



# Tetraspanin CD9: A Key Regulator of Cell Adhesion in the Immune System

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The tetraspanin CD9 is expressed by all the major subsets of leukocytes (B cells, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, natural killer cells, granulocytes, monocytes and macrophages, and immature and mature dendritic cells) and also at a high level by endothelial cells. As a typical member of the tetraspanin superfamily, a prominent feature of CD9 is its propensity to engage in a multitude of interactions with other tetraspanins as well as with different transmembrane and intracellular proteins within the context of defined membranal domains termed tetraspanin-enriched microdomains (TEMs). Through these associations, CD9 influences many cellular activities in the different subtypes of leukocytes and in endothelial cells, including intracellular signaling, proliferation, activation, survival, migration, invasion, adhesion, and diapedesis. Several excellent reviews have already covered the topic of how tetraspanins, including CD9, regulate these cellular processes in the different cells of the immune system. In this mini-review, however, we will focus particularly on describing and discussing the regulatory effects exerted by CD9 on different adhesion molecules that play pivotal roles in the physiology of leukocytes and endothelial cells, with a particular emphasis in the regulation of adhesion molecules of the integrin and immunoglobulin superfamilies.

**Keywords:** CD9, tetraspanins, integrins, ICAM1, activated leukocyte cell adhesion molecule, ADAM17, lymphocyte function-associated antigen 1, very late activation antigen 4

## INTRODUCTION

CD9, or Tspan 29 in the systematic nomenclature, is a 21–24 kDa member of the tetraspanin protein family. Tetraspanins are structurally characterized by containing four transmembrane domains, which delimit a small extracellular loop (known as SEL or EC1), a large extracellular loop (termed LEL or EC2), and short intracellular N- and C-terminal tails. Within the LEL domain five  $\alpha$ -helices (A-E) can be distinguished, with helices A, B, and E defining a region well conserved among different members (“constant region”) that is involved in tetraspanin dimerization and oligomerization, whereas helices C and D define the “variable region” of the LEL, involved in most lateral interactions of tetraspanins with other membrane proteins. The presence of a CCG motif after the B

**Abbreviations:** ADAM, a disintegrin and metalloproteinase; ALCAM, activated leukocyte cell adhesion molecule; BBB, blood-brain barrier; CAMs, cell adhesion molecules; CNS, central nervous system; cSMAC, central supramolecular activation cluster; DCs, dendritic cells; EAPs, endothelial adhesive platforms; ER, endoplasmic reticulum; ICAM-1, intercellular adhesion molecule 1; Ig-SF, immunoglobulin superfamily; IL-2, interleukin-2; IS, immune synapse; LDL, low density lipoproteins; LFA-1, lymphocyte function-associated antigen 1; LPS, lipopolysaccharide; mAb, monoclonal antibody; MMP, matrix metalloproteinase; NK, natural killer cells; PBMCs, peripheral blood mononuclear cells; PHA, phytohemagglutinin; pSMAC, peripheral supramolecular activation cluster; TEMs, tetraspanin-enriched microdomains; TLR4, toll like receptor-4; TNF- $\alpha$ , tumor necrosis factor-alpha; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late activation antigen 4.

helix in the LEL domain, with its two cysteines engaged in the formation of intradomain disulfide bonds with other conserved cysteines, membrane-proximal palmitoylations, and hydrophilic residues within transmembrane domains are also defining features of tetraspanin members. Tetraspanins are glycoproteins which generally contain several N-glycosylation sites in the LEL domain (1), and in this regard, CD9 is peculiar as it contains only one N-glycosylation site that is located in its SEL domain (2).

CD9 shows a wide cellular and tissue distribution and was initially identified as a lymphohematopoietic marker (3) and then implicated in a range of cellular functions, including motility, proliferation, differentiation, fusion, and adhesion [reviewed in Ref. (1, 4–6)]. Not surprisingly, given its involvement in such a range of crucial cell activities, CD9 plays a major role in critical physiological and pathological processes, including sperm–egg fusion, neurite outgrowth, myotube formation, viral infections, tumorigenicity, and metastasis [reviewed in Ref. (7–9)]. As for other members of the tetraspanin family, the biological functions of CD9 greatly depend on the multitude of dynamic interactions that this molecule is able to establish with other transmembrane and cytoplasmic proteins within the context of a specific type of membrane domains, called tetraspanin-enriched microdomains (TEMs) (10, 11). CD9 in TEMs can thus affect, either directly or indirectly, the activity of numerous transmembrane and intracellular proteins such as metalloproteinases and other enzymes, ion channels, receptors for growth factors, cytokines and chemokines, transporters, signaling transducers, and cytoskeletal linkers (Table 1). CD9 can potentially alter the activity of these molecules through different mechanisms including their selective confinement in TEMs or segregation into separate microdomain compartments, which would hinder their access to their cognate substrates or binding of their extracellular or intracellular ligands (10, 11).

It is striking that the basal expression of CD9 in the major types of leukocytes from freshly drawn blood [monocytes, natural killer (NK) cells, B cells, CD8<sup>+</sup> T cells, and CD4<sup>+</sup> T cells] is usually low (49, 50). However, its expression in these leukocyte subpopulations increases following their culture, being particularly high in monocytes (8, 49, 50). Likewise, CD9 is also clearly detected in activated lymphoblasts derived from PHA/interleukin-2-stimulated PBMCs (21). CD9 is also expressed by the different subsets of human and murine dendritic cells (DCs), with the exception of mouse plasmacytoid DCs (51). In contrast to the relatively low expression on resting leukocytes, the expression of CD9 is particularly high on endothelial cells (52), in keeping with the crucial role this tetraspanin plays in regulating the firm adhesion and transendothelial migration of leukocytes (24, 25).

Several excellent reviews have already covered the topic of how tetraspanins, including CD9, participate in the regulation of specific functions in different cells of the immune system (4, 8, 53, 54). In this review, however, we will focus particularly on discussing the regulatory effects exerted by CD9 on different adhesion molecules that play pivotal roles in the physiology of leukocytes and endothelial cells, with a particular emphasis in the regulation of adhesion molecules of the integrin and immunoglobulin superfamilies.

**TABLE 1** | List of molecules reported to associate with CD9.

CD9-associated molecules	Other associated tetraspanins	Reference
<b>Adhesion molecules</b>		
<b>Integrins</b>		
α1β1		(12)
α2β1	CD151	(13, 14)
α3β1	CD63, CD81, CD82, CD151, NAG-2, Co-029	(13, 14)
α4β1 [very late activation antigen 4 (VLA-4)]	CD53, CD81, CD82, CD151	(15, 16)
α5β1 (VLA-5)	CD151	(16)
α6β1	CD63, CD81, CD82, CD151, NAG-2, Co-029	(15, 17)
α7β1	CD151	(18)
αIIbβ3	CD151	(19, 20)
α6β4	CD151	(13)
αLβ2 (lymphocyte function-associated antigen 1)	CD81, CD82	(21–23)
<b>Immunoglobulin-SF members</b>		
Intercellular adhesion molecule 1 (CD54)	CD151	(24, 25)
EpCAM (CD326)	Co-29, Tspan8, D6.1A	(26)
B-CAM/Lu (CD239)		(27)
Activated leukocyte cell adhesion molecule (cD166)		(28, 29)
Vascular cell adhesion molecule-1 (CD106)	CD151	(24)
<b>Other adhesion receptors</b>		
CD42		(20, 30)
CD44	Co-29, Tspan8, D6.1A	(13)
CD47		(20)
Claudin-1		(31)
Syndecan		(13)
<b>Immune system molecules</b>		
HLA-DR	CD153, CD81, CD82	(15)
CD2	CD53	(32)
CD3	CD81, CD82	(33)
CD4	CD81, CD82	(33)
CD5		(33)
CD19	CD81	(34)
CD46		(12)
<b>Growth factors</b>		
Pro-TGF-α		(35)
Pro-HB-EGF		(36)
<b>Signaling molecules</b>		
CD117	CD63, CD81	(37)
GPCR56	CD81	(38)
PI4K	CD81, CD151, CD63	(39)
PKC	CD53, CD81, CD82, CD151	(27, 40)
<b>Other proteins</b>		
ADAM2	CD81	(41)
ADAM10	CD81, CD82	(42, 43)
ADAM17		(42, 44)
CD36		(45)
CD26 (DPPIV)		(27, 46)
CD224	CD37, CD81, CD53, CD82	(27)
CTL1/CD92		(27)
CTL2		(27)
EWI-2	CD63, CD81, CD82, CD151	(47)

(Continued)

TABLE 1 | Continued

CD9-associated molecules	Other associated tetraspanins	Reference
EWI-F	CD81	(48)
Hem-1		(27)
TADG-15		(27)
Syntaxins 3 and 4A		(27)

## CD9 REGULATES ADHESION MOLECULES AT THE IMMUNE SYNAPSE (IS) AND T LYMPHOCYTE ACTIVATION

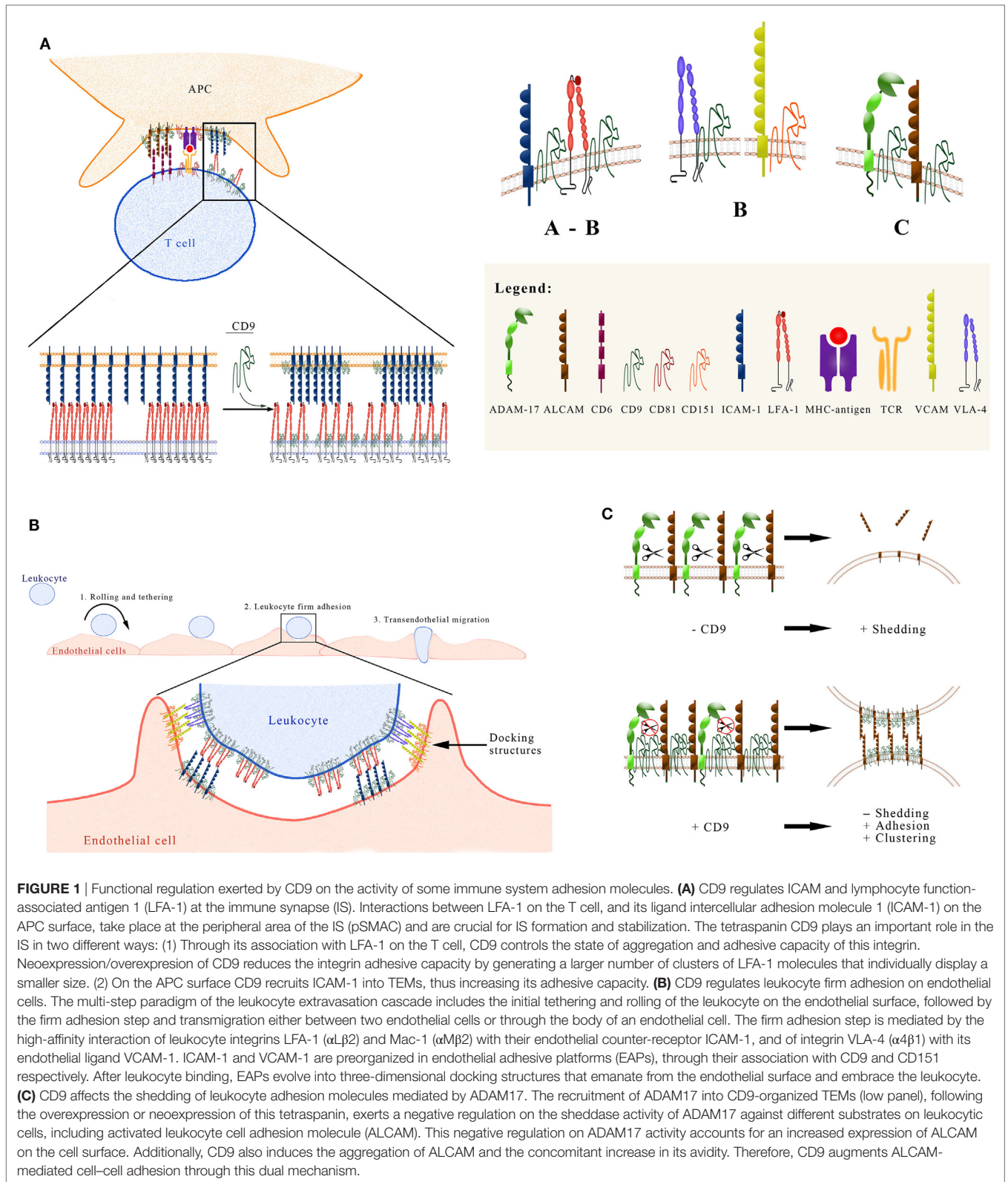
Recognition of antigenic-peptides bound to MCH-I and MCH-II molecules on the surface of antigen presenting cells (APCs) is essential for activation of CD8<sup>+</sup> and CD4<sup>+</sup> T cells, respectively, and triggers the initiation of T cell-mediated immune responses. This process requires the establishment of a dynamic structure at the APC-T cell contact area, termed as IS. In its mature form, the IS contains a clearly distinguishable central region (cSMAC), where clusters of TcR/CD3, costimulatory and signaling molecules concentrate, and a peripheral region (pSMAC) highly enriched in integrins and other adhesion molecules (55, 56). The establishment of IS proceeds through a series of spatiotemporal segregated events, including the initial scanning of antigen peptide-loaded MHC molecules, the specific recognition of antigen-loaded MHC, and signaling by the TcR/CD3 complex and stabilization of the IS. The interaction of different adhesion receptors on either side of the IS (i.e., on the APC or on the T cell) with their specific ligands on the opposing side is essential during the initial scanning and stabilization of the IS. In particular, interactions of integrin lymphocyte function-associated antigen 1 (LFA-1) ( $\alpha$ L $\beta$ 2) on the T cell with its ligands intercellular adhesion molecule 1 (ICAM-1) and ICAM-3 on the APC surface are crucial for IS formation and stabilization. During the initial scanning of MHC-peptide complexes on the surface of APCs, the affinity of T cell LFA-1 for its ligands is low/intermediate, but inside-out signaling triggered from the TcR/CD3 complex upon recognition of the cognate MHC-peptide induces the high-affinity state of this integrin, leading to strong ligand binding and stabilization of the IS (57, 58). TcR and LFA-1 molecules on immune cells are preorganized in nanoclusters that coalesce into larger aggregates following ligand binding (59–62). CD9 has been shown to engage through its LEL domain in direct interactions with LFA-1 on the surface of different types of leukocytes, including T lymphocytes and monocyte/macrophage-like cells (21). Through its association with LFA-1, CD9 controls the state of aggregation and adhesive capacity of this integrin. Through the use of different strategies, such as monoclonal antibody (mAbs) specific for CD9, as well as genetic approaches based on its silencing or neoexpression, it was demonstrated that CD9 exerts a negative regulation on the adhesive capacity of LFA-1. The mechanism involved in this inhibitory effect of CD9 on LFA-1 activity does not rely upon changes in the integrin affinity state, as inferred from the unaltered expression of the activation-reporter 24 epitope (21). These findings concur with the observation that talin relocalization, which is

required for the induction of conformational changes in LFA-1 and acquisition of increased affinity through inside-out signaling [reviewed in Ref. (63)], is not altered in T cells with silenced CD9 and CD151 (64). Thus, the negative regulation exerted by CD9 on the adhesive capacity of LFA-1 is rather related to alterations in its state of aggregation and the control of its binding valency (avidity). Interestingly, confocal and TIRF microscopy analyses showed that expression of CD9 in lymphocytic and monocytic cells induces LFA-1 molecules to become organized in a larger number of clusters but individually displaying a smaller size, which would account for the reduced integrin adhesive capacity (21) (Figure 1A). The association of LFA-1 with the actin cytoskeleton has long been known to control the avidity of this integrin through regulation of its dynamic reorganization into microclusters (65). Thus, although the exact mechanism by which CD9 alters the state of aggregation of LFA-1 molecules has not been resolved, it likely relies upon the linkage of this tetraspanin with the microfilaments of the actin cytoskeleton through the Ezrin–Radixin–Moesin (ERM) proteins (66).

In addition to LFA-1, VLA-4 ( $\alpha$ 4 $\beta$ 1) is another integrin predominantly expressed on hematopoietic cells, which also concentrates at the pSMAC playing a role in the stabilization of the IS and in T cell co-stimulation, though the precise ligand for this integrin on the APC is still unknown (67). The two classic ligands of integrin  $\alpha$ 4 $\beta$ 1, alternatively spliced fibronectin and VCAM-1, are not expressed on APCs. Alternative ligands of this integrin are members of the junctional adhesion molecules (JAM) family, but it has not been demonstrated so far whether these molecules play a role in the IS. Contrary to CD81, which strongly colocalizes with CD3 and contributes to cSMAC formation, becoming segregated from LFA-1 which is mainly found at the pSMAC, CD9 (along with CD151) shows less colocalization with CD3, indicating that it is not involved in the reorganization and clustering of CD3 at the IS (64). In contrast, CD9 does associate with integrin  $\alpha$ 4 $\beta$ 1 on T cells and drives both the accumulation of the high-affinity form of this integrin at the IS and the subsequent downstream signaling (64). CD9 (or CD151) silencing results in diminished relocalization of integrin  $\alpha$ 4 $\beta$ 1 to the IS, reduced accumulation of high-affinity  $\beta$ 1 integrins at the cell–cell contact area, and decreased downstream integrin signaling.

Besides their adhesive function, integrins also work as efficient bidirectional signaling molecules (68). In T cells, LFA-1 costimulation lowers the TcR-mediated activation threshold (69, 70). Similarly, ligation of integrin  $\alpha$ 4 $\beta$ 1 also provides costimulatory signaling (71). Downstream signaling from these adhesion receptors is mediated through interactions with a number of adaptor and signaling molecules (72, 73), whose activity, location, and aggregation may be regulated by tetraspanins through their selective inclusion in, or exclusion from, specific TEMs. CD9 and CD151 have been shown to increase integrin-dependent ERK1/2 signaling, and the knockdown of these tetraspanins reduces the phosphorylation of focal adhesion kinase (FAK) and ERK1/2 integrin downstream targets and impairs the enrichment of phosphorylated FAK at the IS (64).

Activated leukocyte cell adhesion molecule (ALCAM) or CD166 is a member of the immunoglobulin superfamily of CAMs that can engage in homophilic (ALCAM–ALCAM) as well



as heterophilic (ALCAM-CD6) interactions. ALCAM-mediated adhesion is crucial in different physiological and pathological situations, with particular relevance in immune responses, collective cell migration, cancer metastasis, neuronal development,

and leukocyte migration across blood-brain barrier (BBB) in multiple sclerosis and autoimmune encephalomyelitis. ALCAM on APCs engages in heterophilic interactions with CD6 on T cells, with both molecules becoming redistributed to the IS in

an antigen-dependent manner, which have been shown to play an important role in the formation and stabilization of the IS (74). They also provide costimulatory signals and thus contribute to T cell activation and proliferation (75–77). ALCAM-mediated APC-T cell adhesion requires upregulation of ALCAM avidity for CD6, which is brought about by the aggregation of ALCAM molecules on the cell surface, a process that is in turn controlled by their dynamic association with the actin cytoskeleton. Such dynamic linkage with the actin cytoskeleton takes place through the interaction of a C-terminal KTEA PDZ-binding motif and a membrane-proximal positively charged stretch in ALCAM's cytoplasmic tail, with the adaptor proteins syntenin-1 and the ERM protein ezrin, respectively (28, 78).

In this context, CD9 has been shown to play an important role in the regulation of ALCAM-mediated cell adhesion and T cell activation. On leukocytes, CD9 acts as a scaffold in ALCAM-containing TEMs, engaging in direct lateral interactions through its LEL domain with the extracellular region of ALCAM, therefore, upregulating its adhesive capacity and co-stimulation in T cells through its ligand CD6 (29). Importantly, this enhancement of ALCAM avidity and functional activity induced by CD9 is mediated by a dual mechanism involving, on the one hand, augmented clustering of ALCAM molecules and, on the other hand, upregulation of ALCAM surface expression due to the inhibition of ADAM17/TACE sheddase activity (the latter will be discussed below) (Figure 1C). As CD9 has been shown to be part of TEMs that contain a number of proteins directly linked to ERM proteins (66), this tetraspanin could contribute an additional level of regulation to the dynamic association with the actin cytoskeleton that ultimately controls ALCAM avidity and costimulatory capacity.

## ENDOTHELIAL CD9 REGULATES LEUKOCYTE ADHESION, EXTRAVASATION, AND INFLAMMATION

Migration across the endothelial cell layer that lines the inner surface of blood vessels is required for leukocyte recirculation during immune surveillance and their accumulation at sites of tissue infection and inflammation. Circulating leukocytes exit the blood vessels at certain preferred sites, such as the high endothelial venules of secondary lymphoid organs or the postcapillary venules at sites of inflammation. Leukocyte transendothelial migration proceeds through a series of steps which are mediated by a set of complex and sequential interactions between adhesion receptors and counter-receptors expressed on the leukocyte and the endothelial apical surface. This sequence of events represents the multi-step paradigm of the leukocyte extravasation cascade (79–81). These steps include the initial tethering and rolling of the leukocyte on the endothelial surface, followed by the firm adhesion, subsequent crawling on the endothelial apical surface in search for an appropriate exit site and the actual transmigration either between two endothelial cells (paracellular route) or through the body of an endothelial cell (transcellular route) and finally, leukocyte migration in the interstitial tissue following specific chemotactic cues. Specifically, the step of firm leukocyte

adhesion to the endothelium is fundamentally mediated by the high-affinity interaction of leukocyte integrins, LFA-1 ( $\alpha L\beta 2$ ) and Mac-1 ( $\alpha M\beta 2$ ), with their endothelial counter-receptor ICAM-1, and of integrin VLA-4 ( $\alpha 4\beta 1$ ) with its endothelial ligand VCAM-1. These high-affinity interactions require the activation of integrins in response to intracellular signals triggered upon recognition by leukocyte chemokine receptors of chemokines immobilized on heparan sulfate proteoglycans on the endothelial apical surface.

Vascular endothelium plays an active role in transendothelial leukocyte migration by controlling the expression level and organization of adhesive molecules. Following exposure to proinflammatory cytokines (such as TNF- $\alpha$  and IL-1 $\beta$ ), an increase in the expression of E- and P-selectins (main mediators of leukocyte tethering and rolling steps) and integrin ligands ICAM-1 and VCAM-1 is induced on the apical surface of endothelial cells, thus facilitating leukocyte adhesion to the endothelium and their subsequent transmigration. Interestingly, ICAM-1 and VCAM-1 on the endothelial surfaces are preorganized in adhesive clusters, so-called endothelial adhesive platforms (EAPs), by their inclusion in tetraspanin nanoplateforms through their association with CD9 and CD151 (24, 25). Analysis by FLIP-FRET microscopy evidenced that specificity exists in these associations, with CD9 preferentially associating with ICAM-1 whereas CD151 preferentially associates with VCAM-1 (24) (Figure 1B). Upon leukocyte binding, EAPs evolve into three-dimensional docking structures formed by elongated microvilli and long filopodia that emanate from the endothelial surface and embrace the adherent leukocytes, preventing their detachment under physiological hemodynamic flow conditions. The formation of these structures requires not only the tetraspanin-mediated clustering of ICAM-1 and VCAM-1, but also an active participation of the actin cytoskeleton and associated proteins, such as ERMs (ezrin–radixin–moesin),  $\alpha$ -actinin, vinculin, filamin, cortactin, and vasodilator-stimulated phosphoprotein (VASP), as well as signaling molecules like Rho A/ROCK, MLCK, src, pyk-2, and PIP<sub>2</sub> (82–84). Importantly, in the absence of CD9 the formation of microvilli required for the development of full docking structures is inhibited, crucially underlying the relevance of this particular tetraspanin in regulating the firm adhesion and transendothelial migration of leukocytes (85).

ALCAM is also abundantly expressed on endothelial cells in central nervous system (CNS) and has been shown to participate in the formation of docking structures (or transmigratory cups) required for leukocyte diapedesis (86). Interestingly, unlike ICAM-1 and VCAM-1, ALCAM mediates the transmigration of both lymphoid and myeloid leukocytes (86), and seems to be particularly relevant in the extravasation of monocytes rather than T cells across the BBB (87). Blockade of ALCAM reduced the transmigration of CD4<sup>+</sup> lymphocytes and monocytes across the BBB and reduced the severity and delayed the time of onset of experimental autoimmune encephalomyelitis in animal models, highlighting the potential usefulness of ALCAM as a therapeutic target in multiple sclerosis (86). ALCAM has also been found to associate with tight junction molecules that maintain BBB integrity (88). The interaction of CD9 with ALCAM on endothelial cells has not been as yet properly explored, but considering that ALCAM participates in the formation of docking structures and

what has been reported on leukocytic cells (29), it is likely that CD9 could also crucially regulate both the organization and the level of expression of ALCAM molecules on the CNS endothelium, critically controlling the transmigration of lymphocytes and monocytes across the BBB.

## CD9 REGULATES THE SHEDDING OF LEUKOCYTE ADHESION MOLECULES MEDIATED BY ADAM10 AND ADAM17 METALLOPROTEASES

Another important mechanism by which CD9 is capable to influence the activity of adhesion molecules with relevance in the immune system is through the modulation of ADAM10 and ADAM17 metalloproteinases. These two closely related members of the a disintegrin and metalloproteinase (ADAM) family are key enzymes responsible for the cleavage and release from the cell surface of the ectodomains of a large variety of transmembrane proteins, a process known as *ectodomain shedding*. Through this process, cells can rapidly alter their surface phenotype by releasing a variety of soluble protein ectodomains that can subsequently trigger autocrine, juxtacrine, paracrine, or endocrine cellular signaling and responses in specific target cells. The list of identified protein substrates that can be shed either by ADAM10 or by ADAM17 has kept on growing over recent years and currently comprises over a hundred different cell surface proteins, including growth factors, cytokines, many types of receptors, and numerous cell adhesion molecules (CAMs). Among the CAMs shed by ADAM10 or ADAM17 there are members of the immunoglobulin (ICAM-1, L1-CAM, Ep-CAM, VCAM-1, ALCAM, and JAM-A), integrin, cadherin, and selectin families as well as other adhesion receptors like CD44 (89–91). Many of the protein substrates of these two proteases are shared, indicating that their function may sometimes be complementary or redundant, but the mechanisms that selectively regulate their individual activities and substrate preference are still poorly understood. Regulation of ADAMs' shedding activities can be achieved at different levels, impacting upon their intracellular trafficking and maturation, metalloprotease activity, and accessibility of substrates [as reviewed in Ref. (92, 93)].

Tetraspanins are emerging as important regulators of ADAMs' shedding activities. By dynamically regulating their inclusion in, or exclusion from specific TEMs, tetraspanins are shown to influence the relative localization of ADAMs and their substrates on the plasma membrane, thus exerting crucial regulatory effects on their sheddase activity [reviewed in Ref. (42, 94)].

CD9 and other tetraspanins (including CD53, CD81, CD82, and CD151), have been reported to associate with ADAM10 under mild solubilization conditions. Additionally, antibody engagement of CD81, CD82, or CD9 did indeed stimulate ADAM10-dependent shedding of its substrates TNF- $\alpha$  and EGF (43). However, subsequent experiments using more stringent detergent conditions indicated that these tetraspanins do not associate directly with ADAM10 (95). Such interactions, instead, seem to be mediated through a distinct subfamily of tetraspanins (TspanC8), whose specific and direct association with ADAM10

regulates its exit from the ER, enzymatic maturation, intracellular trafficking, subcellular localization, and its metalloprotease activity against different cell surface protein substrates (94, 96, 97).

Importantly, CD9 is the only tetraspanin that has so far been reported to associate directly with ADAM17 on the surface of leukocytes and endothelial cells. This direct association was demonstrated by image and biochemical techniques, including confocal microscopy, proximity ligation, co-immunoprecipitation, covalent crosslinking, and pull-down assays. Heterologous expression/overexpression of CD9 or treatment with agonist CD9-specific antibodies inhibited ADAM17-mediated shedding of different substrates from leukocytic cells, including TNF- $\alpha$  and the adhesion molecules ICAM-1 and ALCAM/CD166. Thus, while anti-CD81 mAbs enhanced the release of TNF- $\alpha$  mediated by ADAM10 (43), anti-CD9 mAbs regulated negatively the stimulated release of that proinflammatory cytokine mediated by the closely related sheddase ADAM17 (44). Additionally, CD9 knockdown increased ADAM17-mediated shedding of its substrates (29, 44). CD9-mediated inhibition of ALCAM shedding by ADAM17 resulted in increased ALCAM levels on the cell surface and augmented ALCAM-mediated cell adhesion (29) (**Figure 1C**). These findings were later confirmed for additional ADAM-17 substrates, like LR11 (SorLa), a member of the low density lipoprotein receptor family which binds ApoE and has a key role in cell migration, adhesion, and drug resistance (98).

## CONCLUDING REMARKS

CD9 is emerging as an important regulatory molecule that controls the expression and activity of different adhesion molecules of crucial importance in the immune system. At the IS, CD9 on the T cell side regulates the clustering and adhesive activity of integrins LFA-1 ( $\alpha$ L $\beta$ 1) and VLA-4 ( $\alpha$ 4 $\beta$ 1), whereas on the APC side, CD9 regulates the expression of ALCAM/CD166 and the avidity of its interaction with CD6. Through the control exerted by CD9 on the expression and/or activity of these adhesive receptors, this tetraspanin regulates the stabilization of the IS and the subsequent activation of T lymphocytes.

On activated endothelial cells exposed to inflammatory cytokines, tetraspanins CD9 and CD151 play a crucial role in the step of firm leukocyte adhesion by arranging endothelial ICAM-1 and VCAM-1 in preorganized adhesive clusters, called EAPs. Upon leukocyte binding to the luminal endothelial surface, EAPs evolve into docking structures that embrace the leukocytes, preventing their detachment under flow conditions.

CD9 is the only tetraspanin that has been shown to associate directly with ADAM17 on leukocytes and endothelial cells. Through this direct interaction with ADAM17, CD9 inhibits the sheddase activity of this metalloproteinase against its substrates, thus regulating the balance between the membrane and soluble forms of crucial adhesion molecules, such as ICAM-1 and ALCAM, which are key in the processes of leukocyte extravasation and recruitment into inflamed tissues and stabilization of the IS.

These important regulatory effects exerted by CD9 on the activity of adhesion molecules with relevance in the immune system are summarized in the three panels of **Figure 1**.

## AUTHOR CONTRIBUTIONS

CC conceived and wrote the manuscript. RR and CC designed the figures. BC, YM-P, and RR commented and edited the manuscript. All authors read and approved the final manuscript.

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