1. INTRODUCTION

1.1 T cell activation

Adaptive immunity is based on the clonal selection of lymphocytes bearing highly diverse antigen-specific receptors that allow the immune system to recognize any foreign antigen. One critical event in the initiation of an adaptive immune response is the activation of T lymphocytes. Antigen-specific T lymphocytes proliferate and differentiate into effector cells that eliminate pathogens. This process is triggered through the interaction of T cell antigen receptors (TCR) with their respective antigenic peptides presented by major histocompatibility molecules (MHC) on the surface of antigen-presenting cells (APC) (Unanue, 1984).

The TCR is a multisubunit complex that consists of clonotypic αβ chains noncovalently associated with the invariant CD3-γ, -δ, -ε and TCR-ζ chains (Weiss, 1993). The initial membrane proximal event triggered by the TCR is activation of protein tyrosine kinases (PTK) (Samelson and Klausner, 1992; Gauen et al., 1994) with the resultant phosphorylation of cellular proteins.

The generally accepted model for the initiation of signaling through antigen-receptors is that upon receptor engagement src family protein tyrosine kinases ($p59^{fyn}$, $p56^{lck}$) are recruited to the receptor. This enhances the phosphorylation of characteristic sequences within the cytoplasmic domains of the antigen-receptor complex, known as immunoreceptor tyrosine-based activation motifs (ITAMs) (Weiss and Littman, 1994). This in turn results in the recruitment and transphosphorylation of ZAP (ζ-associated protein)-70/Syk tyrosine kinases (Iwashima et al., 1994; Weil et al., 1995), which subsequently phosphorylate downstream substrates of the signal transduction cascade.

The substrates of these activated PTKs include well-known effector enzymes such as phospholipase Cγ (PLCγ) and Vav. Tyrosine phosphorylation of these and other

molecules is required for their activation. The class of intracellular molecules known collectively as adaptor proteins, such as Grb2, SLP76 and pp36/LAT, some themselves substrates of activated PTKs, serve to couple proximal biochemical events initiated by TCR ligation with more distal signaling pathways. Such pathways include those regulated by small G-proteins, lipid kinases (i.e. Phosphatidylinositol 3-hydroxyl kinase (PI3-kinase)) and members of the Erk family of serine/threonine protein kinases. In turn, activation of these pathways leads to the regulation of gene transcription, granule release and cytoskeletal changes.

TCR ligation in the context of appropriate costimuli results in transcriptional activation of numerous genes, and the best-characterized example to date is the regulation of the interleukin 2 (IL-2) gene. Transcription of the IL-2 gene is dependent on the formation and activation of a number of transcription factors, including AP-1, NF-κB, and the nuclear factor of activated T cells (NF-AT). The products of the growing NF-AT gene family have been implicated in the regulation of many additional genes, including those encoding numerous cytokines as well as the pro-apoptotic protein CD95 ligand. Ultimately, the intracellular signals elicited by e.g. binding of IL-2 to the IL-2 receptor result in T-cell proliferation, differentiation and/or effector functions (Perlmutter et al., 1993; Cantrell 1996).

In previously activated T cells or hybridoma cell lines, T cell stimulation may also induce apoptosis, a process which has been termed activation-induced cell death (AICD). The early signal transduction cascades which are involved in T cell proliferation and activation-induced apoptosis are overlapping. The interface between the early inductive phase and the effector phase in T cell death appears to be regulated by the interaction of CD95/CD95L (Fas/FasL) the expression of which is tightly regulated by T cell stimulation. Antigen-mediated apoptosis is clearly important in the development and homeostasis of the immune system and is particularly critical for the establishment of immune tolerance.

Signals generated through the T-cell receptor (TCR) alone are not sufficient for full activation of T cells, and therefore, additional signals are required. These signals are

provided by co-receptors and a large number of co-stimulatory molecules expressed on the surface of T lymphocytes.

The specific interaction between antigen and the TCR is accompanied by interactions between non-polymorphic regions of the MHC class I or class II molecules with CD8 and CD4, respectively, which function as co-receptors and associate with activated PTKs (as reviewed by Weiss and Littman, 1994; Chan and Shaw, 1996; Wange and Samelson, 1996). These MHC-binding co-receptors stabilize the TCR:ligand association during a process of structural adaption, affecting TCR occupancy. The resulting signals are critical for T cell development and activation of peripheral T cells (Garcia et al., 1996; Jelonek et al., 1998).

Additional costimulatory signals are required for an optimal generation of functional T cell responses such as interleukin 2 (IL-2) production, proliferation and differentiation to effector cells. However, the mechanisms by which costimulatory molecules facilitate T cell activation and accomplish TCR signaling remain largely elusive. Current models suggest that costimulatory molecules exert their costimulatory action either by transducing distinct signaling pathways leading to gene transcription (Pages et al., 1994; Reif and Cantrell 1998), or alternatively, by acting as a general amplifier of early TCR signaling (Viola and Lanzavecchia, 1996; Bachman et al., 1997; Tuesto and Acuto, 1998).

The best-studied and most prominent co-stimulatory molecule is CD28, which interacts with its ligands CD80 (B7-1) or CD86 (B7-2/B70) on the APC. While originally CD28 was regarded as an adhesion receptor (Linsley et al., 1990; Shimizu et al., 1992), more recent studies have focused on the elucidation of its signaling ability (Rudd, 1996). Many studies have shown that CD28 decisively determines the destiny of the T cell response (reviewed by Jenkins, 1994; Bluestone, 1995; Thompson, 1995). In most T cells, CD28 lowers the threshold (e.g. the number of triggered TCR) needed for T cell activation and increases response longevity, effects linked to increased transcription and stability of mRNAs. In addition, CD28 has also been described to influence the cytoskeleton, which is important for cytokinesis, cell shape, and adhesion processes (Crew and Erickson, 1993). CD28 induces signaling events that are initiated by tyrosine phosphorylation of CD28 at the cytoplasmic motif (pYMNM), followed by recruitment of the signaling proteins

phosphatidylinositol 3-kinase (PI3-kinase), growth factor receptor-bound protein-2 (GRB-2), and T cell specific protein-tyrosine kinase (ITK). In support of this, tyrosine kinase inhibitors can block CD28 costimulation (June, et al., 1987; Atluru, S., and Atluru D., 1991).

Another important costimulatory molecule is CD2, which is expressed by T lymphocytes and natural killer cells. Several lines of evidence indicate that CD2, by binding to its ligands CD48, CD58 (lymphocyte function-associated molecule 3; LFA-3) and CD59 is involved in T cell adhesion and activation (Meuer et al., 1984; Shaw et al., 1986; Selvaraj et al., 1987; Hahn et al., 1992; Kato et al., 1992). Depending on the mode of its ligation, CD2 can deliver either stimulatory or inhibitory signals to T cells (Meuer et al., 1984; Siliciano et al., 1985; Clark et al., 1988; Brown et al., 1989; Beyers et al., 1992). Stimulation of T lymphocytes through CD2 initiates intracellular signaling pathways including a rise in intracellular calcium, an increase in phosphatidylinositol turnover, the activation of tyrosine-specific protein kinases, and the production of cyclic AMP (Bockenstedt et al., 1988; Jin et al., 1990; Ley et al., 1991). However, the CD2 molecule has not been shown to harbor endogenous enzymatic activity, nor does the cytoplasmic region contain overt homology to functional domains of other receptor- and signaling molecules. Recent studies indicate that CD2 initiates a process of cytoskeletal rearrangements in T lymphocytes (Dustin et al., 1998).

T cell activation requires the formation of a specialized junction (Fig. 1), termed the immunological synapse, at the interface between T cell and APC (Shaw and Dustin, 1997; Grakoui et al., 1999).

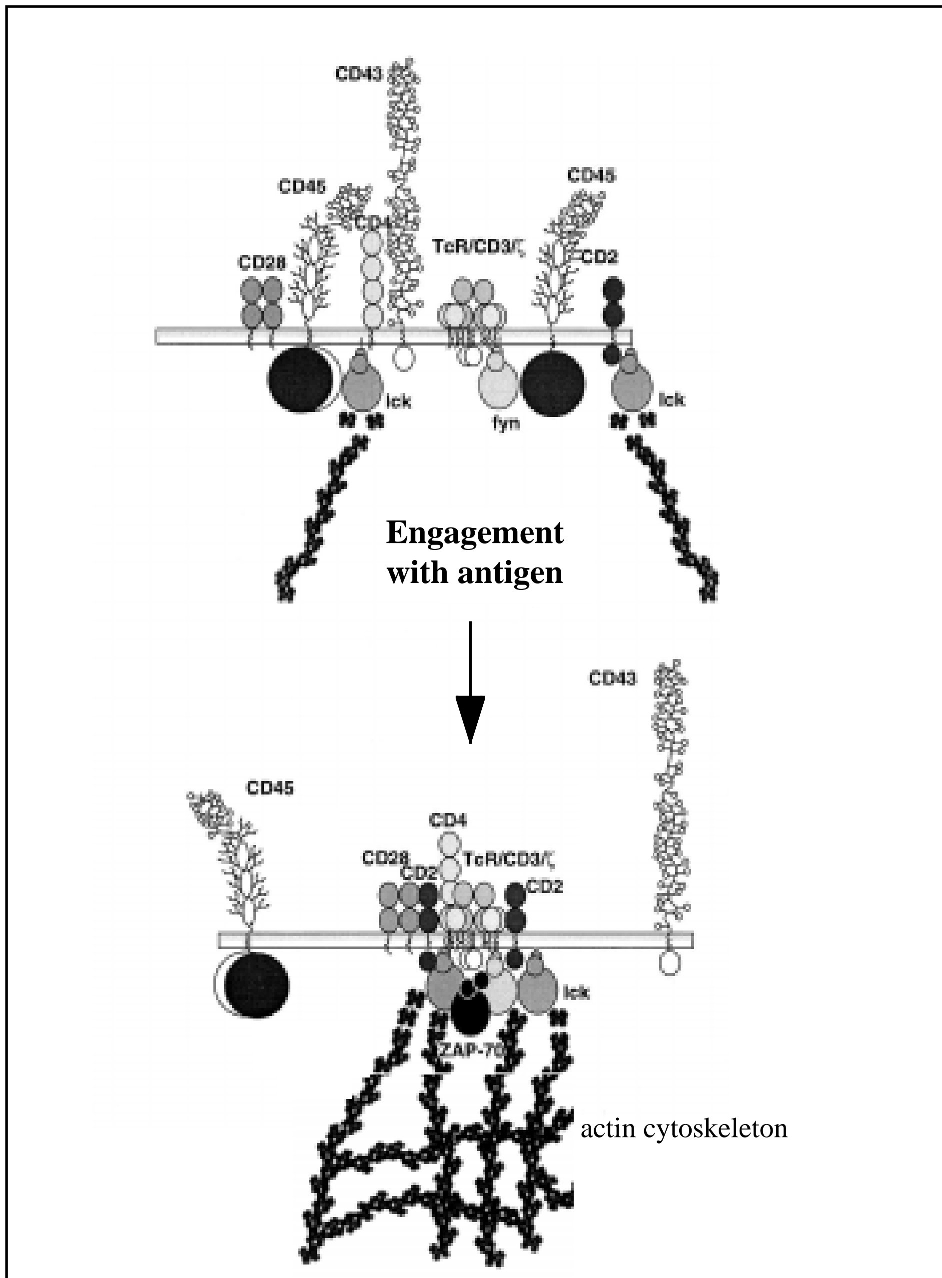


Figure 1

A modified topological model of T cell activation that considers the schematic depiction of T cell membrane proteins and their arrangement before and after engagement with antigen

In resting T cells, surface receptors and signaling molecules on the T cell membrane are randomly distributed (upper panel). Antigen engagement (lower panel) results in the recruitment of co-receptor, co-stimulatory molecules and intracellular signaling molecules to the T cell activation cap containing the TCR-complex. Assembly of this immunological synapse is accompanied by actin polymerization and leads to association of the signaling complex with the actin cytoskeleton. Such a cytoskeletally mediated molecular reorganization is required to sustain TCR engagement and to induce complete T cell activation.
Adapted from models and figures from Shaw and Dustin (1997), Harder and Simons (1997) and Moran and Miceli (1998).

The formation of such delicate contacts is facilitated by the recruitment of surface receptors and intracellular signaling molecules including antigen receptors, co-receptors, co-stimulatory molecules, and signaling molecules. The mature immunological synapse is defined by a specific pattern of receptor segregation with a central cluster containing the TCR, CD3, $p56^{lck}$ and $p59^{fyn}$ kinases, and protein kinase C (PKC)θ surrounded by a ring enriched with the adhesion molecule LFA-1 and the cytoskeletal protein talin (Monks et al., 1998). Assembly of this immunological synapse is accompanied by actin polymerization and leads to the association of this signaling complex with the actin cytoskeleton (Fig. 1). Although the exact function of this receptor patterning for T cell activation is unclear, the formation of the immunological synapse is likely to represent a determinative event for T cell proliferation. Recently, the assembly of the immunological synapse has been shown to be a highly dynamic active process which depends on T cell receptor-ligand interaction kinetics and cluster stabilization (Grakoui et al., 1999).

Recent studies suggest an as yet unrecognized mechanism for costimulation by contributing to the formation of the immunological synapse. Co-stimulation in T cells initiates an active directional transport of receptors and lipid domains to the area of T cell/APC interface (W lfing and Davis, 1999) and recruits membrane microdomains to cell-cell contact sites (Viola et al., 1999). In addition, CD28 signaling leads to the rearrangement of the actin cytoskeleton in the region of a focal adhesion like cell contacts between T cells and APCs (Kaga et al., 1998).

Engagement of the accessory receptor CD2 initiates a similar process of protein segregation, receptor clustering, and polarization of the T cell cytoskeleton (Dustin et al, 1998). Thus, costimulation initiates an active transport mechanism, which appears to be actin driven, however, the transport process and the key cargo are still indiscriminate and not clearly identified. Nonetheless, the actin-dependent reorganization in the T cell membrane is an essential event during the activation of T lymphocytes.

1.2 Actin cytoskeleton

The term cytoskeleton was originally applied to describe the complex set of protein fibres in the cytoplasm. Eucaryotic cells contain three major classes of cytoskeletal fibres known as actin microfilaments, microtubules, and intermediate filaments (Darnell et al., 1986; Amos, L. A., 1991). The three major types of cytoskeletal filaments are connected to one another and their functions are coordinated. Modulation of the cytoskeletal network changes the mechanical properties of the cell that are essential for functions such as locomotion (Stossel, T.P. 1994; Mogliner and Oster, 1996) and cytokinesis (Fishkind and Wang, 1995). Independent of its mechanical strength, the filaments of the cytoskeleton form a continuous, dynamic connection between many cellular structures and represent an enormous surface area on which proteins and other cytoplasmic components can dock. The cytoskeleton is involved in the regulation of numerous intracellular functions, including cell activation processes (Maness and Walsh, 1982).

Actin is a highly conserved cytoskeletal protein that is present at high concentrations in eucaryotic cells. The actin cytoskeleton is central to locomotion, phagocytosis, contractility, shape changes, cytokinesis and maintenance of polarity. The varied forms and functions of actin in eucaryotic cells depend on a versatile repertoire of actin-binding proteins that cross-link actin filaments into loose gels, bind them into stiff bundles, attach them to the plasma membrane, or forcibly move them relative to one another. The set of actin-binding proteins is thought to act cooperatively in generating the change in the actin cytoskeleton which is a fundamental part of the motile response of cells to extracellular ligands. In the cortex of animal cells, actin molecules continually polymerize and depolymerize to generate cell-surface protrusions. The dynamic alterations in both the structural and biochemical properties of the actin network mediate changes in cell shape and migration (Cooper, 1991; Gips et al., 1994). However, many questions regarding the mechanisms that connect signals at the plasma membrane to the assembly of actin filaments into an integrated well orchestrated response remain, so far, unanswered.

Actin reorganization is controlled by the concurrent metabolism of membrane inositol phospholipids. Phosphatidylinositol-4,5-bisphosphate (PtdIns (4,5)P_2) regulates the interaction of actin with its actin-binding proteins. PtdIns (4,5)P_2 causes filament uncapping by a removal of a capping protein from the end of actin filaments that allows the polymerization of actin to resume (Aderem, 1992; Theriot and Mitchison, 1993). Thus, high concentrations of PtdIns (4,5)P_2 can lead to the dissociation of actin-binding proteins, such as profilin (Goldschmidt-Clermont et al., 1990) and gelsolin (Janmey and Stossel, 1989), and promote interactions between actin and the cytoskeletal proteins vinculin and talin. This could represent one mechanism for local actin polymerization and anchoring of the actin cytoskeleton to the cell membrane.

Phosphatidylinositol (PI)-3 kinase (PI3-kinase), which phosphorylates phosphatidyl inositol on the D-3 position, has been shown to bind to proteins involved in actin polymerization, such as α-actinin, gelsolin and profilin (Shibasaki et al., 1994; Wyman and Arcaro, 1994). Although no direct role for PI3-kinase in actin polymerization has been demonstrated, a number of lines of evidence have implicated PI3-kinase in the regulation of the actin cytoskeleton. Inhibition of PI3-kinase blocks growth factor induced actin rearrangements (Wymann and Arcaro, 1994) and the stimulus-dependent activation of integrins and cell adherence (Kinashi et al., 1995; Serve et al., 1995). PI3-kinase activity is also necessary for the formation of actin-rich membrane ruffles and cell motility (Wennstrom et al., 1994; Kotani et al., 1994; Heldman et al., 1996). Consistent with these data is the recent finding that PI3-kinase is the effector enzyme by which Ras induces membrane ruffling (Rodriguez-Viciana et al., 1997). This function involves the small GTPase Rac, which is directly bound to and controlled by substrates and products of PI3-kinase, suggesting that PI3-kinase is an upstream effector of Rac (Hawkins et al., 1995).

Members of the Rho subfamily of small GTPases, including Rho, Rac and Cdc42 are evolutionary conserved regulators of the actin cytoskeleton in a variety of different cell systems (Schlessinger and Ullrich, 1992; Fantl et al., 1993; Hall, 1994). Specifically, Rac has been implicated in the formation of lamellipodia and membrane ruffles, Rho is required for the formation of focal adhesions and actin stress fibers, and Cdc 42 has been described

to induce the formation of filapodia (Hall, 1992; Barry and Critchley, 1994). Among all the Cdc42 interacting proteins identified to date, the Wiskott-Aldrich syndrome protein (WASP) family proteins, especially WASP and N-WASP, are the best candidates for mediating the effects of Cdc42 on the actin cytoskeleton (Rohatgi et al., 1999). WASP controls the assembly of actin filaments and their polymerization by binding PtdIns (4,5)P_2 through the pleckstrin-homology (PH) domain (Miki et al., 1996).

Additionally, the guanine-nucleotide exchange factor Vav, which is expressed specifically in hematopoetic cells (Katzav et al., 1989), is an activator of Rho family GTPases. The GDP/GTP exchange activity of Vav is regulated by tyrosine phosphorylation (Crespo et al., 1997) and by substrates and products of PI3-kinase (Han et al., 1998). Vav is a potential regulator of the actin cytoskeleton in lymphocytes as demonstrated by its essential role for TCR receptor capping and effects on actin polymerization in response to antigen receptor activation (Fischer et al., 1998). Vav has been shown to associate with protein tyrosine kinases, adaptor proteins such as Grb2, SLP-76, Nck, as well as cytoskeletal proteins (Collins et al., 1997) suggesting its potential role in the linkage of antigen receptor signaling to the actin cytoskeleton.

All stages in T lymphocyte life and activation are associated with profound changes in cell morphology that depend on a functional actin cytoskeleton. T lymphocytes migrate through blood and lymph vessels, transgress vessel walls and extracellular matrix spaces, home into lymphoid organs and adhere to target cells.

Upon encountering an antigen-presenting cell (APC), T cells rapidly undergo cytoskeletal polarization, which includes the formation of polymerized actin at the T cell/APC interface and the reorientation of the microtubule-reorganizing center (MTOC) towards the bound APC (Geiger et al., 1982; Ryser et al., 1982). This cytoskeletal polarization directs the delivery of cytokines and cytotoxic mediators to the APC and contributes to the highly selective and specific action of T cell functions.

Until recently, the functional significance of the actin cytoskeleton focused primarily on receptor recycling, receptor internalization, cell-to-cell contacts and cell interaction with the

extracellular matrix in a mechanistic capacity affecting cell motility. However, recent studies have begun to assess the role of the actin cytoskeleton in the regulation of receptor-mediated signal transduction affecting cellular activation of T cells. An increasing number of reports have demonstrated that many surface receptors expressed on T cells, including the TCR ζ chain (Caplan et al., 1995; Rozdzial et al., 1995), CD11a/18 (Pardi et al., 1992; Luna et al., 1992), CD2, CD4, CD8, CD44, class I MHC (Geppert and Lopsky, 1991) and the tyrosine phosphatase CD45 (Lokeshwar et al., 1992) interact with actin-based cytoskeletal components. The cytoskeletal association of surface proteins can be altered by cross-linking of receptors or by the state of activation and /or differentiation of the cells.

The notion that the actin cytoskeleton might be involved in intracellular signaling was raised by studies which investigated specific membrane architectures termed receptor caps in T cell activation. The cap is an asymmetric assembly of receptors and signaling molecules that is formed on the surface of lymphocytes following receptor stimulation. Cap formation is an active process which is dependent on a functional actin cytoskeleton (De Petris, 1974). Given that the cap includes many signaling molecules that are involved in lymphocyte activation, it has been suggested that the cap might be required for conveying signals to the cell interior. In line with this view are current data, which demonstrate that in Vav- and WASP-deficient T cells cap formation is defective and T cell responses such as T cell proliferation and cytokine production are strongly impaired (Holsinger et al., 1998; Snapper et al., 1998). Moreover, inhibition of actin polymerization by cytochalasins resembles defects in Vav- or WASP-deficient T cells and prevents TCR-induced changes in T cell morphology and TCR-mediated signaling events (Holsinger et al, 1998; Kong et al., 1998; Snapper et al., 1998). T cell polarization and actin polymerization, which are important for efficient T cell signaling, require TCR-ITAMs and lck (Lowinkroph et al., 1998). In addition, recent data extend the knowledge about the molecular basis for TCR-mediated actin rearrangements by showing that the assembly of SLP-76, Vav and nck are involved in these processes (Wardenburg et al., 1998).

Taken together, clustering of surface receptors and signaling molecules is an essential step in T lymphocyte activation. The cytoskeleton may serve as a matrix that allows the recruitment of various signaling molecules and enables the efficient formation of new protein-protein interactions. Antigen receptor activation leads to focal reorganization of the actin cytoskeleton whose function is to sustain TCR signaling and to coordinate downstream signaling events. Thus complete activation is achieved and late events such as proliferation and cytokine secretion can occur.

Although it is clear that the actin cytoskeleton is important for the regulation of T lymphocyte activity, however the molecular mechanisms underlying this function are still not well understood. Identification of cytoskeletal regulatory elements and signaling molecules will add substantial new information on the molecular processes required for effective lymphocyte activation in the immune response.

1.3 Cofilin

As the reorganization of the actin cytoskeleton is a basic cellular process of T lymphocytes, the signaling molecules that link external signals to the regulation of the actin cytoskeleton need to be characterized. Cofilin is a potent candidate for these processes. In human peripheral blood T lymphocytes, this 19 kDa protein is constitutively phosphorylated in the cytoplasma (pp19). Cofilin has been found to be dephosphorylated on phosphoserine residue following engagement of several T cell accessory receptors including CD2, CD4, CD8 and CD28 (Samstag et al., 1991, 1992, 1994). Note that in autonomously proliferating T-lymphoma cells, cofilin is spontaneously dephosphorylated (Samstag et al., 1994; Samstag et al., 1996). This constitutive dephosphorylation may represent a mechanism, which supports unregulated proliferation and the prolonged lifespan of malignant cells.

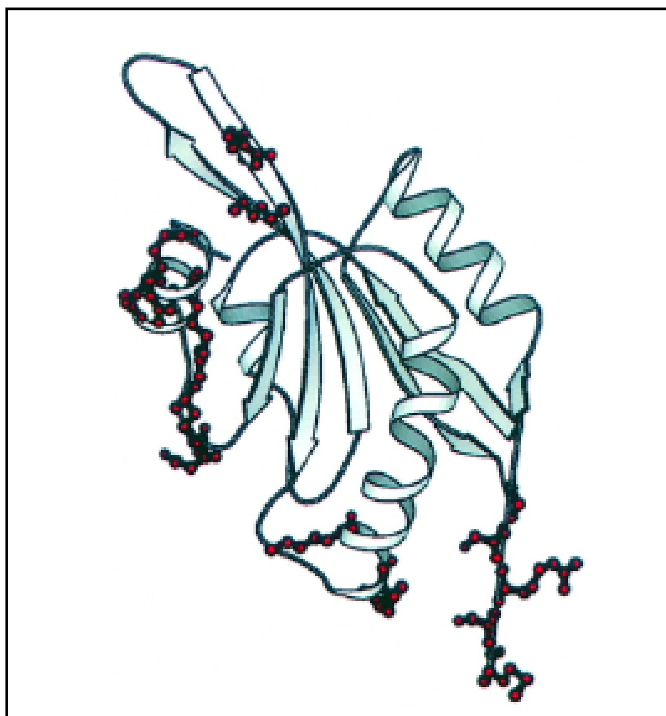
Cofilin is a member of the cofilin/ADF family of small actin-binding proteins with a molecular mass of 15-19 kDa depending on the species (Moon and Drubin, 1995). It is ubiquitously expressed and every eukaryotic cell type contains at least one member of this family, suggesting that cofilin performs a generally important function (Moon and Drubin, 1995). This conclusion is further supported by the observations that in *Saccharomyces cerevisiae, Drosophila melanogaster* and *Caenorhabditis elegans*, null mutations in the cofilin genes are lethal (Moon et al., 1993; McKim et al., 1994; Gunsalus et al., 1995).
The phenotypes resulting from genetic studies indicate that cofilin plays a critical role in the cytoskeletal dynamics that underlie cytokinesis, endocytosis, cell motility and other cell processes which require rapid turnover of actin filaments (Mckim et al., 1994; Aizawa et al., 1995; Gunsalus et al., 1995). Additionally cofilin proteins typically localize to regions of cells characterized by high actin dynamics, including neuronal growth cones, ruffling membranes, cleavage furrows and yeast cortical actin patches (Bamburg and Bray, 1987; Yonezawa et al., 1987; Moon et al., 1993; Nagaoka et al., 1995). Moreover, recent studies demonstrated that cofilin promotes rapid actin filament turnover in the *Listeria* tail (Carlier et al., 1997; Rosenblatt et al., 1997) and in the cortical actin cytoskeleton in yeast cells (Lappalainen and Drubin, 1997).

In vitro, cofilin binds to both globular (G-actin) and filamentous (F-actin) actin with high affinity ($K_d < 1\mu M$) (Nishida et al., 1984; Yonezawa et al., 1985). However, the mechanisms by which cofilin accelerates actin filament dynamics *in vivo* is still controversial.
Until recently, cofilin was classified as an actin filament-severing protein based on visualization by light microscopy (Hawkins et al., 1993). Its actin depolymerizing activity has been shown to be regulated by pH, being favored in an alkaline environment (pH>7.2) *in vitro* (Yonezava et al., 1985; Hawkins et al., 1993; Hayden et al., 1993). Recent work has caused doubts about the idea that disassembly is solely due to filament severing: cofilin rather appears to modulate the rate constants of assembly and disassembly at the ends of filaments (Carlier et al., 1997; Theriot, J. A. 1997). Moreover, it has been shown that

cofilin binds cooperatively between two actin subunits along the filaments which leads to alterations in the F-actin filament twist thereby influencing the interaction of other actin-binding molecules with F-actin (McGough et al., 1997). This could explain why cofilin and phalloidin do not bind simultaneously to F-actin, although their binding sites are distinct. Alteration of the F-actin structure by cofilin appears to be a novel mechanism through which the actin cytoskeleton may be regulated or remodeled.

In vitro studies predicted, that, residues 122-128 and 104-115 of the human cofilin sequence are involved in actin filament binding (Yonezawa et al., 1989; Yonezawa et al., 1991) (Fig. 2B). Moreover, in porcine brain cofilin, genetic studies have shown that the residues Lys 112 and Lys 114 are important F-actin binding sites (Moriyama et al., 1992). Recently, site-directed mutagenesis studies have suggested that the N-terminal Ser-3 in chicken ADF (Agnew et al., 1995) and in yeast cofilin (Lappalainen et al., 1997) are essential for its binding to F-actin. Biochemical characterization of interactions between genetically engineered mutant yeast cofilin and yeast actin have identified further cofilin residues that appear to be important for F-actin binding *in vivo* (Lappalainen et al., 1997) (Fig. 2A).

A



B

M A S G V A V S D G V I K V F N D M K V
phosphorylation site
R K S S T P E E V K K R K K A V L F C L
S E D K K N I I L E E G K E I L V G D V
G Q T V D D P Y A T F V K M L P D K D C
R Y A L Y D A T Y E T K E S K K E D L V
F I F W A P E S A P L K S K M I Y A S S
actin binding site
K D A I K K K L T G I K H E L Q A N C Y
actin binding site
E E V K D R C T L A E K L G G S A V I S
L E G K P L

Figure 2

A: Ribbon diagram of yeast cofilin. The side chains of cofilin residues that appear to be important for F-actin binding are indicated in red. Adapted from Lappalainen et al., 1997.
B: Amino acid sequence of human cofilin. The residues 104-115 and 122-128 which are suggested to be important F-actin bindig sites, are indicated in blue. Serine-3 is the phosphorylation site (orange). Adapted from Ogawa et al., 1990.

However, relatively little is known about the molecular mechanisms by which cofilin stimulates F-actin turnover *in vivo. In vitro* studies indicate that the interaction of cofilin with actin can be inhibited by phosphoinositides (Yonezawa et al., 1990), a property shared with other actin-associated proteins, such as gelsolin, villin and profilin (Rozycki et al., 1994). In addition, *in vitro* studies showed that the actin-binding capacity of cofilin is negatively regulated by phosphorylation of the serine residue at position 3 in many vertebrate cell types (Morgan et al., 1993). In contrast yeast cofilin is not phosphorylated at this serine residue, nevertheless, the mutation of this residue in yeast disrupted the interaction between cofilin and F-actin (Lappalainen et al., 1997). This indicates that Ser-3 itself could be positioned within a critical actin-binding sequence and therefore the introduction of a phosphate group at this position would prevent this region from interacting with actin.

Most recently, LIM-kinase 1 has been identified as an inactivator of cofilin due to its capacity to phosphorylate Ser-3 (Arber et al., 1998; Yang et al., 1998). The enzymes which are responsible for activation of cofilin through dephosphorylation *in vivo* - thereby enabling this protein to promote the disassembly of actin filaments - are as yet unknown.

The processes which regulate the interaction of cofilin with actin filaments in response to receptor stimulation, as well as the functional significance of cofilin dephosphorylation in T lymphocytes are largely unknown.

1.4 Aim of the study

Actin cytoskeletal dynamics are essentially involved in the activation of T lymphocytes. However, as yet only little information existed regarding the molecular elements which link cell surface receptor stimulation directly to the resulting changes of the actin cytoskeleton during T cell activation. Therefore, the aim of the present study was to investigate the involvement of the small actin binding protein cofilin in the process of T cell activation. The interaction of cofilin with the actin cytoskeleton following accessory receptor stimulation as well as the role of cofilin for receptor clustering were analyzed. Moreover, the functional importance of cofilin/actin interactions for the activation of untransformed human peripheral blood T lymphocytes as well as for the activation induced apoptosis of preactivated human T cell clones were characterized.