytic receptor in human granulocytes will surely contribute to this ambitious endeavour.

CRediT authorship contribution statement

Klein Jule: Visualization, Writing – original draft. **Gregg Helena L.:** Visualization, Writing – original draft. **Schüber Meike:** Visualization, Writing – original draft. **Kuiper Johannes W.P.:** Conceptualization, Writing – original draft. **Hauck Christof R.:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors are indebted to Michael Laumann (Electron Microscopy Service; University of Konstanz, Germany) for scanning electron microscopy. Work by the authors has been supported by the DFG (HA 2856/10-1 and HA 2856/11-1) and Baden-Württemberg Stiftung (BWST_WSF-020) to CRH.

References

Abram, C.L., Lowell, C.A., 2007. The expanding role for ITAM-based signaling pathways in immune cells. Sci. 'S. STKE: Signal Transduct. Knowl. Environ. 2007 re2.

Aderem, A., Underhill, D.M., 1999. Mechanisms of phagocytosis in macrophages. Annu. Rev. Immunol. 17, 593–623.

Adrian, J., Bonsignore, P., Hammer, S., Frickey, T., Hauck, C.R., 2019. Adaptation to host-specific bacterial pathogens drives rapid evolution of a human innate immune receptor. Curr. Biol. 29, 616–630 e615.

Araki, N., Johnson, M.T., Swanson, J.A., 1996. A role for phosphoinositide 3-kinase in the completion of macropinocytosis and phagocytosis by macrophages. J. Cell Biol. 135, 1249–1260.

Backert, S., Haas, R., Gerhard, M., Naumann, M., 2017. The *Helicobacter pylori* Type IV secretion system encoded by the cag pathogenicity Island: architecture, function, and signaling. Curr. Top. Microbiol Immunol. 413, 187–220.

Baker, E.P., Sayegh, R., Kohler, K.M., Borman, W., Goodfellow, C.K., Brush, E.R., Barber, M.F., 2022. Evolution of host-microbe cell adherence by receptor domain shuffling. eLife 11, e73330. https://doi.org/10.7554/eLife.73330.

Beauchemin, N., Draber, P., Dveksler, G., Gold, P., Gray-Owen, S., Grunert, F., Hammarstrom, S., Holmes, K.V., Karlsson, A., Kuroki, M., Lin, S.H., Lucka, L., Najjar, S.M., Neumaier, M., Obrink, B., Shively, J.E., Skubitz, K.M., Stanners, C.P., Thomas, P., Thompson, J.A., Virji, M., von Kleist, S., Wagener, C., Watt, S., Zimmermann, W., 1999. Redefined nomenclature for members of the carcinoembryonic antigen family. Exp. Cell Res. 252, 243–249.

Behrens, I.K., Busch, B., Ishikawa-Ankerhold, H., Palamides, P., Shively, J.E., Stanners, C., Chan, C., Leung, N., Gray-Owen, S., Haas, R., 2020. The HopQ-CEACAM interaction controls CagA translocation, phosphorylation, and phagocytosis of *Helicobacter pylori* in neutrophils. mBio 11, e03256-19. https://doi.org/10.1128/mBio.03256-19.

Berger, C.N., Billker, O., Meyer, T.F., Servin, A.L., Kansau, I., 2004. Differential recognition of members of the carcinoembryonic antigen family by Afa/Dr adhesins of diffusely adhering *Escherichia coli* (Afa/Dr DAEC). Mol. Microbiol. 52, 963–983.

Bernstein, B.W., Bamburg, J.R., 2003. Actin-ATP hydrolysis is a major energy drain for neurons. J. Neurosci. 23, 1–6.

Bieling, P., Rottner, K., 2023. From WRC to Arp2/3: collective molecular mechanisms of branched actin network assembly. Curr. Opin. Cell Biol. 80, 102156 https://doi.org/10.1016/j.ceb.2023.102156.

Bonsor, D.A., Zhao, Q., Schmidinger, B., Weiss, E., Wang, J., Deredge, D., Beadenkopf, R., Dow, B., Fischer, W., Beckett, D., Wintrode, P.L., Haas, R., Sundberg, E.J., 2018. The *Helicobacter pylori* adhesin protein HopQ exploits the dimer interface of human CEACAMs to facilitate translocation of the oncoprotein CagA. EMBO J. 37, e98664 https://doi.org/10.15252/embj.201798664.

Booth, J.W., 2006. Phosphoinositides in FCgamma receptor signaling. Front. Biosci. 11, 1264–1274.

Bos, M.P., Grunert, F., Belland, R.J., 1997. Differential recognition of members of the carcinoembryonic antigen family by Opa variants of *Neisseria gonorrhoeae*. Infect. Immun. 65, 2353–2361.

Boulton, I.C., Gray-Owen, S.D., 2002. Neisserial binding to CEACAM1 arrests the activation and proliferation of CD4+ T lymphocytes. Nat. Immunol. 3, 229–236.

Brewer, M.L., Dymock, D., Brady, R.L., Singer, B.B., Virji, M., Hill, D.J., 2019. *Fusobacterium spp.* target human CEACAM1 via the trimeric autotransporter adhesin CbpF. J. Oral. Microbiol. 11, 1565043.

Buntru, A., Zimmermann, T., Hauck, C.R., 2009. FRET-based subcellular visualization of pathogen-induced host receptor signalling. BMC Biol. 7, 81.

Buntru, A., Kopp, K., Voges, M., Frank, R., Bachmann, V., Hauck, C.R., 2011. Phosphatidylinositol-3′ kinase activity is critical for initiating the oxidative burst and bacterial destruction during CEACAM3-mediated phagocytosis. J. Biol. Chem. 286, 9555–9566.

Buntru, A., Roth, A., Nyffenegger-Jann, N.J., Hauck, C.R., 2012. HemITAM signaling by CEACAM3, a human granulocyte receptor recognizing bacterial pathogens. Arch. Biochem Biophys. 524, 77–83.

Castellano, F., Chavrier, P., Caron, E., 2001. Actin dynamics during phagocytosis. Semin. Immunol. 13, 347–355.

Catton, E.A., Bonsor, D.A., Herrera, C., Stalhammar-Carlemalm, M., Lyndin, M., Turner, C.E., Soden, J., van Strijp, J.A.G., Singer, B.B., van Sorge, N.M., Lindahl, G., McCarthy, A.J., 2023. Human CEACAM1 is targeted by a *Streptococcus pyogenes* adhesin implicated in puerperal sepsis pathogenesis. Nat. Commun. 14, 2275.

Chen, L., Chen, Z., Baker, K., Halvorsen, E.M., da Cunha, A.P., Flak, M.B., Gerber, G., Huang, Y.H., Hosomi, S., Arthur, J.C., Dery, K.J., Nagaishi, T., Beauchemin, N., Holmes, K.V., Ho, J.W., Shively, J.E., Jobin, C., Onderdonk, A.B., Bry, L., Weiner, H. L., Higgins, D.E., Blumberg, R.S., 2012. The short isoform of the CEACAM1 receptor in intestinal T cells regulates mucosal immunity and homeostasis via Tfh cell induction. Immunity 37, 930–946.

Crowley, M.T., Costello, P.S., Fitzer-Attas, C.J., Turner, M., Meng, F., Lowell, C., Tybulewicz, V.L., DeFranco, A.L., 1997. A critical role for Syk in signal transduction and phagocytosis mediated by Fcgamma receptors on macrophages. J. Exp. Med 186, 1027–1039.

Daniel, J.L., Molish, I.R., Robkin, L., Holmsen, H., 1986. Nucleotide exchange between cytosolic ATP and F-actin-bound ADP may be a major energy-utilizing process in unstimulated platelets. Eur. J. Biochem 156, 677–684.

Desjardins, M., Houde, M., Gagnon, E., 2005. Phagocytosis: the convoluted way from nutrition to adaptive immunity. Immunol. Rev. 207, 158–165.

Diakonova, M., Bokoch, G., Swanson, J.A., 2002. Dynamics of cytoskeletal proteins during Fcgamma receptor-mediated phagocytosis in macrophages. Mol. Biol. Cell 13, 402–411.

Fischer, W., Tegtmeyer, N., Stingl, K., Backert, S., 2020. Four chromosomal type IV secretion systems in *Helicobacter pylori*: composition, structure and function. Front. Microbiol. 11, 1592.

Flannagan, R.S., Jaumouille, V., Grinstein, S., 2012. The cell biology of phagocytosis. Annu. Rev. Pathol. 7, 49–86.

Fumagalli, M., Sironi, M., Pozzoli, U., Ferrer-Admetlla, A., Pattini, L., Nielsen, R., 2011. Signatures of environmental genetic adaptation pinpoint pathogens as the main selective pressure through human evolution. PLoS Genet. 7 e1002355.

Galaski, J., Shhadeh, A., Umana, A., Yoo, C.C., Arpinati, L., Isaacson, B., Berhani, O., Singer, B.B., Slade, D.J., Bachrach, G., Mandelboim, O., 2021. *Fusobacterium nucleatum* CbpF mediates inhibition of T cell function through CEACAM1 activation. Front Cell Infect. Microbiol 11, 692544.

Gibbs, R.A., Rogers, J., Katze, M.G., Bumgarner, R., Weinstock, G.M., Mardis, E.R., Remington, K.A., Strausberg, R.L., Venter, J.C., Wilson, R.K., Batzer, M.A., Bustamante, C.D., Eichler, E.E., Hahn, M.W., Hardison, R.C., Makova, K.D., Miller, W., Milosavljevic, A., Palermo, R.E., Siepel, A., Sikela, J.M., Attaway, T., Bell, S,, Bernard, K.E., Buhay, C.J., Chandrabose, M.N., Dao, M., Davis, C., Delehaunty, K.D., Ding, Y., Dinh, H.H., Dugan-Rocha, S., Fulton, L.A., Gabisi, R.A., Garner, T.T., Godfrey, J., Hawes, A.C., Hernandez, J., Hines, S., Holder, M., Hume, J., Jhangiani, S.N., Joshi, V., Khan, Z.M., Kirkness, E.F., Cree, A., Fowler, R. G., Lee, S., Lewis, L.R., Li, Z., Liu, Y.S., Moore, S.M., Muzny, D., Nazareth, L.V., Ngo, D.N., Okwuonu, G.O., Pai, G., Parker, D., Paul, H.A., Pfannkoch, C., Pohl, C.S., Rogers, Y.H., Ruiz, S.J., Sabo, A., Santibanez, J., Schneider, B.W., Smith, S.M., Sodergren, E., Svatek, A.F., Utterback, T.R., Vattathil, S., Warren, W., White, C.S., Chinwalla, A.T., Feng, Y., Halpern, A.L., Hillier, L.W., Huang, X., Minx, P., Nelson, J. O., Pepin, K.H., Qin, X., Sutton, G.G., Venter, E., Walenz, B.P., Wallis, J.W., Worley, K.C., Yang, S.P., Jones, S.M., Marra, M.A., Rocchi, M., Schein, J.E., Baertsch, R., Clarke, L., Csürös, M., Glasscock, J., Harris, R.A., Havlak, P., Jackson, A.R., Jiang, H., Liu, Y., Messina, D.N., Shen, Y., Song, H.X., Wylie, T., Zhang, L., Birney, E., Han, K., Konkel, M.K., Lee, J., Smit, A.F., Ullmer, B., Wang, H., Xing, J., Burhans, R., Cheng, Z., Karro, J.E., Ma, J., Raney, B., She, X., Cox, M.J., Demuth, J.P., Dumas, L.J., Han, S.G., Hopkins, J., Karimpour-Fard, A, Kim, Y.H., Pollack, J.R., Vinar, T., Addo-Quaye, C., Degenhardt, J., Denby, A., Hubisz, M.J., Indap, A., Kosiol, C., Lahn, B.T., Lawson, H.A., Marklein, A, Nielsen, R., Vallender, E. J., Clark, A.G., Ferguson, B., Hernandez, R.D., Hirani, K., Kehrer-Sawatzki, H., Kolb, J., Patil, S., Pu, L.L., Ren, Y., Smith, D.G., Wheeler, D.A., Schenck, I., Ball, E.V., Chen, R., Cooper, D.N., Giardine, B., Hsu, F., Kent, W.J., Lesk, A., Nelson, D.L., O'brien, W.E., Prüfer, K., Stenson, P.D., Wallace, J.C., Ke, H., Liu, X.M., Wang, P., Xiang, A.P., Yang, F., Barber, G.P., Haussler, D., Karolchik, D., Kern, A.D., Kuhn, R. M., Smith, K.E., Zwieg, A.S., 2007. Evolutionary and biomedical insights from the rhesus macaque genome. Science 316, 222–234. https://doi.org/10.1126/science.1139247. PMID: 17431167.

Goob, G., Adrian, J., Cossu, C., Hauck, C.R., 2022. Phagocytosis mediated by the human granulocyte receptor CEACAM3 is limited by the receptor-type protein tyrosine phosphatase PTPRJ. J. Biol. Chem. 298, 102269.

Goodridge, H.S., Reyes, C.N., Becker, C.A., Katsumoto, T.R., Ma, J., Wolf, A.J., Bose, N., Chan, A.S., Magee, A.S., Danielson, M.E., Weiss, A., Vasilakos, J.P., Underhill, D.M., 2011. Activation of the innate immune receptor Dectin-1 upon formation of a 'phagocytic synapse. Nature 472, 471–475.

Gray-Owen, S.D., Blumberg, R.S., 2006. CEACAM1: contact-dependent control of immunity. Nat. Rev. Immunol. 6, 433–446.

Gray-Owen, S.D., Lorenzen, D.R., Haude, A., Meyer, T.F., Dehio, C., 1997. Differential Opa specificities for CD66 receptors influence tissue interactions and cellular response to *Neisseria gonorrhoeae*. Mol. Microbiol. 26, 971–980.

Greenberg, S., 1995. Signal transduction of phagocytosis. Trends Cell Biol. 5, 93–99.

Heinrich, A., Heyl, K.A., Klaile, E., Muller, M.M., Klassert, T.E., Wiessner, A., Fischer, K., Schumann, R.R., Seifert, U., Riesbeck, K., Moter, A., Singer, B.B., Bachmann, S., Slevogt, H., 2016. *Moraxella catarrhalis* induces CEACAM3-Syk-CARD9-dependent activation of human granulocytes. Cell Microbiol. 18, 1570–1582.

Hermiston, M.L., Zikherman, J., Zhu, J.W., 2009. CD45, CD148, and Lyp/Pep: critical phosphatases regulating Src family kinase signaling networks in immune cells. Immunol. Rev. 228, 288–311.

Hill, D.J., Virji, M., 2003. A novel cell-binding mechanism of *Moraxella catarrhalis* ubiquitous surface protein UspA: specific targeting of the N-domain of carcinoembryonic antigen-related cell adhesion molecules by UspA1. Mol. Microbiol 48, 117–129.

Hill, D.J., Whittles, C., Virji, M., 2012. A novel group of *Moraxella catarrhalis* UspA proteins mediates cellular adhesion via CEACAMs and vitronectin. PLoS One 7, e45452.
Huang, Z.Y., Barreda, D.R., Worth, R.G., Indik, Z.K., Kim, M.K., Chien, P., Schreiber, A. D., 2006. Differential kinase requirements in human and mouse Fc-gamma receptor phagocytosis and endocytosis. J. Leukoc. Biol. 80, 1553–1562.
Isakov, N., 1997. ITIMs and ITAMs. The Yin and Yang of antigen and Fc receptor-linked signaling machinery. Immunol. Res 16, 85–100.
Islam, E.A., Anipindi, V.C., Francis, I., Shaik-Dasthagirisaheb, Y., Xu, S., Leung, N., Sintsova, A., Amin, M., Kaushic, C., Wetzler, L.M., Gray-Owen, S.D., 2018. Specific binding to differentially expressed human Carcinoembryonic Antigen-related cell adhesion molecules determines the outcome of *Neisseria gonorrhoeae* infections along the female reproductive tract. e00092-18 Infect. Immun. 86. https://doi.org/10.1128/IAI.00092-18.
Javaheri, A., Kruse, T., Moonens, K., Mejias-Luque, R., Debraekeleer, A., Asche, C.I., Tegtmeyer, N., Kalali, B., Bach, N.C., Sieber, S.A., Hill, D.J., Koniger, V., Hauck, C.R., Moskalenko, R., Haas, R., Busch, D.H., Klaile, E., Slevogt, H., Schmidt, A., Backert, S., Remaut, H., Singer, B.B., Gerhard, M., 2016. *Helicobacter pylori* adhesin HopQ engages in a virulence-enhancing interaction with human CEACAMs. Nat. Microbiol. 2, 16189 https://doi.org/10.1038/nmicrobiol.2016.189.
Johswich, K.O., McCaw, S.E., Islam, E., Sintsova, A., Gu, A., Shively, J.E., Gray-Owen, S. D., 2013. In vivo adaptation and persistence of *Neisseria meningitidis* within the nasopharyngeal mucosa. PLoS Pathog. 9, e1003509.
de Jonge, M.I., Hamstra, H.J., van Alphen, L., Dankert, J., van der Ley, P., 2003. Mapping the binding domains on meningococcal Opa proteins for CEACAM1 and CEA receptors. Mol. Microbiol. 50, 1005–1015.
Kiefer, F., Brumell, J., Al-Alawi, N., Latour, S., Cheng, A., Veillette, A., Grinstein, S., Pawson, T., 1998. The Syk protein tyrosine kinase is essential for Fcgamma receptor signaling in macrophages and neutrophils. Mol. Cell Biol. 18, 4209–4220. https://doi.org/10.1128/MCB.18.7.4209.
Königer, V., Holsten, L., Harrison, U., Busch, B., Loell, E., Zhao, Q., Bonsor, D.A., Roth, A., Kengmo-Tchoupa, A., Smith, S.I., Mueller, S., Sundberg, E.J., Zimmermann, W., Fischer, W., Hauck, C.R., Haas, R., 2016. *Helicobacter pylori* exploits human CEACAMs via HopQ for adherence and translocation of CagA. Nat. Microbiol. 2, 16188 https://doi.org/10.1038/nmicrobiol.2016.188.
Kopp, K., Buntru, A., Pils, S., Zimmermann, T., Frank, R., Zumbusch, A., Hauck, C.R., 2012. GRB14 is a negative regulator of CEACAM3-mediated phagocytosis of pathogenic bacteria. J. Biol. Chem. 287, 39158–39170.
Kuderna, L.F.K., Gao, H., Janiak, M.C., Kuhlwilm, M., Orkin, J.D., Bataillon, T., Manu, S., Valenzuela, A., Bergman, J., Rousselle, M., Silva, F.E., Agueda, L., Blanc, J., Gut, M., de Vries, D., Goodhead, I., Harris, R.A., Raveendran, M., Jensen, A., Chuma, I.S., Horvath, J.E., Hvilsom, C., Juan, D., Frandsen, P., Schraiber, J.G., de Melo, F.R., Bertuol, F., Byrne, H., Sampaio, I., Farias, I., Valsecchi, J., Messias, M., da Silva, M.N. F., Trivedi, M., Rossi, R., Hrbek, T., Andriaholinirina, N., Rabarivola, C.J., Zaramody, A., Jolly, C.J., Phillips-Conroy, J., Wilkerson, G., Abee, C., Simmons, J.H., Fernandez-Duque, E., Kanthaswamy, S., Shiferaw, F., Wu, D., Zhou, L., Shao, Y., Zhang, G., Keyyu, J.D., Knauf, S., Le, M.D., Lizano, E., Merker, S., Navarro, A., Nadler, T., Khor, C.C., Lee, J., Tan, P., Lim, W.K., Kitchener, A.C., Zinner, D., Gut, I., Melin, A.D., Guschanski, K., Schierup, M.H., Beck, R.M.D., Umapathy, G., Roos, C., Boubli, J.P., Rogers, J., Farh, K.K., Marques Bonet, T., 2023. A global catalog of whole-genome diversity from 233 primate species. Science 380, 906–913.
Kuespert, K., Pils, S., Hauck, C.R., 2006. CEACAMs - their role in physiology and pathophysiology. Curr. Opin. Cell Biol. 18, 565–571.
Kuiper, J.W., Pluk, H., Oerlemans, F., van Leeuwen, F.N., de Lange, F., Fransen, J., Wieringa, B., 2008. Creatine kinase-mediated ATP supply fuels actin-based events in phagocytosis. PLoS Biol. 6, e51.
Kuiper, J.W.P., Krause, J., Potgeter, L., Adrian, J., Hauck, C.R., 2023. A genome-wide genetic screen identifies CYRI-B as a negative regulator of CEACAM3-mediated phagocytosis. J. Cell Sci. 136 https://doi.org/10.1242/jcs.260771.
Kuroki, M., Arakawa, F., Matsuo, Y., Oikawa, S., Misumi, Y., Nakazato, H., Matsuoka, Y., 1991. Molecular cloning of nonspecific cross-reacting antigens in human granulocytes. J. Biol. Chem. 266, 11810–11817.
Lobo, E.O., Zhang, Z., Shively, J.E., 2009. Pivotal advance: CEACAM1 is a negative coreceptor for the B cell receptor and promotes CD19-mediated adhesion of B cells in a PI3K-dependent manner. J. Leukoc. Biol. 86, 205–218.
Lu, R., Pan, H., Shively, J.E., 2012. CEACAM1 negatively regulates IL-1beta production in LPS activated neutrophils by recruiting SHP-1 to a SYK-TLR4-CEACAM1 complex. PLoS Pathog. 8, e1002597.
May, R.C., Machesky, L.M., 2001. Phagocytosis and the actin cytoskeleton. J. Cell Sci. 114, 1061–1077.
McCaw, S.E., Schneider, J., Liao, E.H., Zimmermann, W., Gray-Owen, S.D., 2003. Immunoreceptor tyrosine-based activation motif phosphorylation during engulfment of *Neisseria gonorrhoeae* by the neutrophil-restricted CEACAM3 (CD66d) receptor. Mol. Microbiol. 49, 623–637.
McCaw, S.E., Liao, E.H., Gray-Owen, S.D., 2004. Engulfment of *Neisseria gonorrhoeae*: revealing distinct processes of bacterial entry by individual carcinoembryonic antigen-related cellular adhesion molecule family receptors. Infect. Immun. 72, 2742–2752.
Mehidi, A., Kage, F., Karatas, Z., Cercy, M., Schaks, M., Polesskaya, A., Sainlos, M., Gautreau, A.M., Rossier, O., Rottner, K., Giannone, G., 2021. Forces generated by lamellipodial actin filament elongation regulate the WAVE complex during cell migration. Nat. Cell Biol. 23, 1148–1162.
Mittal, M., Siddiqui, M.R., Tran, K., Reddy, S.P., Malik, A.B., 2014. Reactive oxygen species in inflammation and tissue injury. Antioxid. Redox Signal 20, 1126–1167.
Mix, A.K., Goob, G., Sontowski, E., Hauck, C.R., 2021. Microscale communication between bacterial pathogens and the host epithelium. Genes Immun. 22, 247–254.
Mocsai, A., Ruland, J., Tybulewicz, V.L., 2010. The SYK tyrosine kinase: a crucial player in diverse biological functions. Nat. Rev. Immunol. 10, 387–402.
Moonens, K., Hamway, Y., Neddermann, M., Reschke, M., Tegtmeyer, N., Kruse, T., Kammerer, R., Mejias-Luque, R., Singer, B.B., Backert, S., Gerhard, M., Remaut, H., 2018. *Helicobacter pylori* adhesin HopQ disrupts trans dimerization in human CEACAMs. EMBO J. 37, e98665.
Muenzner, P., Hauck, C.R., 2020. *Neisseria gonorrhoeae* blocks epithelial exfoliation by nitric-oxide-mediated metabolic cross talk to promote colonization in mice. Cell Host Microbe 27, 793–808 e795.
Muenzner, P., Dehio, C., Fujiwara, T., Achtman, M., Meyer, T.F., Gray-Owen, S.D., 2000. Carcinoembryonic antigen family receptor specificity of *Neisseria meningitidis* Opa variants influences adherence to and invasion of proinflammatory cytokine-activated endothelial cells. Infect. Immun. 68, 3601–3607.
Muenzner, P., Rohde, M., Kneitz, S., Hauck, C.R., 2005. CEACAM engagement by human pathogens enhances cell adhesion and counteracts bacteria-induced detachment of epithelial cells. J. Cell Biol. 170, 825–836.
Muenzner, P., Bachmann, V., Kuespert, K., Hauck, C.R., 2008. The CEACAM1 transmembrane domain, but not the cytoplasmic domain, directs internalization of human pathogens via membrane-microdomains. Cell Microbiol. 10, 1074–1092.
Muenzner, P., Bachmann, V., Hentschel, J., Zimmermann, W., Hauck, C.R., 2010. Human-restricted bacterial pathogens block shedding of epithelial cells by stimulating integrin activation. Science 329, 1197–1201.
Muenzner, P., Kengmo Tchoupa, A., Klauser, B., Brunner, T., Putze, J., Dobrindt, U., Hauck, C.R., 2016. Uropathogenic *E. coli* Exploit CEA to promote colonization of the urogenital tract Mucosa. PLoS Pathog. 12, e1005608.
Niedergang, F., Chavrier, P., 2005. Regulation of phagocytosis by Rho GTPases. Curr. Top. Microbiol Immunol. 291, 43–60.
Nordenfelt, P., Tapper, H., 2011. Phagosome dynamics during phagocytosis by neutrophils. J. Leukoc. Biol. 90, 271, 84.
Pechous, R.D., 2017. With Friends Like These: The Complex Role of Neutrophils in the Progression of Severe Pneumonia. Front Cell Infect. Microbiol 7, 160.
Pils, S., Kopp, K., Peterson, L., Delgado-Tascon, J., Nyffenegger-Jann, N.J., Hauck, C.R., 2012. The adaptor molecule Nck localizes the WAVE complex to promote actin polymerization during CEACAM3-mediated phagocytosis of bacteria. PLoS One 7, e32808.
Reth, M., 1989. Antigen receptor tail clue. Nature 338, 383–384.
Rosales, C., Uribe-Querol, E., 2017. Phagocytosis: a fundamental process in immunity. Biomed. Res. Int. 2017, 9042851.
Roth, A., Mattheis, C., Münzner, P., Unemo, M., Hauck, C.R., 2013. Innate recognition by neutrophil granulocytes differs between *Neisseria gonorrhoeae* strains causing local or disseminating infections. Infect. Immun. 81, 2358–2370.
Rottner, K., Stradal, T.E., 2016. How distinct Arp2/3 complex variants regulate actin filament assembly. Nat. Cell Biol. 18, 1–3.
Rottner, K., Stradal, T.E.B., Chen, B., 2021. WAVE regulatory complex. Curr. Biol. 31, R512–R517.
Sarantis, H., Gray-Owen, S.D., 2007. The specific innate immune receptor CEACAM3 triggers neutrophil bactericidal activities via a Syk kinase-dependent pathway. Cell Microbiol 9, 2167–2180.
Schmitter, T., Agerer, F., Peterson, L., Muenzner, P., Hauck, C.R., 2004. Granulocyte CEACAM3 is a phagocytic receptor of the innate immune system that mediates recognition and elimination of human-specific pathogens. J. Exp. Med. 199, 35–46.
Schmitter, T., Pils, S., Sakk, V., Frank, R., Fischer, K.D., Hauck, C.R., 2007. The granulocyte receptor CEACAM3 directly associates with Vav to promote phagocytosis of human pathogens. J. Immunol. 178, 3797–3805.
Shang, W., Jiang, Y., Boettcher, M., Ding, K., Mollenauer, M., Liu, Z., Wen, X., Liu, C., Hao, P., Zhao, S., McManus, M.T., Wei, L., Weiss, A., Wang, H., 2018. Genome-wide CRISPR screen identifies FAM49B as a key regulator of actin dynamics and T cell activation. Proc. Natl. Acad. Sci. USA 115, E4051–E4060.
Sheikh, A., Fleckenstein, J.M., 2023. Interactions of pathogenic *Escherichia coli* with CEACAMs. Front. Immunol. 14, 1120331.
Sintsova, A., Wong, H., MacDonald, K.S., Kaul, R., Virji, M., Gray-Owen, S.D., 2015. Selection for a CEACAM receptor-specific binding phenotype during Neisseria gonorrhoeae infection of the human genital tract. Infect. Immun. 83, 1372–1383.
van Sorge, N.M., Bonsor, D.A., Deng, L., Lindahl, E., Schmitt, V., Lyndin, M., Schmidt, A., Nilsson, O.R., Brizuela, J., Boero, E., Sundberg, E.J., van Strijp, J.A.G., Doran, K.S., Singer, B.B., Lindahl, G., McCarthy, A.J., 2021. Bacterial protein domains with a novel Ig-like fold target human CEACAM receptors. EMBO J. 40, e106103.
Steffen, A., Rottner, K., Ehinger, J., Innocenti, M., Scita, G., Wehland, J., Stradal, T.E., 2004. Sra-1 and Nap1 link Rac to actin assembly driving lamellipodia formation. EMBO J. 23, 749–759.
Swanson, J.A., Baer, S.C., 1995. Phagocytosis by zippers and triggers. Trends Cell Biol. 5, 89–92.
Tchoupa, A.K., Schuhmacher, T., Hauck, C.R., 2014. Signaling by epithelial members of the CEACAM family - mucosal docking sites for pathogenic bacteria. Cell Commun. Signal 12, 27. https://doi.org/10.1186/1478-811X-12-27.
Tchoupa, A.K., Lichtenegger, S., Reidl, J., Hauck, C.R., 2015. Outer membrane protein P1 is the CEACAM-binding adhesin of *Haemophilus influenzae*. Mol. Microbiol. 98, 440–455.
Tegtmeyer, N., Neddermann, M., Asche, C.I., Backert, S., 2017. Subversion of host kinases: a key network in cellular signaling hijacked by *Helicobacter pylori* CagA. Mol. Microbiol. 105, 358–372.
Tegtmeyer, N., Harrer, A., Schmitt, V., Singer, B.B., Backert, S., 2019. Expression of CEACAM1 or CEACAM5 in AZ-521 cells restores the type IV secretion deficiency for translocation of CagA by *Helicobacter pylori*. Cell Microbiol. 21, e12965.
Thompson, J.A., Grunert, F., Zimmermann, W., 1991. Carcinoembryonic antigen gene family: molecular biology and clinical perspectives. J. Clin. Lab Anal. 5, 344–366.

Underhill, D.M., Ozinsky, A., 2002. Phagocytosis of microbes: complexity in action. Annu. Rev. Immunol. 20, 825–852.
van Valen, L. , 1973. A new evolutionary law. In: Evolutionary Theory.
Virji, M., Makepeace, K., Ferguson, D.J.P., Watt, S.M., 1996. Carcinoembryonic antigens (CD66) on epithelial cells and neutrophils are receptors for Opa proteins of pathogenic *Neisseriae*. Mol. Microbiol. 22, 941–950.
Winterbourn, C.C., Kettle, A.J., Hampton, M.B., 2016. Reactive oxygen species and neutrophil function. Annu. Rev. Biochem. 85, 765–792.
Yelland, T., Le, A.H., Nikolaou, S., Insall, R., Machesky, L., Ismail, S., 2021. Structural basis of CYRI-B direct competition with Scar/WAVE Complex for Rac1. Structure 29, 226–237 e224.
Yuki, K.E., Marei, H., Fiskin, E., Eva, M.M., Gopal, A.A., Schwartzentruber, J.A., Majewski, J., Cellier, M., Mandl, J.N., Vidal, S.M., Malo, D., Dikic, I., 2019. CYRI/FAM49B negatively regulates RAC1-driven cytoskeletal remodelling and protects against bacterial infection. Nat. Microbiol. 4, 1516–1531.
Zhu, J.W., Brdicka, T., Katsumoto, T.R., Lin, J., Weiss, A., 2008. Structurally distinct phosphatases CD45 and CD148 both regulate B cell and macrophage immunoreceptor signaling. Immunity 28, 183–196.