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Role of ERM (ezrin-radixin-moesin) proteins in T lymphocyte polarization, immune synapse formation and in T cell receptor-mediated signaling

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. The ERM family of proteins
3.1. Activation of ERM proteins
3.2. Involvement of ERM proteins in cell cortex organization
3.3. Involvement of ERM proteins in intracellular signaling
4. Involvement of ERM proteins in T lymphocyte physiology
4.1. Involvement of ERM proteins in T cell polarization during lymphocyte migration
4.2. Involvement of ERM proteins during the formation of the immunological synapse
4.3. Involvement of ERM proteins in T cell intracellular signaling
5. Conclusions and perspectives
6. Acknowledgements
7. References

1. ABSTRACT

Following antigen recognition, T lymphocytes undergo strong actin cytoskeletal rearrangements. These play a crucial role in the molecular reorganization at the contact site between the T lymphocyte and the antigen presenting cell, termed the immunological synapse. Moreover, they are necessary for T cell activation that leads to cytokine secretion, T cell proliferation and effector function. Little is known on how membrane and signaling molecules interact with the actin cytoskeleton during these processes. Here we review the function of the ERM family of membrane-microfilament linkers, making emphasis on the role of these proteins in T lymphocyte physiology. We discuss how ERM proteins are involved in membrane reorganization during T lymphocyte polarization and immune synapse formation, and how these proteins may contribute to T cell receptor-mediated intracellular signaling that leads to T cell activation.

2. INTRODUCTION

Antigen recognition and subsequent T cell activation require the appropriate interaction between T cells and antigen presenting cells (APC). This interaction involves a series of events that depend on membrane and actin cytoskeleton dynamics, such as cell motility, cell-cell adhesion, and molecular relocalization that leads to cell polarization. Initial T cell receptor (TCR) signaling induces actin cytoskeleton rearrangements, which in turn are necessary for the stability of T cell-APC interactions, the organization of the contact zone between T cell and the APC, termed the immunological synapse, and for T cell activation. The immunological synapse appears as a complex molecular reorganization at the contact site between T cells and APCs. There, T cell receptors (TCR), co-receptors, adhesion molecules, as well as intracellular signaling and cytoskeleton components concentrate and eventually segregate into distinct supramolecular clusters

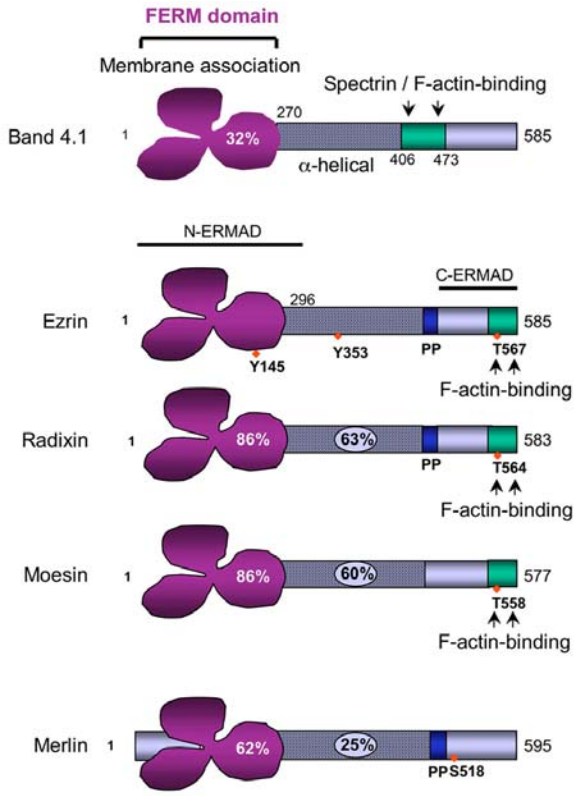

Figure 1. Structure of ERM family proteins**.** Schematic representation of the structure of ERM family proteins, as compared with that of the band 4.1 of erythrocytes. The percentage of homology between each protein and ezrin is shown inside each domain. The domains of intra- and inter-molecular interactions of ERM proteins are shown as N-ERMAD (N-terminal ERM association domain) and C-ERMAD (C-terminal ERM association domain). Tyrosines and Threonine residues important in ERM functions are depicted.

that redistribute following a precise relative topology. Immune synapse formation requires a functional actin and myosin cytoskeleton and agonistic TCR stimulation (reviewed in (1-4)).

Depending on the type and state of maturation of the T cell, the type and state of activation of the APC, and the stimulatory capacity of the antigen, the extent and dynamics of T cell-APC interactions, as well as the physical organization of immune synapses may be different. Thus, the molecular patterning forming central and peripheral supra-molecular activation clusters (5, 6) may not be always observed. However, common features appear clear, such as the spatial and temporal concentration of a variety of receptors, cytoskeletal and signaling proteins in the area of cell-cell contact. Immunological synapses are thought to serve to structure in time and space the complex communication between the T lymphocyte and the APC, in a way to ensure efficient antigen recognition and controlled T cell activation, and to provide stimuli to the APC (reviewed in (7, 8)).

The role of the actin cytoskeleton in T cell-APC interactions and subsequent T cell activation has been the object of active investigation during the last decade. An important question in the field is how interactions between the plasma membrane and the actin cytoskeleton take place and control the formation of the immunological synapse, as well as T cell activation. Various actin cytoskeleton-associated proteins were found to be involved. Talin was the first to be described as being recruited to the T cell-APC contact (9), and to localize in the peripheral zone of the immunological synapse (5). Talin can interact with integrins and with the actin cytoskeleton and may be involved in the stabilization of adhesion between the T cell and the APC (10). Moreover, the adaptor protein CD2AP links CD2 signals to the actin cytoskeleton *via* WASP (Wiscott-Aldrich Syndrome Protein) (11, 12). However, the role of CD2AP appears more complex, being also involved in TCR downregulation at the synapse (13). Recently, several laboratories including ours turned their attention to the ERM (ezrin-radixin-moesin) family of membrane-cytoskeleton crosslinkers. The data cumulated during the last several years indicate that these proteins may be important regulators of T cell functions. We briefly review here the functions and the putative importance of these proteins in T lymphocytes and other cell types, and we focus our discussion on aspects concerning T cell polarization, immune synapse formation and T cell activation.

3. THE ERM FAMILY OF PROTEINS

Ezrin, radixin and moesin are highly homologous proteins that link membrane components with the actin cytoskeleton. They were originally characterized as structural components of the cell cortex. They form, together with merlin/schwannomin, the ERM family of proteins (reviewed in (14-16)). Ezrin radixin and moesin share a high degree (about 75%) of amino acid identity and are highly similar to the single forms of this protein expressed in Drosophila (Dmoesin) and Caenorhabditis elegans (ERM-1). The fourth member, merlin/schwannomin displays a lower degree of homology with the other members of the family (49% identity with ezrin) and appears to have distinct functions. Merlin is the product of the tumor suppressor gene mutated in the neurofibromatosis type 2 syndrome. ERM proteins belong to the erythrocyte protein 4.1 super-family that are characterized by a conserved ~300-residue globular N-terminal domain, the FERM domain (Four.1-Ezrin-Radixin-Moesin). The FERM domain is also found in a wide variety of proteins including the erythrocyte band 4.1 protein, talin, as well as several tyrosine kinases and phosphatases. The FERM domain is followed by an alpha-helical domain and a charged C-terminal region with a binding site to filamentous actin (F-actin) at its extremity. Ezrin, radixin and merlin also contain a polyproline region between the helical and C-terminal domains. The FERM domain interacts with membrane components and most signaling molecules, whereas the C-terminus binds with the actin cytoskeleton (Figure 1).

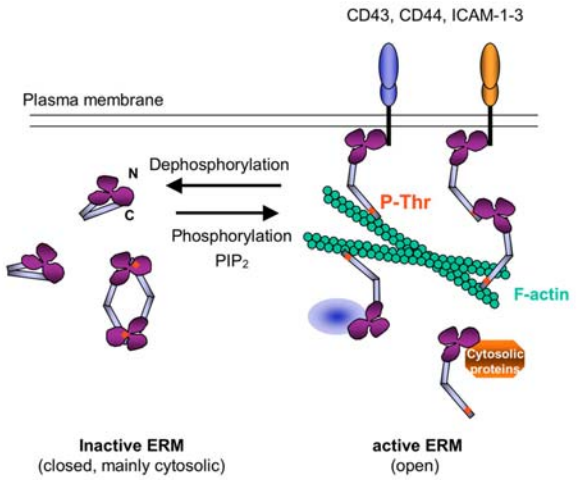

Figure 2. Activation of ERM proteins. ERM proteins exist under two conformations: a "dormant state", in which the protein presents a folded conformation, and an "active" state, in which the protein is unfolded and fully capable to interact with membrane components and with the cytoskeleton. The folded conformation is stabilized through the intramolecular interaction of the FERM domain with a 100 amino acid region at the C-terminal end of the protein. This interaction masks membrane and F-actin-binding site. Intermolecular association of these two opposite ERM regions may also occur leading to ERM homo- or hetero-dimerization. The activation of ERM proteins results in the unmasking of their membrane and cytoskeleton binding sites. This occurs through conformational changes induced by the binding of phosphoinisitides to the FERM domain and by the phosphorylation of a conserved threonine residue in the C-terminal domain.

ERMs can mediate the anchoring of some transmembrane proteins to the actin cytoskeleton, either directly, or through adapter molecules, such as EBP50. They control cell shape, cytokinesis and cell adhesion in various cell types including lymphocytes. In addition, ERMs are involved in intracellular signaling. ERM proteins appear therefore as important molecular integrators in a variety of cell functions, and may be therefore involved in tumor development (14-16).

ERM proteins are widely expressed throughout the organism, being, at least two of them, co-expressed in numerous cell types. However, their relative expression varies among the different tissues, indicating some specific functions. For instance, ezrin is found primarily in the apical region of epithelial cells, whereas moesin is enriched in endothelial and hematopoietic cells. Ezrin and moesin were found co-expressed, and radixin absent, in human lymphocytes, monocytes and neutrophils. Moesin is predominant ERM protein in these cells and the only one detected in platelets. In contrast, in human natural killer cells all three ERMs were present. The specific function of ERM proteins co-expressed in a particular cell type is not well defined. In stimulated lymphocytes, ezrin was cleaved by calpain, whereas moesin was not, suggesting that these two ERM proteins may play distinct functions (17-20). Moreover, the study of mice deficient in individual ERM proteins reveal that these proteins may have overlapping functions in many tissues, since these mice do not have general alterations. Nevertheless, some of these deficient mice reveal very defined functions of these proteins in particular tissues (21-24).

3.1. Activation of ERM proteins

ERM proteins exist under two conformations: a "dormant" state, in which the proteins present a head to tail folded conformation, and an "active" state, in which the protein is unfolded and fully capable to interact with membrane components and with the actin cytoskeleton (Figure 2). The folded conformation is stabilized through the intramolecular interaction of the FERM domain with the 100 amino acid C-terminal end of the protein (25). This interaction masks membrane and F-actin-binding sites (26, 27). Intermolecular association of these two opposite ERM regions may also occur leading to ERM homo- or hetero-dimerization. This is important in cellular morphogenesis (28).

The activation of ERM proteins results in the unmasking of their membrane and cytoskeleton binding sites. This occurs through conformational changes induced by the binding of phosphoinisitides to the FERM domain and by the phosphorylation of a conserved threonine residue in the C-terminal domain (29-32). Protein kinase C (PKC)-theta and Rho-kinase were reported to phophorylate this threonine residue. However, the particular serine/threonine kinase that phosphorylates ERM proteins *in vivo* are poorly defined and may vary among different cells and with the type of stimulation (18, 30, 33, 34).

3.2. Involvement of ERM proteins in cell cortex organization

The role of ERM proteins in cell cortex organization has been extensively studied in epithelial cells. ERM proteins, and in particular ezrin, are enriched in the apical regions of polarized cells, where they play an important role in cell morphogenesis and in the localization of some transmembrane proteins in particular areas of the cell surface (14, 15).

The FERM domain can bind directly to the cytosolic tail of various transmembrane proteins (Table 1). They can also indirectly interact with other proteins *via* the adaptor protein EBP50. In T lymphocytes, the transmembrane proteins CD43, CD44, L-selectin, P-selectin glycoprotein ligand-1 (PSGL-1), intercellular adhesion molecules (ICAM-1, ICAM-2 and ICAM-3) and CD95 (APO-1/Fas), have been identified as ERM partners (Table 1). Although most transmembrane proteins appear to bind all three ERM proteins, the death receptor Fas/CD95 binds to ezrin, but not moesin, in T lymphocytes.

ERM proteins are involved in cell cortex organization at two important stages of T lymphocyte physiology: during the polarization and migration in response to chemokines, and during the formation of the immunological synapse upon antigen recognition (chapter 4).

3.3. Involvement of ERM in intracellular signaling

In addition to their capacity to interact with the cytoskeleton and with membrane components, ERM

Table 1. ERM-associated proteins

Transmembrane proteins	References	Cytoplasmic proteins	References
CD43	56, 97	Crk	40
CD44	97-100	Dbl	35
CD46	101	EBP50/NHE-RF	102
CD93	103	E3KARP	104
CD95 APO/Fas	60, 88	FAK	45
ICAM-1	68, 105	Hamartin	39
ICAM-2	97, 105	Lck	94
ICAM-3	55, 59	N-WASP	41
L-selectin	106	Palladin	107
PSGL-1	57, 58	PI3K	44
Syndecan-2	108, 109	PKA	110
VCAM-1	68	PKC-alpha	46
NHE-1	111	Src	48
		Syk	89
		Pak-1	43
		PALS1	113

proteins were shown to interact with an increasing number of effectors of intracellular signaling (Table 1). Some interactions may be direct, while others are mediated by EBP50. ERM proteins are substrates of protein kinases that phosphorylate ERM proteins on tyrosine, or serine and threonine residues. These phosphorylation events are crucial for ERM function. Finally, the binding of phosphoinositides produced during the activation of phospholipid kinases, such as phosphatidylinositol-4,5-bisphosphate (PIP2) modulates ERM activity. Therefore, ERM proteins are themselves effectors of some intracellular signaling pathways (14, 15).

ERM proteins are important players in signaling pathways regulated by Rho-family GTPases. These pathways control cell morphogenesis, adhesion, motility and proliferation in response to a variety of cellular stimuli. Modulation of Rho family pathways may occur in part through interaction of ERM proteins with regulators or effectors of Rho family GTPases, such as RhoGDI, Dbl, Hamartin, Crk, Pak-1, N-WASP, etc. (34-43). Through interaction with the phosphatidylinositol-3 kinase (PI3K)/Akt pathway and with focal adhesion kinase, ezrin controls survival signals induced by adhesion molecules in epithelial cells (44, 45). Moreover, ezrin interaction with protein kinase C (PKC)-alpha modulates cell motility induced by this kinase (46). Ezrin interacts with c-Src, undergoes tyrosine phosphorylation, and cooperates with this kinase in deregulating cadherin-dependent cell-cell contacts in mammary carcinoma cells (47). This phenomenon is important for adhesion-mediated proliferation in epithelial cells (48). Interesting, phosphorylation of distinct tyrosine residues of ezrin appear to control the involvement of ezrin in different adhesion-dependent signaling pathways that lead to cell survival or proliferation (44, 48). ERM proteins could also participate in c-AMP-dependent protein kinase (PKA) pathways, since interaction between ERM and PKA, as well as ERM serine phosphorylation by PKA were reported. (49-53).

4. INVOLVEMENT OF ERM PROTEINS IN T LYMPHOCYTE PHYSIOLOGY

T lymphocytes are highly dynamic cells that migrate through the blood stream, the lymphoid tissues to ensure adaptive immune responses. To accomplish their functions, T cells interact in various manners with a variety of cells in different tissues, such as endothelial cells in blood vessels or antigen presenting cells (dendritic cells, B cells and macrophages) in lymphoid organs. In addition, cytotoxic T lymphocytes interact with virus-infected target cells or tumor cells in order to eliminate them. The actin cytoskeleton plays a crucial role in all lymphocyte functions. ERM proteins, in particular, appear to perform key functions in cell polarity during lymphocyte migration and in T cell-APC interactions during the formation of the immunological synapse. The role of ERM proteins in intracellular T cell signaling starts to be elucidated, but it is still poorly explored.

4.1. Involvement of ERM proteins in polarization during T lymphocyte migration

ERM proteins play a crucial role in cell polarization during T lymphocyte migration. T cells polarize in response to adhesion or chemotactic stimuli displaying two poles: a lamellipodium-like structure at the front edge, which adheres to the extracellular substratum, and a posterior protrusion called the uropod. ERM proteins are involved in the generation of the uropod and in the anchoring of various transmembrane proteins (ICAMs, CD43, CD44, PSGL-1 and the death receptor CD95/Fas) to that area of the cell (54-60). The integrin LFA-1 is also concentrated in the uropod, although in the low affinity state (61). The precise function of the uropod in T cell physiology remains to be elucidated. However, the localized anchoring of adhesion molecules and some observations under the microscope suggest that the uropod could be an adhesive protrusion involved in the recruitment of bystander lymphocytes to areas of lymphocyte migration and in other immune cell interactions. Moreover, it may also facilitate the triggering of apoptosis by the Fas receptor (60, 62-64).

How do ERM proteins contribute to uropod formation is not completely elucidated. The activation of ERM proteins enhances the size of the uropod indicating a role of these proteins in the generation of this structure (54). However, inhibitors of the Rho kinase ROCK

inhibited uropod formation, without inhibiting ERM protein threonine phosphorylation, or the polarization of CD44 (54). This indicates that uropod formation depends on ROCK activity but is not the consequence of ERM activation by ROCK phosphorylation. It is worth noting that ERM activation through phosphorylation by the Rho kinase was previously reported (30). However, the threonine kinase that phosphorylates ERM in polarized T lymphocytes *in vivo* remains undetermined. PKC-theta could be a candidate although it has not been formally proven (33, 34, 54). ERM proteins may therefore be important for the maintenance of uropod integrity, perhaps through local activation of the Rho signaling pathway (36), but not for uropod generation.

The mechanism that drives ERM protein localization to the uropod is not known. In epithelial cells, ERM proteins concentrate in areas rich in F-actin (15, 65). This suggests that ERM positioning in these cells might be controlled, at least in part, by actin polymerization. In contrast, in migrating lymphocytes, ERM proteins strongly concentrate at the uropod, whereas F-actin is mainly concentrated at the opposite side, in the leading edge (63). This indicates that ERM subcellular localization is not necessarily driven by the localization of F-actin and may be differently regulated following the physiological needs of each cell type.

ERM protein activation is modulated during T cell polarization and cell migration. Thus, threonine phosphorylation of ezrin and moesin is rapidly down-modulated upon chemokine stimulation. This correlates with loss of microvilli and polarization of chemokine-activated T cells. Moreover, expression of an ERM T/D mutant, which mimics the phosphorylated active form of ezrin (66), retards the loss of microvilli and the polarization induced by chemokines (67). This indicates that ERM proteins need to be desactivated in order to facilitate T cell migration and polarization. This is likely an important mechanism to facilitate morphological changes during cell extravasation. Interestingly, ERM proteins are also recruited within endothelial cells, together with the adhesion proteins VCAM-1 and ICAM-1 at the sites of contact with leukocytes, forming a docking structure for leukocyte adhesion (68). Therefore, ERM proteins seem to be crucial for forming adhesive structures on both the migrating leukocyte and the endothelial cell (69).

What is the relationship between Rho family GTPases and ERM proteins during T cell polarity and cell migration ? Rho family GTPases are key regulators of cell polarity (70). Consistently, these GTPases regulate T cell polarization and uropod formation in T lymphocytes. Thus, over-expression of constitutive active mutants of these GTPases inhibited T cell polarization in cells displaying motile cell morphology. Conversely, dominant negative mutants induce a polarized phenotype in round shaped cells (71). Moreover, chemokines activate the guanine nucleotide exchange factor for Rho GTPases Vav, and over-expression of dominant negative mutant of Vav inhibit lymphocyte polarization (72). ERM dephosphorylation, loss of microvilli and polarization in chemokine-activated T lymphocytes involves Rac1, rather than Rac2, Rho, or Cdc42, as assessed using inhibitory peptides and dominant negative mutants. Interestingly, constitutive active mutants of Rac1 and Cdc42 induced ERM protein dephosphorylation and loss of microvilli, whereas constitutive active Rho has the opposite effect, increased ERM phosphorylation and the size of microvilli (73). Therefore, ERM proteins seem to cross-talk with Rho-family GTPases in T cells and control lymphocyte morphological changes in response to chemokines.

4.2. Involvement of ERM proteins during the formation of the immunological synapse

T cells recognize antigens as molecular fragments displayed at the surface of APCs. To this end, lymphocytes screen the surface of numerous APCs in lymphoid organs. Transient interactions occur at this stage needing dynamic morphological changes. ERM proteins and their associated transmembrane proteins seem to play important roles during the different stages of T cell-APC interactions.

The ERM-associated adhesion molecule ICAM-3 on the surface of T cells plays a key role in establishing the initial interactions between the T lymphocyte and the APC. Thus, relocalization of this adhesion molecule to the sites of contact rapidly occurs, even in the absence of antigenic signal. Upon antigen recognition and stabilization of the T cell-APC interaction, ICAM-3 is mainly observed at the peripheral zone of the synapse. Interestingly, another ERM-binding protein, PSGL-1, which co-localizes with ICAM-3 in the uropod of migrating cells (57), relocalizes to the pole of the cell opposite to the immune synapse, only in antigen-stimulated cells (74). Although it is tempting to speculate that ERM binding to ICAM-3 and to PSGL-1 might be responsible for their respective subcellular translocation, it is at present unknown how ERM-interactions could mediate the translocation of these two proteins to opposite poles of the T cell. Perhaps, dynamic interaction between ICAM-3 and PSGL-1 with ERM proteins, as well as with other partners, might account for their specific relocalization during T cell-APC interactions.

In human T cells, we observed that ezrin and moesin concentrate in the F-actin rich membrane protrusions that establish the contact with the APC. Ezrin is first observed at the peripheral area of the immune synapse, covering, at later times, also the center of the T cell-APC contact (75-77). In mouse T cells, however, the accumulations of ERM proteins at the T cell-APC contact site appear difficult to observe. Ezrin and moesin were reported to be excluded from the T cell-APC contact site with no accumulation in the contact zone, or even found accumulated in the antipodal region of the APC contact site (78, 79).

Ezrin and moesin appear to be important to move their partner surface molecules CD43 out of the center of the immunological synapse (78, 79). CD43 is an abundant highly glycosylated and sialylated surface protein. Studies of CD43-deficient mice lead to propose that CD43 plays a negative regulatory role in T cell activation (80). It was therefore proposed that the large size of the CD43

extracellular domain (81) was impeding, by steric hindrance and negative charge, the effective interactions of other surface receptors. Consistent with this idea, CD43 was shown to be excluded from the T cell-APC contact site (82). CD43 exclusion was active and dependent on CD43 interaction with ERM proteins and the actin cytoskeleton. Thus, over-expression of CD43 mutated in the ERM binding stretch, or the FERM domain of ezrin, inhibited CD43 exclusion from the immune synapse and further T cell activation (78, 79). Additional work showed, however, that the steric barrier model of CD43 was not completely correct and that the presence of CD43 extracellular domain in the T cell-APC contact site may not be inhibitory. In contrast, the intracellular region of CD43 may negatively regulate T cell activation (83, 84). An apparent paradox to the CD43 inhibitory role is the observation that CD43 may also display co-stimulatory properties when cross-linked alone or together with the TCR by means of antibodies (85). However, since CD43 does not have an identified ligand on the APC, the co-stimulatory role of CD43 remains questionable.

Antigenic stimulation induces the rapid dephosphorylation of ezrin and moesin on the C-terminal threonine residue that controls ERM activation (79, 86). ERM dephosphorylation is controlled by Vav1 and Rac1. Interestingly, both Rac1 and Cdc42 constitutive active mutants induced ERM dephosphorylation, whereas the Rho active mutant had the opposite effect. However, only the Rac1 dominant negative mutant inhibited ERM dephosphorylation induced by TCR engagement. ERM inactivation appears to modulate cellular rigidity and facilitate T cell-APC conjugation (86). ERM proteins also influence molecular clustering at the synapse. Thus, perturbing ERM function by over-expression of the FERM domain has an inhibitory effect on TCR clustering (75). Moreover, ERM proteins through their associated adaptor EBP50 interact with Cbp-PAG, a Csk-associated signaling adaptor localized in membrane rafts. Through this interaction, ERM proteins link raft components with the actin cytoskeleton, modulating the dynamics of membrane rafts in T cells and the formation of the immune synapse. T cell activation downregulates Cbp-EBP-moesin interaction, indicating that this complexes may play a negative regulatory role in raft dynamics (87).

4.3. Involvement of ERM proteins in intracellular signaling in T lymphocytes

Although much less explored than in epithelial cells, ERM proteins were also shown to be involved in signaling pathways in leukocytes. Thus, ERM proteins appear involved in signaling triggered by the death receptor CD95, or those triggered by the adhesion proteins PSGL-1 or ICAM-1. Very little is known, however, whether ERM proteins are involved in antigen-triggered signal transduction.

The interaction of ezrin with CD95 (APO/Fas) is important for triggering the intracellular signaling cascade leading to apoptosis induced by this receptor. Interestingly, contrary to other receptors that can interact with several ERM proteins, Fas specifically interacts with ezrin on a region of the FERM domain. CD95 is polarized in the uropod of migrating lymphocytes. Moreover, CD95-mediated apoptosis requires the integrity of the actin cytoskeleton. Therefore, the interaction of CD95 with ezrin and its polarization appear to be key events for rendering human T lymphocytes sensitive to CD95-mediated apoptosis (60, 88).

Lymphocyte adhesion may also induce activation signals through ERM proteins. Thus, ezrin and moesin mediate signal transduction of PSGL-1, a leukocyte adhesion molecule involved in tethering and rolling on endothelium. ERM proteins interact with PSGL-1 and with the protein tyrosine kinase Syk *via* the ITAM (immuno-receptor tyrosine-based activation motif)-based motif contained in the FERM domain (89). In this way ezrin and moesin can mediate PSGL-1-induced Syk activation that leads to transcriptional activation. Moreover, ezrin appears to be involved in an ICAM-2-mediated cell survival signaling pathway (90). This pathway involves Src kinases, ROCK, PI3K and AKT (90) and is reminiscent of that described in epithelial cells (44).

Ezrin and moesin interact *via* EBP50 with the signaling adaptor Cbp/PAG (87). This protein is mainly localized in membrane rafts, and binds and modulates the activity of Csk, a protein tyrosine kinase that negatively regulates Src-family kinases (91). Although not formally proven, it is tempting to speculate that ERM proteins could, through their interaction with this adaptor, be involved in the regulation of the activity of Src family kinases in T cells.

Ezrin was shown to be a substrate of tyrosine kinases activated by TCR and CD4 stimulation (92, 93). Lck appears responsible for this phosphorylation (94). The importance of phosphorylated tyrosine residues in ezrin and moesin was previously shown in epithelial cells (44, 48), or in T cells activated by PSGL-1 (89). However, it remains unknown whether the TCR signaling cascade involves ERM proteins similarly to adhesion molecules.

Although the role of ERM proteins in antigen-triggered signaling cascades is unknown, it is tempting to speculate that ERM proteins could play a role in the spatio-temporal positioning of key signaling molecules that polarize upon TCR engagement. For instance, we have shown that in response to antigenic stimulation human T cells form transient membrane protrusions that partially engulf the APC and occupy the peripheral area of the immune synapse. These membrane protrusions are enriched in F-actin and ezrin (75, 76). Interestingly, we also observed that some key effectors of the TCR signaling cascade, such as Vav1 and Ly-GDI, or NEMO, also transiently accumulate in those cellular protrusions (95, 96). This suggests that these, and likely other signaling molecules, may interact with the actin cytoskeleton in those areas. ERM proteins might be candidates for these interactions. They could favor polarized interactions between signaling molecules in that pole of the cell (Figure 3 A). In addition, ERM could maintain signaling molecules anchored to the cytoskeleton, preventing their spontaneous interaction thereby buffering the intensity of the signaling

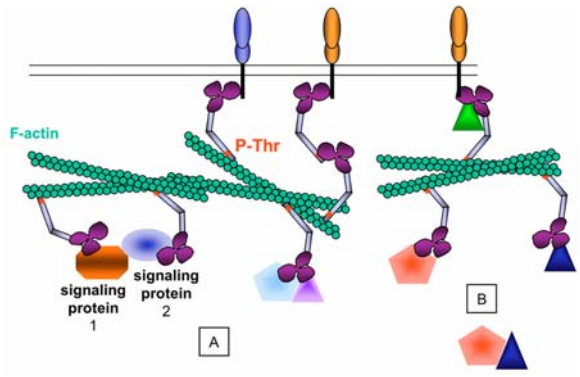

Figure 3. Involvement of ERM proteins in signal transduction. ERM proteins could play a role in the spatio-temporal positioning of key signaling molecules that polarize upon TCR engagement. They could favor local interactions between signaling molecules due to cytoskeleton-mediated localization of ERM proteins (A). In addition, ERM could maintain signaling molecules anchored to the cytoskeleton, preventing their spontaneous interaction, thereby buffering the intensity of the signaling cascade (B).

cascade (Figure 3 B). Clearly, the role of ERM proteins in T cell signaling is still largely unexplored and it will need deeper investigation in the near future.

5. CONCLUSION

ERM proteins appear as key organizers of the cell cortex during different stages of T cell physiology. In addition, their role in signal transduction just starts to be elucidated. These proteins will likely play unexpected roles in T lymphocytes, due to the dynamics and the multiplicity of processes at which membrane and cytoskeleton rearrangements are crucial in T cell physiology.

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