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Review

The Immunological Synapse

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Abstract. The induction of a proper adaptive immune response is dependent on the correct transfer of information between antigen-presenting cells (APCs) and antigen-specific T cells. Defects in information transfer may result in the development of diseases, e.g. immunodeficiencies and autoimmunity. A distinct 3-dimensional supramolecular structure at the T cell/APC interface has been suggested to be involved in the information transfer. Due to its functional analogy to the neuronal synapse, the structure has been termed the "immunological synapse" (IS). Here, we review molecular aspects concerning IS formation, appearance, and cessation. In addition, proposed functions of the IS are discussed. The process of IS formation occurs in a sequential manner, initially causing a remarkable large-scale redistribution of a number of integral membrane and cytosolic proteins. At the T cell/APC interface the structure comprises in its nascent stage a non-random pattern of protein distribution. The protein pattern is regulated during development of the mature IS and is finally organized into concentric rings of co-receptors and adhesive molecules surrounding the T cell antigen receptor (TCR). The relocations of proteins are influenced by passive as well as active mechanisms. Considering the IS as a device enabling cell-cell communication, clarification of its exact function is of huge general as well as therapeutic interest.

Key words: immunological synapse; signaling; T cell; TCR; MHC.

Introduction

Living organisms sense and respond to their environment by a set of mechanisms referred to as cell signaling. These mechanisms are part of a complex system of communication that directs basic cellular activities and coordinates the action of cells. Since errors in cellular signaling often contribute to diseases such as cancer and autoimmunity, it is important to understand the basic mechanisms of cell signaling. The neuronal synapse is a well-known example of a highly developed structural organization for cell-cell communication. The interface is easily distinguished from other areas of the neuron by a distinct 3-dimensional protein distribution and a polarized secretion of transmitter molecules. This structure provides an efficient and specific way of neuronal cross-talking used to generate a response in the postsynaptic partner[63].

Abbreviations used: APC – antigen-presenting cell, cSMAC – central supramolecular activation cluster, CTLA – cytotoxic T lymphocyte antigen, DC – dendritic cell, DC-SIGN – dendritic cell-specific ICAM-3 grapping nonintegrin, ERM – ezrin, radixin and moesin adaptor protein family, ICAM – intercellular adhesion molecule, IS – immunological synapse, LFA – leukocyte functional antigen, MHC – major histocompatibility complex, MHCp – major histocompatibility complex peptide, MTOC – microtubule-organizing center, PKC-θ – protein kinase C theta, PSGL – P-selectin glycoprotein ligand, pSMAC – peripheral supramolecular activation cluster, TC – cytotoxic T lymphocyte, TCR – T cell antigen receptor, TH – T helper lymphocyte.

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The immune system represents another system, which relies on cell-cell communication, especially during induction of the adaptive immune response. This process is initiated by dendritic cells (DCs), which are specialized antigen-presenting cells (APCs) capable of engulfing antigens, process them into ~1–2 kDa peptides, and present these to T cells by use of tissue antigen families encoded by the major histocompatibility complexes (MHCs) gene[4, 5, 40]. Both self and non-self peptides are presented via MHC molecules for interaction with the T lymphocyte antigen receptor (TCR). The majority of presented MHC peptide (MHCp) complexes comprises self peptides. Under normal conditions these MHCp complexes are non-activating. In contrast, foreign peptides bound to self MHC molecules induce T cell activation and proliferation, resulting in either of two distinct types of effector cells: 1) the cytotoxic T lymphocytes (TCs) that induce target cell apoptosis through the perforin/granzyme- or FASL/FAS-mediated pathways[77], or 2) the T helper lymphocytes (THs) that facilitate activation of antibody-producing B cells, TCs and natural killer cells and regulate pathogen destruction by macrophages[8, 101]. TCs recognize MHC class I molecules using the TCR and CD8 co-receptors, whereas THs recognize MHC class II molecules employing the TCR and CD4 co-receptors[50].

The onset of signaling and hence T cell activation involves the formation of a synaptic structure at the T cell/APC interface, with many features similar to those of the neuronal synapse[31, 72]. In addition to the TCR, this structure includes several co-stimulatory and adhesion molecules which interact with counter-receptors on the APC. Together, these different molecules organize themselves into a specific 3-dimensional pattern known as the immunological synapse (IS), composed of the peripheral and central supramolecular activation clusters (pSMAC and cSMAC)[69].

Considering the IS as a device enabling communication between cells of the immune system, this review mainly aims at describing molecular aspects concerning the formation, appearance, and cessation of the IS. The review is structured chronological in regard to the various stages that apparently emerge during IS genesis. Finally, different theories of IS function are discussed. The focus will mainly be on T cell ISs since these are the best studied, although antibody-producing B cells and natural killer cells also form ISs with APCs and target cells, respectively[7, 17].

T Cell Polarization during Migration and APC Encounter

Naive, circulating T cells are relatively undifferentiated and without any obvious asymmetrical planes[78]. Immobilization of integrins such as leukocyte functional antigen (LFA)-1 due to actin cytoskeleton attachment furthermore makes the cells non-adhesive and maintained in a low-avidity state[24, 27]. Upon activation the lymphocytes enhance adhesiveness and show cytoskeletal polarization and morphological asymmetry[21]. T cells in this crawling state establish two asymmetrical poles referred to as the leading edge and the uropod. The leading edge serves as a sensory and propulsive front pole containing components involved in the development of contractive forces, such as F-actin and myosin light chain. In addition, the chemokine receptors CCR2, CCR5 and CXCR4, responsible for lymphocyte chemotaxis, are located in this front lamella. The front pole, furthermore, contains the nucleus[71, 76, 78]. During the migrating state, the uropod tends to contain many critical surface components, including the intercellular adhesion molecules (ICAM)-1, -2, and -3, the larger trans-membrane adhesion molecules CD43, CD44 and P selectin glycoprotein ligand (PSGL)-1, the TCRs and the co-receptors. In addition, the cytoplasm and most of the cytoplasmic organelles, i.e. the endoplasmic reticulum/Golgi apparatus, the microtubule-organizing center (MTOC) and secretory vesicles, are piled up in the uropod. Members of the ezrin, radixin and moesin (ERM) adaptor protein family, which facilitate linkage of a number of membrane proteins to the cytoskeleton, are also found in this posterior appendage[21, 22, 54, 78, 79, 83, 84].

The encounter of the T cell and the APC initiates a number of cellular responses. The MTOC and the Golgi apparatus relocate to the area between the nucleus and the APC. The TCRs as well as co-receptors and several adhesion receptor/ligand molecules, e.g. LFA-1/ICAM-1, also accumulate at the T cell/APC junction. Additionally, a number of adaptor proteins, including ERMs and talin, are detectable at this leading edge[69, 78]. This large-scale relocation of T cell membrane molecules has been assumed to rely on a passive, diffusion-limited, co-capping mechanism suggesting initial binding of a few ligands to their counter receptors. This causes a diffusional recruitment and interaction between increasing numbers of ligand-receptor pairs, resulting in stable intercellular adhesion[86]. However, several recent observations suggest that the receptor accumulation at the T cell/APC interface ap-

pears to depend on an active cytoskeleton-associated mechanism.

To study whether the T cell cytoskeleton could account for the polarized accumulation of receptors, WÜLFING and DAVIS[104] used a technique where the general movement of cortical actin cytoskeletal and attached receptors could be monitored by linking specific antibody-coated beads to surface receptors of antigen-specific T cells. They showed that 4±1 min after the initial increase in the intracellular calcium concentration – an early marker of T cell activation – the receptors were translocated from the posterior part to the anterior part of the T cells. This indicates that the process of receptor repositioning is active rather than passive, as it takes place immediately after T cell activation. Further studies support the idea that receptor translocation rely on an active process involving the actin cytoskeleton. SERRADOR et al.[84] investigated the dynamics of ERM family proteins and its influence on the cellular redistribution of membrane adhesion molecules. The ERM proteins contain three conserved domains: an N-terminal domain of ~300 residues, a central helical domain of ~200 residues, and a C-terminal tail of ~100 residues. The N- and C-terminal domains are responsible for binding to the cytoplasmic part of adhesion molecules and F-actin, respectively. In this way ERM proteins function as adaptor molecules, capable of connecting a number of integral membrane proteins, including PSGL-1 and ICAM-1, -2, and -3, to the actin cytoskeleton[43, 44, 64, 82]. ERM proteins appear in an inactive and an active state, depending on the phosphorylation state of a conserved threonine residue in the C-terminal domain. Only activated ERM proteins bind the actin cytoskeleton[64, 95]. During T cell migration, activated ERM proteins are found in the uropod, corresponding to the stacking of adhesion molecules[21, 78]. However, during T cell/APC encounter, the active ERM proteins accumulate at the leading edge. This suggests that ERM proteins, and hence the actin cytoskeleton, may be involved in redistribution of integral membrane adhesion molecules.

Blocking actin filament assembly with cytochalasin D has, in addition, been shown to cause a reduced relocation of ICAM-1 and a 10-fold reduction in T cell activation frequency[105]. Nevertheless, specific inhibition of the actin cytoskeletal motor protein myosin with butanedione monoxime yields a much more pronounced reduction of receptor translocation[104]. This implies that the receptor/cytoskeletal movements to a certain degree are dependent on myosin motor proteins.

Initial Adhesive Contact and Stopping of the Migrating T Cell

Which molecules and mechanisms could account for the initial adhesive contact between the migrating T cell and the APC? To answer this question a number of biophysical phenomena must be taken into consideration. Both cell types have a negatively charged glycocalyx located at their surfaces mediating an electrostatic repulsion at close contact[88, 89]. To overcome this repulsion, only surface molecules of adequate length, to stand out from the negatively charged glycocalyx, are able to create the preliminary contact. Additionally, the molecules should interact with low affinities in order to create a loose contact between the two cell types, which provides the background for the TCR-mediated scanning for distinct MHCp molecules at the surface of different APCs[97]. It is in general believed that the TCR/MHCp complex is unable to be involved in the initial contact due to several features. First of all, this receptor/ligand couple interacts with extremely weak affinity[1, 65, 66]. Furthermore, a very low concentration of specific ligands for a particular TCR are found on the APC surface[23, 42]. In addition, the extracellular portion of the TCR and MHC is small (~7 nm) and for that reason simply buried and suppressed by the surface glycocalyx[6, 88]. Taken together, these facts imply that a TCR/MHCp-mediated initial adhesion would require too many MHC molecules and too much time in practice.

Instead, a number of adhesion molecule pairs have been proposed to initiate an antigen-independent adhesion of T cells and APCs, the most notable being the integrin LFA-1 and its major immunoglobulin superfamily ligands and the non-integrins CD2/LFA-3 and ICAM-3/DC-specific ICAM-3 grapping nonintegrin (DC-SIGN). CD2/LFA-3 pairs interact with relatively low affinities, making them appropriate candidates permitting scanning of APCs by T cells[33, 98]. However, the molecules are quite small and therefore tend to interfere with the glycocalyx during interaction[18, 47, 52, 102]. The LFA-1/ICAM-1 adhesion pairs are, on the other hand, not inhibited by the glycocalyx, as they contain large extracellular domains[6]. LFA-1 is normally found on the cell membranes in an inactive state, activation being facilitated by, for example, certain chemokines or TCR occupancy[10]. Even though the LFA-1/ICAM-1 adhesion pairs fulfill many of the expected properties necessary to initiate adhesion contact, much discussion has taken place due to the high-affinity interaction between the two molecules[94]. Rather than allowing T cell

scanning of the APC surface, this seems to be optimal for conjugate stabilization, which follows initial contact and TCR/MHCp engagement. Much evidence suggests that ICAM-3 adhesion molecules may be involved in the initial exploratory contact of T cells and APCs. The molecules are highly constitutively expressed on resting T cells[20] and cluster during APC encounter in the contact region, where they are able to facilitate LFA-1/ /ICAM-1 adhesion[16]. ICAM-3 adheres through low- and high-affinity interactions with the APC ligands LFA-1 and DC-SIGN, respectively[19, 36, 37], and has in addition been shown to exert some co-stimulatory effects. These include phosphorylation and dephosphorylation of a set of signaling molecules involved in T cell activation and promotion of the cytoskeletal rearrangement causing MTOC translocation[45, 53, 70, 90]. ICAM-3 may have a key role in initiating cell adhesion, but there is still no direct evidence.

Assuming that all of the above-mentioned adhesion molecules are involved in the initial T cell/APC contact, the most reasonable explanation would be that ICAM-3 mediates the primary weak adhesive interaction with LFA-1, allowing T cells to scan the APC surface. At this point ICAM-3 may as well contribute to membrane receptor relocation by co-stimulatory effects. Subsequently, CD2/LFA-3 interaction may bring the membranes in close proximity due to their small size, permitting the TCR/MHCp complex to form. It appears that TCR, CD2 and ICAM-3 engagement induce LFA-1/ICAM-1 adhesion[16, 34, 99], which at this stage of interaction would function to reinforce the conjugate. High-affinity bonds between ICAM-3 and DC-SIGN may further stabilize the complex[37].

The "stop" signal is possibly the most important event during T cell/APC recognition, responsible for detaining the T cell after encounter with the APC. In an *in vitro* system, DUSTIN et al.[29] have shown that this stop signal may be mediated by TCRs interacting with MHCps. By using harvested and stimulated T cells containing TCRs for a particular epitope, they examined the migratory behavior of the cells on planar phospholipid bilayers. The planar bilayers were coated with ICAM-1 and MHCp molecules. The T cells crawled on the ICAM-1 substrate, but obviously stopped on encounter with a relevant MHCp ligand. On encounter with an irrelevant MHCp molecule they did not stop their migration. Thus, in contrast to the initial antigen--independent adhesive contact, their stop signal, is an antigen-dependent process. The antigen-dependent stop signal, shown to be facilitated *in vitro* by interactions between a limited number of molecules, i.e. TCR/MHCp and LFA-1/ICAM-1, questions whether a complete IS is necessary to activate T cells. By measuring the calcium level in T cells simultaneously to the movements of IS-involved proteins, IRVINE et al.[48] observed that a single MHCp complex is sufficient to stop migrating T cells, forming a tight interface and elevate the calcium level. However, at least 10 MHCp complexes are needed to facilitate IS genesis. Cross--linking T cell membrane receptors by antibodies or tetramers are in addition known to facilitate T cell activation without forming an IS, assuming that the calcium level reflects the activation state. This indicates that only some components of the IS are needed during the activation process, at least *in vitro*. The situation is probably more complex *in vivo*, requiring further levels of activation regulation and thereby the need for additional IS components.

The initial antigen-dependent stop signal seems to compete with chemokine gradients, which influence the T cell to continue chemotaxis. By establishment of a liquid culture system, in which T cells could be simultaneously exposed to antigen and chemokine gradients, BROMLEY et al.[15] demonstrated that only certain chemokines were able to reverse or prevent the TCR/MHCp-mediated stop signal. They divided the chemokines into two groups according to their ability to interfere with T cell stopping: the dominant and subordinate groups, capable of suppressing the stop signal and not, respectively. Included in the dominant group are chemokines engaging CXCR3 and CCR7 receptors, whereas the subordinate group comprises chemokines which affect CXCR4, CCR2, CCR4 and CCR5 receptors. Furthermore, it has been demonstrated that APCs with appropriate MHCps do not stop T cells in 3-dimensional gels in contrast to liquid culture models[41]. This suggests that other environmental factors, in addition to the chemokine forces, may influence the initial antigen-dependent interaction of the T cell and APC. The mechanism may involve T cell interaction with collagen fibers of the extracellular matrix[28].

Molecular Remodeling and Segregation at the T Cell/APC Interface

At the T cell/APC interface a number of morphological changes and molecular micro-scale redistributions occur prior to IS formation[13, 32, 56]. MONKS et al.[69] were the first to describe the segregation of molecules at the interface, taking advantage of digital imaging. They were able to show a redistribution of molecules by staining cell-surface receptors with specific antibodies in fixed T cell/APC couples, isolated at various

times after cell mixture. Later, the process described by MONKS et al.[69] was confirmed in other types of experiments which, furthermore, have provided knowledge about the dynamics of IS formation. By seeding Oregon-green-labeled MHCp molecules and Cy5-labeled ICAM-1 molecules into artificial lipid bilayers that lacked other APC molecules, GRAKOUI et al.[38] could follow the IS formation consecutively in real time using live cells. These experiments also revealed that the strong agonist MHCp complexes yielded a more pronounced high-density MHCp accumulation at the T cell/APC interface compared with the weak agonist MHCps. Simultaneous measurement of the intracellular Ca^{2+} concentration has shown that the receptor dynamics follows the onset of Ca^{2+} signaling. This technique, used by GRAKOUI et al.[38] has been utilized to clarify the redistribution of a number of surface molecules, e.g. TCR/MHCp and LFA-1/ICAM-1. More recently it has been possible to collect entire 3-dimensional data-sets of IS formation, taking advantage of flourescently conjugated receptors and improvements in high-speed video microscopy[54, 93].

The observations by MONKS et al.[69] and GRAKOUI et al.[38] suggest that, following the initial T cell/APC engagement, a distinct molecular pattern is created. The adhesion molecules LFA-1 and ICAM-1 accumulate in the center of this pattern being surrounded by a sphere of TCRs/MHCps. However, by taking advantage of more sophisticated methods, KRUMMEL et al.[54] demonstrated that, previous to this phenomenon, dispersed microclusters (<1 μm^2) of TCRs and CD4 appear at the T cell/APC interface. These structures are very dynamic, constantly moving and forming/reforming over time, and occur almost at the onset of intracellular Ca^{2+} signaling. This indicates that the initial event in T cell activation is mediated by these microclusters. Nevertheless, it seems clear that the formation of TCR microclusters follows the relocation of membrane proteins from the posterior to the anterior of the T cell. This suggests that another mechanism is responsible for the large-scale movement of proteins to the leading edge. If this process has to be antigen dependent, it would require TCR engagement before formation of TCR microclusters. Perhaps even smaller TCR aggregates are the primary initiators. Another possible explanation would be that this large-scale relocation is antigen independent. REVY et al.[75] have shown that membrane proteins may accumulate at the intercellular interface in the absence of any antigen-specific activation. These proteins may facilitate the initial signaling, e.g. a small Ca^{2+} respons and some tyrosine phosphorylation, and thereby recruitment of other membrane proteins. ICAM-3 could be suggested as a candidate, accumulating at the interface either in the presence or absence of an antigen[70].

Within 5–10 min, the microclusters typically arrange into the structure observed by MONKS et al.[69] and GRAKOUI et al.[38]. At this early stage the molecular organization is referred to as the nascent IS. The nascent organization is inverted within minutes and finally consists of a central cluster of TCRs/MHCps surrounded by a ring of LFA-1/ICAM-1 adhesion molecules. At this stage the formation is known as the mature IS, which consists of the cSMAC and pSMAC areas[13, 38, 69]. Non-random patterns of protein distribution for a number of adhesion and accessory receptors have now been documented. CD4 co-localizes as previously described with the TCR at the initial stage of IS formation. However, during maturation of the IS, the CD4 co-receptor migrates to the pSMAC, while the TCR tends to coalesce in the cSMAC. The purpose of this dissociation is not fully understood, but suggests that the CD4 co-receptor is only needed to "boost" recognition of MHCp and is not required during the following activation[38, 54, 69]. The heavily glycosylated membrane protein, CD43, is totally excluded from the cSMAC and pSMAC areas[87], whereas there is still much discussion about the location of the tyrosine phosphatase CD45[51, 59]. At time of contact, before TCRs condensate into the cSMAC, CD45 is interspersed within the microclusters. Subsequently, CD45 seems to migrate to the pSMAC, although a discrete pool of CD45 is observed in the cSMAC[59]. The CD2/LFA-3 adhesion molecules have been recognized between the cSMAC and the outer LFA-1/ICAM-1 ring[85]. Another set of molecules with well-known strong impact on activation of naive T cells are the co-stimulatory receptor CD28 and its ligands CD80/CD86, localized on T cells and APCs, respectively[12, 39]. Recently, it has been observed that CD80 and CD86 are exported to the plasma membrane of DCs linked to MHCps molecules. At the surface they remain associated within the cSMAC area[96]. Since the antigen receptors of MHCp ligands are located in the cSMAC area on T cells, CD28 presumably accumulate at the same location.

A number of cytoplasmic adaptor and signaling molecules are in addition found in a distinct pattern in the mature IS. MONKS et al.[69] demonstrated that talin and protein kinase C (PKC)-θ localize to the pSMAC and cSMAC, respectively. Talin is a cytoskeletal adaptor protein which connects actin filaments and integrins, including LFA-1[60]. Thus, the observation seems reasonable, as LFA-1 localizes in the same area of the mature IS. PKC-θ is involved in signaling transduction

through the TCR in response to stimulation and co-localization of TCR, and PKC-θ in the cSMAC is for that reason anticipated[3]. Other proteins facilitating TCR-mediated signaling, e.g. the tyrosine kinases Lck and Fyn, have also been detected in the cSMAC area[69].

Looking at the IS development over time, it is revealed that various molecules are uniformly distributed at the T cell/APC interface at the pre-contact stage. However, at initial cell-cell contact, the TCR/MHCp complexes redistribute and form microclusters within seconds. At this stage the T cell membrane protrudes in order to allow the small TCR/MHCp complexes to combine. Within several minutes the cSMAC is developed by migration of, for example TCR/MHCp and CD28/CD80-86 towards the central area. The mature IS, consisting of the cSMAC and pSMAC areas, are observed within 30 min of T cell/APC contact[38, 54].

Mechanisms Contributing to Molecular Remodeling at the T Cell/APC Interface

The mechanisms leading to this reorganization and segregation of the surface molecules at the T cell/APC interface can be divided according to whether the mechanisms rely on passive or active forces, respectively. The passive mechanisms depend on a number of factors, which include, for example, interaction of a receptor with its counter-receptor at the contact interface and biophysical aspects. In contrast, the active mechanisms are driven by cellular processes as, for instance, remodeling of the cortical actin cytoskeleton.

The passive mechanisms

One notable observation is that the plasma-membrane molecules as mentioned vary considerably in size. The extracellular part of the TCR and MHCp is only ~7 nm, which makes it unusually small compared with other highly abundant surface molecules, as for instance CD43 (~43 nm) and CD45 (~23–50 nm). The adhesion molecule family of integrins is about 21 nm[6, 88]. Significant reorganization must therefore occur in order to allow TCR/MHCp engagement. A mechanism of receptor relocation within the synapse has been proposed which is based on the differences in the dimensions of the receptor/ligand interactions[18, 85]. According to this theory, the smaller molecules will cluster together in membrane regions that are intimately connected with each other upon T cell/APC interaction. Larger molecules will also tend to cluster together, but in other, separate membrane regions, where the membranes are more distantly situated. The process is connected to the membrane protrusions, which are observed on the T cell surface at the same time. Whether the membrane protrusions are promoted by passive biophysical mechanisms or by an active actin cytoskeleton-mediated mechanism is uncertain. The theory proposes further arguments that the relative shortness of the TCR/MCHp complex (~14 nm) compared with other membrane molecules, is responsible for the subsequent translocation of TCRs/MHCps into the center of the interface. Larger molecules, e.g. LFA-1, ICAMs and CD45, would migrate to the exterior of the synapse, simply due to steric mechanisms.

A study done by WILD et al.[103] support the idea that passive mechanisms based on molecule dimensions are responsible for the receptor/ligand reorganization at the T cell/APC interface. In an *in vitro* system they expressed modified variants of the CD2 ligand, CD48, in transfected Chinese hamster ovary cells. CD48 is the rodent analog of the human CD58 or LFA-3. The immunoglobulin loop present in CD48 molecules was either truncated or extended, using parts of CD2 or CD22. Under normal conditions, the function of CD2/CD48 is to enhance MHC-restricted T cell antigen recognition and to deliver a co-stimulatory signal during T cell activation[9, 46, 68, 81]. WILD et al.[103] demonstrated that whereas wild-type or shortened CD48 enhanced the CD2-bearing T cell antigen recognition, the extended form exerted an inhibitory effect, preventing the TCR engagement of MHCp. The authors concluded that alteration in CD48 dimension may cause “mis-placing” in the T cell/APC interface and hence prevention of co-stimulation. Thus, it looks like the final location of each surface molecule in the IS depends on a well-defined design in regard to dimension, and that a close membrane contact is necessary for T cell antigen recognition. Taking advantage of mathematical models, other authors have also proposed that the molecular reorganization is based on spontaneous self-assembly mechanisms. This further supports the idea of a passive molecular segregation at the cell surface[74].

The active mechanisms

If the molecular reorganization only relied on passive mechanisms it would require a relatively high lateral mobility[49], allowing the molecules to diffuse freely in the plasma membrane. Roughly 70% of the CD2 molecules located at the T cell surface have been shown to be mobile[61]. Similar observations have been found for other surface molecules[80]. However, only about 20% of the TCRs on mature T cells are mobile[38].

This indicates that the TCRs either have a basal association with the cytoskeleton, which stabilizes them, or are inhibited by diffusion barriers in the membrane involving, for example, other membrane proteins or lipid rafts. One possible link between the TCRs and the actin-based cytoskeleton may be formed via the actin filament-coupling protein VASP. The TCRs are linked to VASP through the adaptor protein Fyb, which interacts with Fyn and VASP. Upon initial antigen receptor triggering, the TCRs activate actin polymerization through Nck, WASP, Vav and Arp2/3. This may force further TCRs to the T cell/APC interface[30, 32]. Additionally, the actin polymerization could account for the T cell membrane protrusions observed during T cell/ /APC encounter, which causes the TCR/MHCp complexes to cluster and expand in areas of close membrane proximity.

VASP protein bonds between the TCRs and the actin cytoskeleton are probably not the only mechanism responsible for forming linkage between the two, as indicated by studies of Fyn-deficient mice[91].

An active mechanism responsible for the transport of engaged TCRs from the pSMAC to the cSMAC has not been identified so far. However, the process may involve activation of myosin II. Myosin II is comprised of two actin filament-binding myosin heads and a neck region containing the essential and regulatory light chains. Ca^{2+}/calmodulin complexes are able to interact with the regulatory light chains. At low Ca^{2+} concentration, interaction between myosin and the actin filaments is inhibited. However, at a sufficiently high Ca^{2+} concentration, the myosin heads are activated due to phosphorylation of the regulatory light chains. This induces a conformational change in the myosin heads, allowing them to bind actin filaments. Due to the bipolar structure of myosin filaments, they are able to cross--link different actin filaments. Thus, activation leads to a gathering of actin filaments and the associated membrane proteins[62].

Several other adaptor proteins are, in addition, involved in linking various surface molecules to the actin cytoskeleton. As described earlier, talin and LFA-1 co--localize in the pSMAC area of the mature IS[69]. This indicates that talin, and hence the actin cytoskeleton, could account for the localization of LFA-1. CD2AP is another example of an adaptor protein which may be responsible for CD2 reorganization[73].

Even though it has been demonstrated that the rearrangement of surface proteins into distinct supramolecular clusters may rely solely on passive self-assembly processes, much indicates that an active actin cytoskeleton-driven mechanism could be responsible for the T cell/APC interface reorganization as well. Probably, the two processes interact with each other, implying that active cellular interventions (e.g. directed cytoskeletal rearrangement) can either amplify or regulate the passive processes.

Duration and Cessation of the T Cell/APC Conjugation

Intense effort has been made to elucidate the basic mechanisms leading to IS formation during T cell antigen recognition. In contrast, detailed information is lacking concerning duration and cessation of the T cell/APC conjugation. An *in vivo* experiment by STOLL et al.[93] has provided some insight into the process of IS termination. Using 4-dimensional confocal imaging they were able to visualize the cessation of the T cell and APC in intact mouse lymph nodes. They showed that upon division of activated T cells, the mother and daughter cells were separated from the APC. This indicates that mitotic-derived cellular factors are responsible for IS termination. Furthermore, factors in the extracellular milieu influence the duration of T cell/APC interaction and hence the duration and cessation of IS formation. Several factors have been proposed, including chemokine gradients[15] and T cell interactions with collagen fibers of the extracellular matrix[41]. The extracellular factors probably act in combination with several cell-derived mechanisms in order to determine the duration of T cell/APC interaction.

The Function of the IS

The main function of the IS is still under discussion. Initially it was believed to serve as a device for initiating, stabilizing, and sustaining TCR signal transduction during APC encounter. This proposal seemed reliable for at least two reasons. First, the surface area of the interface is substantial (~50 μm^2), allowing a considerable number of adhesion molecules to interact, thus stabilizing the area. Second, the postulate made sense considering the prolonged TCR-mediated stimulation required in order to fully activate T cells. However, recent investigations have demonstrated that a role for the IS in initiating TCR signaling is very unlikely. TCR triggering and hence activation of proximal tyrosine kinases, as for instance Lck and Zap-70, occurs within seconds of antigen encounter, while the assembly of the mature IS may take several minutes[58]. Thus, as TCR

signaling precedes IS assembly, the IS cannot be responsible for initiating TCR signaling.

Although not required for initial TCR signaling, IS establishment is essential to intensify TCR-mediated signaling and achieve proper T cell activation[35]. One possible mechanism would be by carefully regulating inclusion and exclusion of various molecules from the T cell/APC interface. Up-concentration of the co-stimulatory molecule CD28 in the cSMAC is one example of including a molecule which in turn will amplify TCR signaling[14]. In contrast, phosphatases may be excluded from the cSMAC in order to avoid interference with the kinase activity required for TCR signaling. Although controversial, the tyrosine phosphatase CD45 is an example of a molecule that is removed from the cSMAC[51, 59, 85]. However, it is still not obvious why physical separation of positive and negative regulators of signaling would require molecular segregation at the T cell/APC interface on such a large scale, i.e. micrometers, as observed for the IS.

The supramolecular organization of the IS has also been linked to directed secretion[11, 55, 92]. This implies that T cell effector molecules are secreted in a localized manner on the APC or target cell surface. In this way the only cell affected is the cell with which the T cell has formed a synapse, thus reducing bystander effects. The effector functions may, for instance, be secretion of cytokines and cytotoxic agents produced by $CD4^+$ and $CD8^+$ T cells, respectively.

As demonstrated by VALITUTTI et al.[97] a few MHCp complexes may be responsible for triggering many TCRs. One possible function of the IS may be to provide a molecular organization which could serve as a site allowing this serial TCR triggering. One could imagine that new TCRs constantly arrive to the cSMAC of the IS, driven either by passive or active forces, and that they subsequently are internalized and degraded after fulfilling their signaling function[2, 57]. This persistent delivery of new TCRs to the cSMAC may possibly occur by different cellular mechanisms. Active lateral movement of TCRs mediated by the actin cytoskeleton has been suggested[32]. Although this mechanism seems realistic, it implies that the TCRs have to pass the pSMAC area, comprised partly of strongly interacting adhesion molecules. If these adhesion molecules form a dense sphere around the cSMAC, they would probably inhibit the translocation of TCRs. To avoid this obvious problem, the TCRs could enter the cSMAC simply by an endo/exocytotic pathway translocating TCRs from the rear of the cell to the cSMAC, as previously suggested by us. At least two different endocytotic pathways exist to regulate TCR expression levels at the T cell surface[57]. One pathway is dependent on tyrosine kinase activity. Following tyrosine phosphorylation the TCRs are endocytosed via clathrin-coated pits and subsequently sorted for lysosomal degradation. Thus, this pathway leads to a diminished total amount of TCRs requiring new synthesis for restoring the previous level. However, another pathway, which relies on recycling of TCRs, is more relevant in this case. This pathway is mediated by the balance between PKC and serine/threonine protein phosphatase activities and is dependent on serine phosphorylation of CD3γ by PKC[25]. Activation by PKC initiates the endo/exocytotic cycling pathway, which may account for the redistribution of TCRs to the cSMAC[26, 67, 100]. A similar exocytotic pathway could be proposed considering directed secretion of, for example, cytokines and cytotoxic agents.

Recently, a model has been suggested that relates some properties of the neuronal synapse to the IS[31]. According to this model it is believed that the IS forms a gasket into which soluble as well as membrane-anchored molecules are exocytozed. The directed exocytosis is facilitated by APCs, which are considered to be the pre-synaptic cells. Newly synthesized MHCp complexes are co-secreted with co-stimulatory molecules, e.g. CD80 and CD86, into the synapse to interact with appropriate receptors located on the post-synaptic T cell side. In this model, it is assumed that a very low number of MHCp complexes are present at the pre-synaptic side on initial encounter with the T cell. However, following the low level of agonist-induced activation, a positive-feedback mechanism is established, causing further delivery of MHCp complexes to the interface. This phenomenon is suggested to provide the basis for sustained TCR signaling.

Clearly, a better understanding of the structure and function of the IS will lead to an improved knowledge of how T cells communicate with other cells and are activated. Advances in this field could open up the prospect of development of new therapeutics for a number of immunological diseases.

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