1. INTRODUCTION

1.1 T cell activation

The activation of T lymphocytes plays an integral role in the development of an adaptive immune response against foreign antigens. Antigenic stimulation of T cells is initiated by the specific interaction of T cell antigen receptors (TCRs) with their respective antigenic peptides presented by major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells (APCs). Optimal generation of a T cell response requires simultaneous engagement of a costimulatory molecule on the T cell surface. Recognition of foreign antigens by the T cell then activates a cascade of intracellular signaling events that culminates in cytokine release, cellular proliferation, and differentiation into effector cells. The latter processes result in a clonal expansion of T cells which is essential for the removal of pathogens.

The TCR is a multisubunit signaling complex composed of clonotypic ligand-binding heterodimers (αβ or γδ) noncovalently associated with the nonpolymorphic CD3 γ, δ, ε and TCRζ-subunits (Weiss, 1993). The capacity of the TCR to transduce signals across the T cell membrane is mediated by the cytoplasmic domains of the CD3 complex and the ζ-chain, which contain critical consensus sequences termed immunoreceptor tyrosine-based activation motifs (ITAMs). This motif, which is found in single copies in each CD3 polypeptide and in triplicate in the ζ-chains (Chan and Shaw, 1996), is crucial for TCR coupling to intracellular tyrosine kinases and plays a crictical role in assembling the signal transducing complex.
The generally accepted model for the initiation of signaling through T cell antigen receptors proposes that upon receptor engagement the src-family protein tyrosine kinases (PTKs) $p56^{lck}$ and $p59^{fyn}$ associate with the receptor and promote phosphorylation of the ITAMs (Straus and Weiss, 1992; van Oers et al., 1996; Gauen et al., 1994). In addition, src-kinases can function as adaptor proteins, which might allow for binding of other signaling components (Xu and Littman, 1993). Tyrosine phosphorylation of the ITAMs provides docking sites for the recruitment of the cytoplasmatic syk-family of PTKs, ZAP (ζ-associated protein)-70 and syk (Chan et al., 1992). The colocalization of ZAP-70 with the phosphorylated receptor permits transphosphorylation of ZAP-70 by lck (or fyn) and upregulation of ZAP-70 enzymatic activity, which in turn is required for the initiation of multiple downstream signaling pathways within the cell. These include those regulated by many small G-proteins, lipid kinases and members of the mitogen-activated protein (MAP) kinase family.

The bridging of the TCR associated PTKs with downstream signaling functions is regulated through tyrosine phosphorylation of two linker proteins, SLP-76 (SH2-domain containing leukocyte protein of 76 kD) and LAT (linker for activation of T cells) (Jackman et al., 1995; Zhang et al., 1998a). These adaptors lack intrinsic enzymatic and transcriptional domains, but the phosphorylation of these proteins facilitates their interaction with other signaling molecules.

LAT is a 36-38 kD transmembrane protein that becomes rapidly tyrosine phosphorylated by ZAP-70 following triggering of the TCR (Zhang et al., 1998b). Upon phosphorylation of its cytoplasmatic tail, LAT associates with Grb2, PLCγ1 and the 85 kD subunit of PI3-kinase. Recent evidence indicates that LAT is essentially required to couple the TCR to the PLCγ/calcineurin/NF-AT and the ras/ERK pathways (Finco et al., 1998). This function requires the targeting of LAT to glycolipid-enriched microdomains (Lin et al., 1999).

In addition to LAT, tyrosine phosphorylation of SLP-76 by ZAP-70/syk-family PTKs is also required for a functional TCR (Bubeck-Wardenburg et al., 1996; Raab et al., 1997). The cytoplasmatic adaptor protein SLP-76 interacts in a phosphotyrosine dependent manner with the adaptor nck, and the guanine nucleotide exchange factor $p95^{vav}$ (Bubeck-Wardenburg et al., 1996). Furthermore, tyrosine phosphorylation of SLP-76 has been shown to be critically involved in the regulation of Ca^{++} and ras signaling pathways (Yablonski et al., 1998).

Thus, the adaptor function of LAT and SLP-76 serves to provide a scaffold for the subsequent docking of well known effector enzymes such as phospholipase Cγ (PLCγ) and $p95^{vav}$. The formation of this multimolecular signaling complex is an essential prerequisite for subsequent activation of diverse signaling routes. Upon activation by tyrosine phosphorylation, PLCγ initiates two important signaling pathways, the IP3/Ca^{++}/ calcineurin/NF-AT- and the DAG/PKC- signaling cascades (reviewed by Alberola-Ila et al., 1997). Involvement of small G proteins of the ras and rho family GTPases also plays critical roles in T cell activation. Ras activation is thought to be mediated by the guanine nucleotide exchange factor sos (son of sevenless), and results in the activation of the ERK (extracellular-regulated kinase) pathway (Cantrell, 1996). Tyrosine phosphorylation of $p95^{vav}$ results in upregulation of its guanine nucleotide exchange factor activity for rho-family GTPases and has been implicated in regulation of JNK and p38 MAPK pathways. T cell activation also involves additional signaling cascades, such as those mediated by lipid kinases (e.g. PI3-kinase).

The cooperation of various signal transduction pathways, which in many cases are interconnected and influence each other, initiates a complex program of gene expression. The best-characterized example to date is the regulation of the interleukin 2 (IL-2) gene. TCR ligation in the context of appropriate costimuli results in the transcriptional activation of the IL-2 gene. Transcription of IL-2 is dependent on the formation and activation of a number of transcription factors, including AP-1, NF-κB, Oct-1 and NF-AT (Rothenberg and Ward, 1996). IL-2 gene transcription requires the action of several signaling pathways that integrate at the level of these transcription factors. The products of the growing NF-AT gene family have been implicated in the regulation of many additional genes, including those encoding numerous cytokines (e.g. IL-4, TNFα) as well as the pro-apoptotic protein CD95 ligand. Ultimately, the signals elicited by ligand binding of the TCR result in expression of new surface molecules, cytokine secretion and cytoskeletal changes leading to T cell proliferation, differentiation and/or effector functions (Perlmutter et al., 1993; Cantrell, 1996).
Alternatively, in immature T lymphocytes or previously activated mature T cells, antigenic stimulation may also induce apoptotic cell death. TCR-induced apoptosis plays a key role in the elimination of self-reactive T cells, thus, in the prevention of autoimmunity, and is clearly important for the establishment of homeostasis (Jenkinson et al., 1989; Lenardo et al., 1995). Apoptosis is a highly regulated process which involves a cascade of specific biochemical and morphologic events characterized by membrane blebbing, activation of proteolytic caspases, nuclear condensation and DNA fragmentation. Antigen-induced apoptosis of T cells consists of an inductive phase, triggered by TCR stimulation and a subsequent effector phase, which features the biochemical means necessary for cell death to occur. The early signal transduction processes which are involved in T cell activation/proliferation and TCR-induced apoptosis are overlapping (Winoto, 1997). The interface between the early inductive phase and the effector phase in T cell death of peripheral T cells and T cell hybridomas appears to be regulated by the interaction of CD95/CD95L, the expression of which is thightly regulated by T cell stimulation (Dhein et al., 1995; Ju et al., 1995; Yang et al., 1995), whereas in negative selection of immature thymocytes CD30/CD30L interactions play a more crucial role (Amakawa et al., 1996).

T cell receptor (TCR) recognition of antigenic peptide presented by major histocompatibility complex (MHC) molecules of antigen presenting cells (APCs) is critical for the process of T cell activation. Although the binding of the TCR to its cognate peptide/MHC complex confers specificity to this process, efficient T cell activation requires additional signals provided by engagement of coreceptors and a large number of costimulatory molecules expressed on the surface of T lymphocytes (Croft and Dubey, 1997).

The specific interaction between antigen and the TCR is accompanied by interactions between non-polymorphic regions of MHC class I or class II molecules with CD8 and CD4, respectively, which function as coreceptors and are associated with the src-family PTK $p56^{lck}$ (as reviewed by Weiss and Littman, 1994; Chan and Shaw, 1996). These MHC-binding coreceptors stabilize the TCR:ligand association during a process of structural adaption, affecting TCR occupancy. Furthermore, they enhance T cell activation by physically juxtaposing TCR complexes with coreceptor associated $p56^{lck}$, which promotes efficient phosphorylation of TCR ITAMs and ZAP-70 (Barber et al., 1989; Garcia et al., 1996). The resulting signals are critical for T cell development and activation of peripheral T cells.

Additional costimulatory signals are required for an optimal generation of functional T cell responses such as IL-2 production, proliferation and differentiation to effector cells. However the mechanism by which costimulatory molecules facilitate T cell activation and accomplish TCR signaling remain largely elusive. Costimulation was originally defined as a distinct signal required in conjunction with the signal transduced by the TCR for the initial activation of naive T cells (Harding et al., 1992). These unique costimulatory signals were supposed to integrate with TCR signals in the nucleus to affect gene expression. However, this view has recently been questioned by studies indicating that costimulatory molecules might act as general amplifiers of early TCR signaling (W lfing and Davies, 1998, Tuesto and Acuto, 1998; Viola et al., 1999). A new concept suggests that costimulatory molecules by their adhesive function stabilize the contact and induce recruitment of protein kinases and/or other signaling molecules at the site of TCR-engagement, thus allowing for the initiation of signal transduction by the TCR/CD3 complex (Shaw and Dustin, 1997).

The best-studied and most prominent costimulatory molecule is CD28, which interacts with its ligands CD80 (B7.1) and CD86 (B7.2) expressed on the APC. Many studies have shown that CD28 decisively determines the destiny of the T cell response (reviewed by Bluestone, 1995; Thompson, 1995). In most T cells, CD28 ligation lowers the threshold (e.g. the number of triggered TCRs) needed for T cell activation and increases response longevity, effects linked to increased transcription and stability of mRNAs. However not all T cell mediated immune responses are CD28-dependent, since CD28 knockout mice are still able of mounting MHC class I and class II restricted T cell responses (Shahinian et al., 1993).

Other important costimulatory receptors on T cells and their ligands are LFA-1/ICAM-1 (Sperling and Bluestone, 1996) and CD2, which can bind to CD58, CD59 or CD48 on the APC (Hahn et al., 1992; Kato et al., 1992). Meanwhile a number of additional molecules have been proposed to possess a costimulatory capacity. Among others, these include CD5, CD9, CD29, and CD44 (Yashiro et al., 1998). Recent evidence suggests that different costimulatory molecules can enhance T cell activation in an additive fashion (Bachmann et al., 1999). This

enables T cells to respond to lower concentrations of antigens and also provides a mechanism for fine-tuning of T cell responses.

A critical event for TCR signaling is the coordinated reorganization of membrane receptors and signaling molecules in the contact area between the T cell and the APC. The formation of this specialized junction, termed immunological synapse, is thought to provide a mechanism for sustained TCR engagement and signaling (Shaw and Dustin, 1997; Grakoui et al., 1999). The assembly of this delicate contact leads to organized focal interaction sites containing antigen receptors, coreceptors, costimulatory molecules, adhesion 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 integrin family adhesion molecules and the cytoskeletal protein talin (Monks et al., 1998). The formation of the immunological synapse is an active process which depends on a functional cytoskeleton and leads to the association of the signaling complex with the actin cytoskeleton. Furthermore, receptor patterning may be accompanied by a concentration of lipid microdomains nearby engaged TCRs (Moran and Miceli, 1998; Thomas, 1999). The assembly of the immunological synapse is a highly dynamic process that allows T cells to distinguish potential antigenic ligands (Grakoui et al., 1999). Stable central cluster formation has been shown to correlate directly with the induction of T cell proliferation. Since the immunological synapse promotes extended cell contact and provides a unique environment around engaged TCRs, it has been proposed that the productive formation of this specialized junction allows to maintain TCR engagement and promotes optimal signal transduction leading to T cell activation (Grakoui et al., 1999).

Recent studies suggest an as yet unrecognized mechanism for costimunation by contributing to the formation of the immunological synapse. Costimulation in T cells initiates an active directional transport of receptors and lipid domains toward the interface between T cell and APC (W lfing and Davis, 1998) and recruits membrane microdomains to the contact area (Viola et al., 1999). In addition, engagement of CD28 induces the rearrangement of the actin cytoskeleton in the region of a focal adhesion-like cell contact between T cells and APCs (Kaga et al., 1998). Engagement of the accessory receptor CD2 initiates a similar process of protein segregation, receptor patterning, and cytoskeletal polarization in T cell contacts (Dustin et al., 1998). Thus, costimulation initiates an active transport mechanism, which appears to be actin driven. However, the transport process appears to be indiscriminate and the mechanism of molecular patterning and cytoskeletal polarization still remains elusive. Nonetheless, the actin dependent reorganization of the T cell membrane is an essential event during the activation of T lymphocytes.

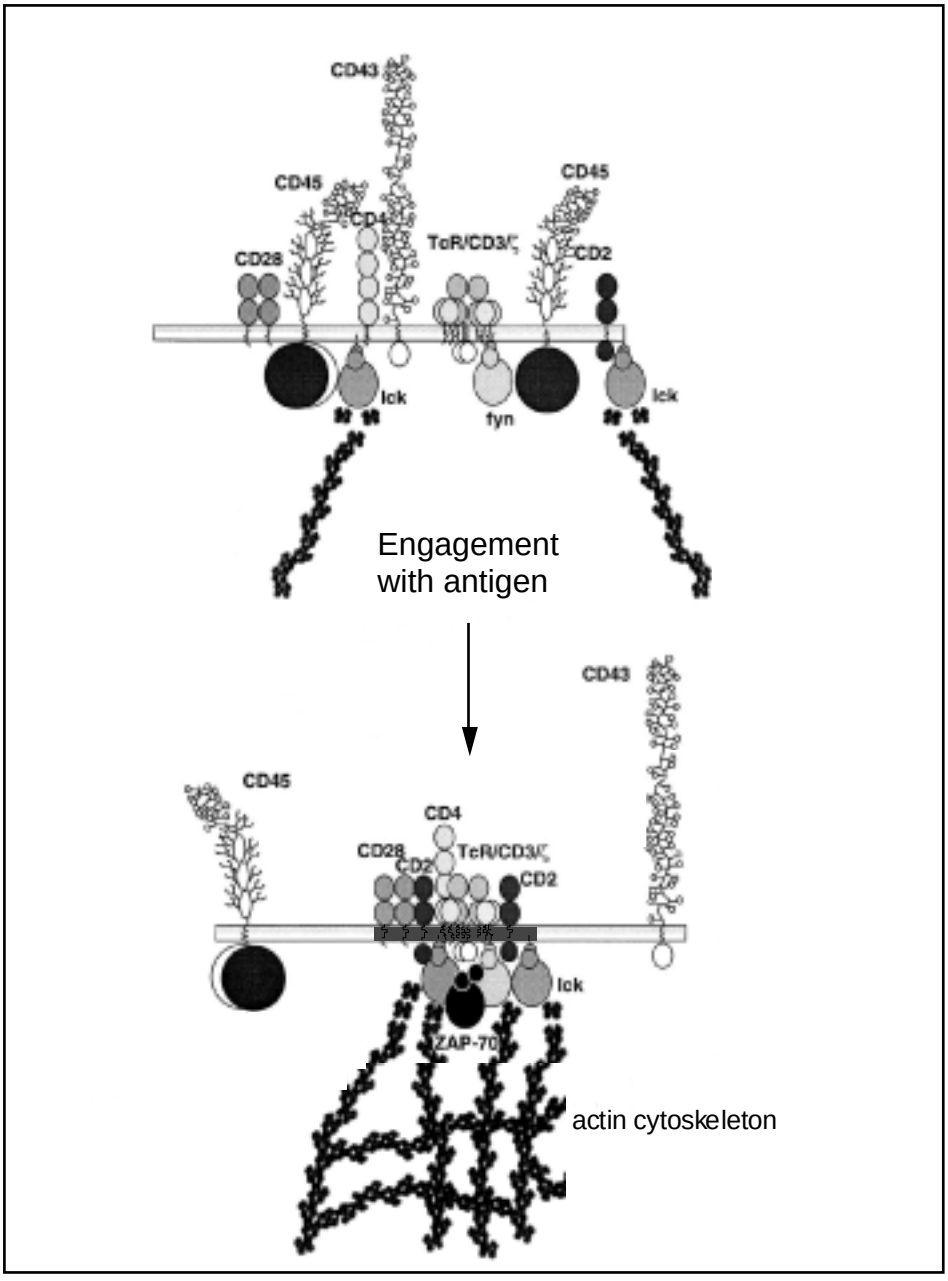

Figure 1

Model of T cell activation that considers the possible contribution of the actin cytoskeleton and lipid rafts
At steady state, cell surface receptors and signaling molecules are randomly distributed (upper panal). Antigenic stimulation results in the stabilization of the receptor complex and the accumulation of lipid rafts (indicated in the figure as darker membrane region) at the T cell contact cap (lower panel). This process is accompanied by the recruitment of coreceptors, costimulatory molecules and intracellular signaling molecules to the activation cap containing the TCR complex. The actin cytoskeleton is supposed to be the driving force of this molecular reorganization and, in addition, provides the scaffold for the recruitment of additional signaling molecules. Clustering of lipid rafts and focal reorganization of the actin cytoskeleton by engagement of the TCR and costimulatory molecules are 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).

1.2 Actin cytoskeleton

The cytoplasm of eucaryotic cells is spatially organized by a network of protein filaments known as the cytoskeleton. This network is formed by three classes of filamentous assemblies - actin filaments, microtubules and intermediate filaments (Darnell et al., 1986; Amos, 1991). The three major types of cytoskeletal filaments are connected to one another, and their functions are coordinated. The cytoskeleton is a highly dynamic structure that reorganizes continuously as the cell changes shape, divides, and/or responds to extracellular stimuli. Independent of its mechanical properties, the filaments of the cytoskeleton form a 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 functions, including cell activation processes (Maness and Walsh, 1982).

The actin cytoskeleton maintains cellular shape and plays a pivotal role in cell motility, cytokineses, contractility, and phagocytosis. 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 each other. Direct morphological changes in plasma membrane topology are produced by polymerization and rearrangement of underlying cortical actin filaments (Stossel, 1993). Actin polymerization can be regulated by extracellular signals binding to cell-surface receptors that act through signaling pathways involving tyrosine kinases, small GTPases of the rho subfamily and/or metabolically active membrane phospho-lipids.
Members of the rho subfamily of small GTPases are evolutionary conserved regulators of the actin cytoskeleton, and furthermore, through their interaction with multiple target proteins they can control other cellular activities such as gene transcription (Hall, 1998; Mackay and Hall, 1998). The rho subfamily consists of several members, including rho, rac, and cdc42, which cycle between the active GTP-bound state and the inactive GDP-bound state. In fibroblasts, it has been demonstrated that activation of rho by extracellular growth factors triggers the formation of actin stress fibers and focal adhesion complexes (Ridley and Hall, 1992), whereas activation of rac elicits actin polymerization at the plasma membrane to produce lamellipodia and membrane ruffles (Ridley et al., 1992). Activation of cdc42 induces the formation of filopodial protrusions and microspikes at the cell periphery (Kozma et al., 1995). Target molecules for rho, rac, and cdc42 differ widlely in their functions and cellular distribution and include such molecules as phosphatidylinositol 4-phosphate 5 kinase (PIP5-kinase), myosin light chain (MLC) phosphatase and the p21-activated kinase (PAK). An

effector of cdc42, the Wiskott-Aldrich Syndrome protein, WASP, has recently been shown to be a critical regulator of actin polymerization in lymphocytes (Miki et al., 1996).
The guanine-nucleotide exchange factor $p95^{vav}$, which is exclusively detected in cells of the hematopoietic system, acts on members of the rho-family of small GTPases. Tyrosine phosphorylation of $p95^{vav}$ results in an augmentation of its GDP/GTP exchange activity (Crespo et al., 1997). The catalytic activity of $p95^{vav}$ is further influenced by products of phosphatidylinositol-3 kinase (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 capping and effects on actin polymerization in response to antigen receptor activation (Holsinger et al., 1998; Fischer et al., 1998).
Actin reorganization is also controlled by the concurrent metabolism of membrane inositol phospholipids. Phosphoinositides are thought to regulate the interaction of actin with actin binding proteins, such as profilin and gelsolin (Stossel, 1993; Kandzari et al., 1996). High concentrations of phosphatidylinositol-4,5-bisphosphate (PIP2) cause uncapping of the fast-growing end of actin filaments, and allows rapid actin polymerization. Regulatory molecules involved in phospholipid metabolism and actin cytoskeletal rearrangements include PI3-kinase and PLCγ, both of which have also been implicated in TCR-signal transduction.

All stages in lymphocyte life and activation are associated with profound changes in cell morphology that depend on a functional actin cytoskeleton. During the maturation of T lymphocytes continuous contact with cells of the thymic microenvironment is essential for differentiation and selection. Mature lymphocytes migrate through blood and lymph vessels, home into lymphoid organs, interact with APCs, and adhere to target cells. Binding of a T cell to an appropriate APC induces the rapid reorientation of the cytoskeleton and secretory apparatus towards the T cell/APC interface in a TCR and peptide/MHC dependent process (Geiger et al., 1982; Ryser et al., 1982). Such T cell polarization, which requires TCR-ITAMs and lck (Lowin-Kropf et al., 1998), directs the delivery of cytokines and cytotoxic mediators towards the APC and contributes to the highly selective and specific action of T cells.
A number suface proteins involved in T cell function, including LFA-1 (Pardi et al.,1992), CD2, CD4, CD8, CD44 (Geppert and Lopsky, 1991) and TCR ζ (Rozdzial et al., 1995) have been reported to 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 diferentiation of the cells. Until recently, the functional significance of this association focused primarily on receptor recycling, internalization, cell-to-cell contacts, and cell interactions with the extracellular matrix in a mechanistic view. However recent studies have begun to assess the role of the cytoskeleton in the regulation of receptor mediated signal transduction affecting cellular activation of T cells. The cytoskeleton provides a large surface

area on which many signaling molecules including protein and lipid kinases, phospholipases and GTPases localize in response to activation of specific surface receptors (Janmey, 1998). Thus, the cytoskeletal scaffold structure might spatially and temporally regulate the molecular dynamics of lymphocyte signaling.

The notion that the actin cytoskeleton might be involved in lymphocyte signaling was raised in 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 upon stimulation is formed on lymphocytes in an actin dependent process (dePetris, 1974). Because the cap includes many molecules involved in lymphocyte activation, it has been suggested that the cap might be required for conveying signals to the cell interior. More recent studies have demonstrated that engagement of the TCR by peptide/MHC complexes on APCs leads to an actin cytoskeleton guided reorganization of T cell surface receptors and signaling molecules forming the immunological synapse. It is believed that cluster dependent signaling processes during the formation of caps or immunological synapse formation are necessary to induce immune resonses such as T cell proliferation, the production of regulatory cytokines and possibly additional lymphocyte effector functions. This idea has been strengthened by current data demonstrating that in vav- and WASP-deficient T cells cap formation is defective and antigen-receptor induced T cell proliferation as well as cytokine production are strongly impaired (Holsinger et al., 1998; Fischer et al., 1998; Snapper et al., 1998). 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). In addition, recent data extend the knowledge about the molecular basis for TCR-mediated actin rearrangements by showing that the formation of a trimolecular SLP-76-vav-nck complex is critically involved in polarized F-actin formation and TCR signaling (Bubeck-Wardenburg et al., 1998).

Taken together, cytoskeleton driven clustering of surface receptors and signaling molecules is an essential step during T lymphocyte activation. The cytoskeleton may serve as a matrix that allows the recruitment of variuos signaling molecules and enables the formation of new protein-protein interactions. The antigen receptor induced focal reorganization of the actin cytoskeleton leads to sustained TCR-signaling and coordinates downstream signaling events in a way that complete activation is achieved and late events such as proliferation and cytokine secretion can occur.

1.3 Lipid microdomains in cell surface membranes

The plasma membrane lipid bilayer appears to be organized into membrane domains with distinct lipid constituents and function. Among them, the glycolipid-enriched membrane microdomains (GEMs) have been proposed to be involved in membrane trafficking, cell morphogenesis and signal transduction mechanisms (Harder and Simons, 1997; Simons and Ikonen, 1997). These so called lipid rafts are rich in cholesterol, glycosphingolipids and glycosylphosphatidylinositol (GPI)-anchored proteins. Some transmembrane proteins (e.g. CD4, CD8, CD26 and CD44) can also bind to GEMs, and, importantly, various signaling molecules are concentrated within lipid rafts, including src-family kinases, monomeric and heterotrimeric G proteins, and lipid kinases (Simons and Ikonen, 1997). GEMs are relatively insoluble in Triton X-100 at 4 ¡C, and can be isolated as low-density complexes by gradient centrifugation (Brown and Rose, 1992). Studies using fluorescence-resonant-energy transfer and chemical cross-linking have shown that lipid microdomains are dynamic structures in living cells, which approximately 70 nm in size (Friedrichson and Kurzchalia, 1998; Varma and Mayor, 1998). In many cells the lipid rafts are associated with caveolin, a marker for caveolae, small bulb-shaped invaginations of the plasma membrane that have been implicated in cellular transport processes and in signal transduction processes (Lisanti et al., 1994). The existence of lipid rafts has also been clearly demonstrated in cells lacking caveolae, such as lymphocytes and certain neuronal cells (Brown and London, 1997; Simons and Ikonen, 1997).

Recent data indicate that lipid rafts may be crucial for affecting TCR signaling. GEMs of unstimulated T cells are enriched with various proteins involved in cell activation, such as $p56^{lck}$, $p59^{fyn}$, CD4, LAT and ras. Although the TCR is not significantly located into the GEMs of resting cells, activation of T cells by engagement of the antigen-receptor results in the specific recruitment of triggered TCRs to the GEMs (Xavier et al., 1998; Montixi et al., 1998; Kosugi et al., 1999). In parallel, upon TCR-stimulation, the lipid rafts become enriched in signal transduction molecules, such as PLCγ, vav, and PI3-kinase (Xavier et al., 1998; Zhang et al., 1998b). The general function of raft association in T cell activation might therefore be to concentrate receptors and effector molecules at the T cell/APC contact site, facilitating sustained TCR signaling. The functional significance of this specific redistribution into lipid rafts has been demonstrated by intervening with raft integrity. Treatment of cells with various agents that disrupt GEM structure inhibits TCR-induced protein tyrosine phosphorylation and Ca^{++} influx (Xavier et al., 1998). Thus, membrane compartmentation is essentially required for T cell activation.

Interestingly, costimulation appears to be intimately associated with the redistribution of membrane microdomains during T cell activation. Engagement of CD28 leads to clustering of

membrane and intracellular kinase-rich microdomains at the site of TCR engagements (Viola et al., 1999). There is evidence indicating that the transport process initiated by triggering of costimulatory molecules is actively driven by the T cell actin cytoskeleton (W lfing and Davis, 1998). The recruitment of lipid rafts to the site of TCR engagement may represent a general mechanism by which costimulation can increase the activation process, an idea which is further supported by the observation that passive clustering of lipid rafts and triggered TCRs provides efficient costimulation (Viola et al., 1999).

1.4 Forms and functions of CD44

CD44 refers to a number of multistuctural and multifunctional transmembrane glycoproteins involved in various cell-cell and cell-matrix interactions. The broad range of physiologic activities in which CD44 has been implicated in, includes embryogenesis, hematopoiesis, as well as lymphocyte activation and homing. In addition, CD44 also plays roles in pathologic processes, such as metastasis formation and autoimmunity (reviewed by Lesley et al., 1993; Z ller, 1995; Naor et al., 1998; Borland et al., 1998). This versatility in function is thought to result from the expression of a large group of CD44 isoforms, generated by both post-translational modification and differential utilization of alternatively spliced exons.

1.4.1 Structure of CD44

The CD44 family of molecules is encoded by a single gene, located on chromosome 11 in humans (Goodfellow et al., 1982) and in chromosome 2 in mice (Colombatti et al., 1982). It occupies 50-60 kb of genomic DNA, and comprises at least 20 exons (Figure 2). The so called standard isoform of CD44 spans a region of seven extracellular exons (exons 1-5, 16-17), a transmembrane exon (exon 18), and a cytoplasmic exon, wich can be short (exon 19) or long (exon 20). The exons between 5 and 16 encode the 10 variant exons (designated v1 to v10), which are not expressed in CD44s, but alternative splicing can introduce these sequences into the membrane proximal extracellular domain of CD44 to produce variant isoforms (Screaton et al., 1992; Cooper et al., 1992; T lg et al., 1993). Consensus splice donor/acceptor sites are also found within exons 5 and 8, resulting in CD44 isoforms containing shortened versions of these exons. An additional exon (called v9a), located between exon v9 and exon v10 and with a restricted expression pattern has been identified recently (Yu and Toole; 1996).

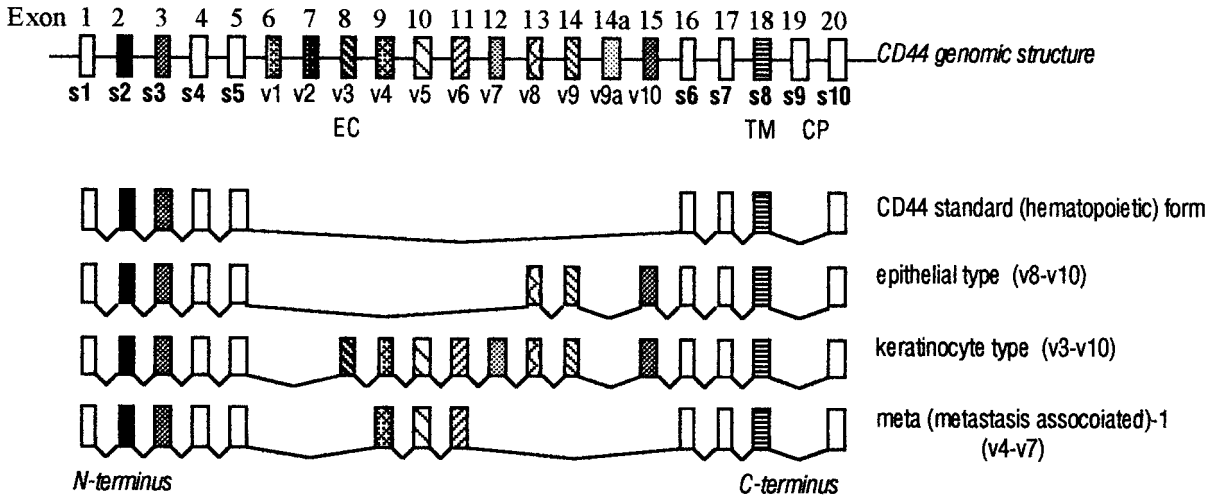

Figure 2

Genomic organization of CD44 and protein structure of some CD44 isoforms
The genomic structure of CD44 is shown at the top with exons 1 to 17 encoding the extracellular domain (EC), exon 18 encoding the transmembrane domain (TM), and exon 19 or 20 encoding the cytoplasmic tail (CP) of CD44. The protein structure of the standard or hematopoietic form of CD44 (CD44s), which lacks the variable region, and some frequent variant isoforms is depicted.

The most abundant version of CD44, the standard isoform (CD44s), which lacks the entire variable region, is, like all membrane-bound forms of CD44, a type I transmembrane glycoprotein. The N-terminal (membrane distal) region of CD44 folds into a globular domain through disulphide bonding of conserved cysteine residues. CD44s is expressed mainly on hematopoietic cells and is therefore also designated hematopoietic CD44 (CD44H). The amino acid sequence of CD44s predicts a molecular weight of the core protein of about 37 kD, which contrasts its apparent size of 80-95 kD. This difference is mainly the result of extensive glycosylation of the extracellular domain, which contains many sites for both N- and O-linked carbohydrates (Zhou et al., 1989; Lokeshwar and Bourguignon, 1991). In addition, the membrane proximal region contains consensus sequences for the attachment of the glycosaminoglycan heperansulfate or chondroitin sulfate (Brown et al., 1991; Jalkanen et al., 1988; Stamenkovic et al., 1991). CD44 can also be modified by the attachment of lipids, such as palmitic acid (Guo et al., 1994). Phosphorylation of the CD44 cytoplasmic tail is another optional posttranslational modification (Kalomiris and Bourguignon, 1988; Neame and Isacke, 1992). Polypeptide isoforms of CD44 are produced by alternative splicing during mRNA processing. Theoretically, more than 700 membrane-bound CD44 isoform can be generated by alternative use of the variant exons. To date, at least 25 different isoforms have been described, some of which are differentially expressed or at least predopminatly found on

defined tissues such as the epithelial form (Cooper et al., 1992) or the keratinocate type (Brown et al., 1991). Cell surface CD44, as well as some variant sequences introduced by alterantive splicing are vulnerable to proteolytic cleavage, giving rise to soluble forms of CD44.

The CD44 adhesion molecule is the principle receptor for hyaluronic acid (Aruffo et al., 1990). Hyaluronic acid (HA), a glycosaminoglycan, is a major component of the extracellular matrix (ECM) of many tissues. The binding of CD44 to HA causes cell adhesion to ECM components and has been implicated in the stimulation of proliferation, cell migration and angiogenesis (Turley et al., 1991; Lokeshwar et al., 1996). Two HA-binding sites have been described in the extracellular domain of CD44 (Peach et al., 1993). HA binding to CD44 is influenced by the cytoplasmatic tail (Neame and Isacke, 1993), while the membrane proximal domain does not appear to be involved (He et al., 1992). Not all CD44-positive cells bind to HA, but in some cells HA binding can be induced by activatory signals (Liao et al., 1993; Liu et al., 1996; Sy et al., 1996). Furthermore, N- and O-glycosylation sites are critically involved in the CD44-HA interaction (Lokeshwar and Bourguignon; 1991; Katoh et al., 1995, Sy et al., 1996).

In addition to HA, CD44 has been reported to recognize a number of other ligands. CD44 can adhere to the ECM components collagen, fibronectin, laminin and chondroitin sulfate (Lokeshwar and Bourguignon, 1991; Jalkanen and Jalkanen, 1992; Aruffo et al., 1990, Peach et al., 1993). Serglycin/gp600 (Toyama-Sorimachi and Miyasaka, 1994, Toyama-Sorimachi et al., 1995), the chemotactic phosphoprotein osteopontin (Weber et al., 1996), and the MHC class II associated invariant chain (Ii) (Naujokas et al., 1993) are additional ECM-unrelated ligands of CD44. Serglycin/gp600 is a small chondroitin-sulfated preoteoglycan stored in the secretory granules of lymphoid, myeloid, and some tumor cells (Stevens et al., 1988). The interaction with serglycin/gp600 allows cytotoxic T lymphocytes activated with anti-CD44 and anti-CD3 mAbs to release granzyme A (Toyama-Sorimachi et al., 1995), suggesting a physiological role for this proteoglycan. The Ii is a nonpolymorphic glycoprotein associated with class II MHC, which prevents the binding of inappropriate peptides to class II molecules during their early transport in APCs. The small amount of chondroitin-sulfated Ii that remains associated with class II molecules on the APC surface membrane markedly enhances its ability to stimulate allogeneic and mitogenic responses in T cells by interacting with CD44 (Naujokas et al., 1993).

According to several reports, the CD44 cytoplasmatic tail can interact cytoskeleton-related components such as actin, ankyrin, or members of the ERM (ezrin/radixin/moesin) family (Kalomiris and Bourguignon, 1988; Bourguignon et al., 1992, 1995; Lokeshwar et al., 1994, 1996; Tsukita et al., 1994, Hirao et al., 1996). A number of modifications, including PKC-mediated phosphorylation, palmitoylation, and GTP binding may regulate the interplay between CD44 and ankyrin. ERM proteins are thought to function as general cross-linkers between plasma membranes and actin-based cytoskleletons. It has been proposed that ERM proteins exist in a closed (inactive) conformation and an open (active) conformation. There is evidence to suggest that this transition can be regulated by rho-GTPases, perhaps through the activation of a protein kinase or a lipid kinase (e.g. through PIP2) (Hirao et al., 1996; Tsukita et al., 1997; Hall, 1998). In the active conformation, the NH_2-terminus of ERM proteins can interact with CD44, whereas the COOH-terminus interacts with F-actin. At least for some functions the interaction of CD44 with the underlying cytoskeleton is essential. The HA binding function of CD44 has been reported to depend on the association of CD44 with ankyrin (Liao et al., 1993; Bourguignon et al., 1993; Liu et al., 1996) and CD44 isoforms are involved in cytoskeleton-mediated tumor cell migration and invasion (Bourguignon et al., 1998).

1.4.2 Functions of CD44 in the immune system

CD44 displays a large array of functional activities, most of them related to the hematopoetic system. As to the immune system, CD44 is essential in hematopoiesis, is known to be involved in lymphocyte homing and has been implicated in lymphocyte activation.

CD44 is known to play important roles in the differentiation and proliferation of hematopoietic progenitor cells in the bone marrow microenvironment (Kobayashi et al., 1994). Administration of anti-CD44 mAb to long-term bone marrow cultures blocks both lymphopoiesis and myelopoiesis, but apparently does not interfere with stroma formation (Miyake et al., 1990; Khaldoyanidi et al., 1996). The observation that anti-CD44 interferes predominantly with the maturation/expansion of stem cells and/or early progenitor cells, indicates that the expression of CD44 is required either for interactions between stem cells/progenitor cells and stromal elements, or for ligand binding of growth promoting factors. According to published evidence, the CD44 standard isoform upon ligand binding promotes proliferation of early progenitor cells, whereas CD44v may be involved primarily in transducing signals between stromal cells and stem cells, which initiate differentiation (Z ller, 1995).

Another function associated with CD44 is its involvement in lymphocyte homing (Shimizu and Shaw, 1991; Z ller, 1996). It has long been known that CD44 facilitates homing of mature lymphocytes into peripheral lymphoid tissues, in particular into lymph nodes. Lymphocytes bind to high endothelial venules via CD44, a function which seems to be restricted to CD44s (Jalkanen et al., 1988; Berg et al., 1989; Miyake et al., 1990; Scheeren et al., 1991). CD44 is also involved in the binding of bone marrow cells to stromal elements (Morimoto et al., 1994; Khaldoyanidi et al., 1996). The migration of prothymocytes into the thymus is also guided by CD44 (Wu et al., 1993). In addition to its function in lymphocyte homing into lymphoid organs, CD44 is also involved in the homing into non-lymphoid organs, which is especially important in infectious and allergic and autoimmune reactions (Frogner and O'Neill, 1992). Leukocytes extravasate from the blood into inflammatory sites through complementary ligand interactions between leukocytes and endothelial cells. Recently, CD44 could be proven to be of major importance for the extravasation of activated T cells into an inflamed site (DeGrendele et al., 1997). Studies on a lymph node endothelial cell line have shown that a CD44-HA interaction is important in lymphocyte rolling (DeGrendele et al., 1996), an essential prelude to the movement of leukocytes across endothelial barriers. Thus, CD44 is involved in the homing of progenitors and naive lymphocytes into hematopoietic organs, and selectively participates in the traffic of activated lymphocytes to sites of inflammation.

The CD44 receptor has also been implicated in lymphocyte activation. The vast majority of peripheral lymphocytes express CD44s. CD44 is upregulated during lymphocyte activation (Hamilton et al., 1991; Lesley and Hyman, 1992; Guo et al., 1996). Upregulation of CD44s during the activation process is permanent, i.e. memory cells are defined by high levels of CD44s expression. Like most adhesion molecules, CD44 may serve as an accessory molecule, cooperating with other molecules in the promotion of cell activation. Several lines of evidence indicate that anti-CD44 mAbs can modulate CD2 or CD3 induced T cell proliferation and/or cytokine production *in vitro* (Huet et al., 1989; Rothman et al., 1991; Guo et al., 1993, Lesley et al., 1993; Galandrini et al., 1993; Galluzzo et al., 1995; Sommer et al., 1995). Depending on the epitope recognized by anti-CD44 mAb and the nature of the other mitogenic signal CD44 has been suggested to deliver either positive or negative signals to the T cell. The underlying mechanism, however, has not yet been clarified. Under more physiological conditions of antigen-specific activation CD44 has been implicated in enhanced binding of dendritic cell to T cells (John et al., 1990) and has been suggested to function as a costimulus in allogeneic and mitogenic T cell responses by binding to the chondroitin sulfate form of the invariant chain (Ii) (Naujokas et al., 1993). While HA seems to be unable to act as a significant costimulus for T cells, B cells respond strongly to stimulation with HA comparable to other B cell mitogens (Rafi et al., 1997).

CD44 also plays a role in lymphokine-activated killer cell cytotoxicity, as demonstrated by genetic studies (Matsumoto et al., 1998). Antibodies to CD44 have been reported to interfere with the lytic activity of cytotoxic T cells and natural killer (NK) cells against target cells (Seth et al., 1991; Galandrini et al., 1993, 1994; Sconocchia et al., 1994). There is evidence that the interaction of serglycin with activated CD44 may augment CD3-dependent degranulation of cytotoxic T lymphocytes (Toyama-Sorimachi et al., 1995).

It has been described that a strong and prolonged Ca^{++} influx will be observed after cross-linking of CD44 on NK cells or on activated T lymphocytes (Galandrini et al., 1994; Galluzzo et al., 1995; Dianzani et al., 1999). It also has been suggested that signaling through CD44 is mediated by protein tyrosine kinases, since CD44 associates with src-family kinases in human T lymphocytes (Taher et al., 1996). Recent data indicate that this interaction occurs in glycolipid-enriched membrane microdomains (GEMs) (Ilangumaran et al., 1998). Cross-linking of CD44 may be accompanied by the tyrosine phosphorylation of intracellular proteins, including lck, fyn and ZAP-70 (Galandrini et al., 1993; Taher et al., 1996; Dianzani et al., 1999).

Taken together, there is ample evidence that CD44 may be involved in the process of lymphocyte activation and the subsequent aquisition of effector functions. However, the mechanism of function which links CD44 receptor engagement to the induction of these activities still remains to be elucidated.

1.5 Aim of the study

The initiation of an optimal T cell response requires the coengagement of the TCR with so called costimulatory molecules. Several lines of evidence indicate that CD44 may exert costimulatory activity, however, like for other costimulatory molecules, the mechanism by which this is accomplished is unclear. Therefore, the aim of the present study was to examine the biochemical and functional consequences of engaging CD44 during TCR stimulation. To this end, the involvement of CD44 in T cell activation was investigated under the physiologic condition of an antigenic stimulation. This should provide a solid starting point to evaluate cross-linking studies, in which the effect of CD44 engegament on TCR-mediated proliferation, as well as on TCR-induced apoptosis should be characterized. To get further insight in the molecular mechanisms of the costimulatory function of CD44, the effective contribution of CD44 to the process of T cell activation should be analyzed on the level of signal transduction. Furthermore, the potential association of CD44 with signal transduction molecules should be investigated. Finally, since recent evidence indicates that the reorganization of the T cell cytoskeleton and the redistribution of membrane lipid rafts is critically involved in the activation of T lymphocytes and might be influenced by costimulatory molecules, the effect of CD44 cross-linking on the actin cytoskeleton and the organization of lipid rafts should be analyzed.