A Predetermined Site for The Immunological Synapse

MED-3950 5th Year Thesis

Cand. med. degree program

Universitetet i Tromsø

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Stavanger, May 31, 2015.

Abstract

Background

Over the last 30 years there has been great advances in understanding the interaction between T cells and antigen presenting cells (APCs). A transient structure called the immunological synapse (IS) has been discovered, functioning as a information center, pulling the strings leading up to T cell activation. Further, a T cell has a marked head-and-tail like configuration when actively seeking its target APC, with the leading edge shown to partake in the first activation events. However it has not been conclusively demonstrated that the IS is exclusively confined to this area. Thus the question whether or not there exists a pre-determined site for the immunological synapse is asked.

Methods

A literature review has been done utilizing the freely available online database front-end PubMed. The terms queried where «immunological synapse», «supra-molecular activation complex» and the two combined with «predetermined site». Further the works of central authors in the field has been sought and read in detail in order to follow the scientific narrative.

Conclusion

No definite proof could be found for a predetermined site on a T cell in the reviewed literature. However it is deemed likely that such a place can exist due to the conformational changes observed upon T cells actively searching its match.

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Work Process

Originally this assignment was planned as an experimental study, to be conducted at the laboratory of the Immunological Research Group, at the Department of Medical Biology at the University of Tromsø. The assignment was planned in relation to my ongoing work as a researching medical student. In short I was to explore the possibility of a cytotoxic CD8+ T lymphocyte response in the disease neonatal alloimmune thrombocytopenia (NAIT).

Due to illness in my near family this plan was altered, as I moved away for the remaining part of the semester. I therefore drafted a literature review proposal for my supervisor. During January and February we had two meetings, further detailing the scope of the paper. Concurrently I started to search the literature, which continued the following months through March and April. At this point I also started writing the thesis. This work continued until the deadline on June 1st.

Research Question

«Is there a predefined site for the immunological synapse on a resting T cell?»

As a researching medical student my work has mainly focused on T cell immunology. Being one of the main orchestrators of the adaptive immune response, T cells are intensely researched. Both in regards to their physiology, but also as perpetrators of pathology in various immune-related disorders and cancer. At the core of their effector function lies the crosstalk with other immune cells, where the field has experienced great advances over the last 30 years. We now know there exists a transient immunological synapse, which functions as an information hub, deciding wether or not the T cell activates. In return this can result in life saving immune responses, and we are now starting to manipulate these using various strategies in the clinic, such as adoptive cell transfer, with promising results [1].

In this paper I have therefore decided to explore the immunological synapse, focusing on its creation and its associated cellular changes leading up to the signaling event. More precisely, the question whether or not there is a predetermined site for the immunological synapse on a resting T cell is asked. Later I want to explore this in further detail, using T cells already stored in the biobank of the Immunological Research Group.

Methods

A literature review was carried out using PubMed and the search terms «immunological synapse», «supra-molecular activation complex» and the two combined with «predetermined site» [2]. A total of 274 hits were returned, of which more than 50 where determined relevant. There was a mix of original reports and review papers, with more of the latter appearing over the last 10 years. Further the works of various central authors, such as Michael L. Dustin and Mark M. Davis, were read in detail in order to follow the scientific narrative. Additionally, a number of papers relevant to other areas of immunology were accessed, together with supporting literature, read over four years as a part of my ongoing work as a researching medical student.

The Human Immune System in Brief

The human body is vulnerable to a multitude of attacks resulting in disease. Both infectious and non infectious threats needs to be eliminated in a quick and sufficient manner, without damaging the host. Through jawed vertebrate evolution to mammals, humans have acquired several different systems, each specialized in fighting various intruders, living or non-living [3]. Roughly we divide the defense of the body in to two arms, the innate and the adaptive immune system. These are not in a binary opposition, thus activating and helping each other in nearly all cases [4]. Nevertheless, the innate part is usually the first responder directed towards a new threat. However, this is a non-specific response, meaning its efficacy over time is low. The invaders have evolved ways to escape a relatively limited set of protective measures [5]. Further, exposure leads to an immediate maximal response, and it does not improve until the next time the body encounters the same pathogen. The opposite is called immunological memory, and is one of the two defining components of the adaptive system, together with a high specificity, which is the ability to target selectively [6].

The main orchestrators of the adaptive response are the T cells, though dependent on other types of immune cells, namely the antigen presenting cells (APCs). These comprise of three different cell types, the dendritic cells, the macrophages and the B cells [7]. Together they search and kill of attackers, engulfing their constitutes, before presenting bits of proteins on their surface - also known as antigens - which subsequently are detected by the T cells. In turn, they recognize these parts as foreign, alerting the immune system to be in a defensive state through the release of various molecules called cytokines. The T cells also signal back to the B cells to develop in to plasma cells, which thereafter start producing antibodies. These are molecules that

circulate in the bodily fluids, clinging to the parts of the aggressor they originally where detected from. Within this lies one aspect of immunological specificity [8]. One T cell recognize specific combinations of amino acids stringed together to a peptide. These are typically derived from a protein expressed by the pathogen, it being a bacterium, helminth, virus or any other parasite attacking the human body. As this happens the involved immune cells multiply, with some of them diverging in to memory cells. These cells survive after the body eradicates the infection, with the purpose of quickly being able to detect and expand their population, upon a future recognition of a recolonization event. By doing this the body can kill off the attacker before its too numerous and overwhelm the immune system. In other words, an immunological memory [9].

The process previously described is greatly simplified, not only disregarding the innate immune system, but also a number of different cell types, humoral components, anatomical compartments, and chain of events. However, the immune system is complex, not even completely mapped out in the simplest invertebrate [10]. This paper will concentrate on the adaptive immune system, focusing on the T cell. More specifically, the biology surrounding the ability of a cell to detect presented antigens, directing attention to the cell surface and the specific area in which the cell-to-cell contact occurs, and later the surrounding intracellular changes. This location is called the immunological synapse.

What Is a T Cell and What Does It Do?

The immune system is comprised of an heterogenous group of cells, all destined from a multipotent hematopoietic stem cell in the bone marrow [11]. The first division decides whether it will belong to the myeloid or lymphoid lineage. The first aforementioned

group includes the red blood cells, platelets and the macrophage, together with the cells of the innate immune system, the granulocytes. The other group, the lymphoid cells, consists of the T and B lymphocytes, and thereby the plasma cell, in conjunction with the natural killer cells (NK cells). The dendritic cells are a special case, with different subsets from both the main two lineages. An overview of the lineage is seen in figure 1.

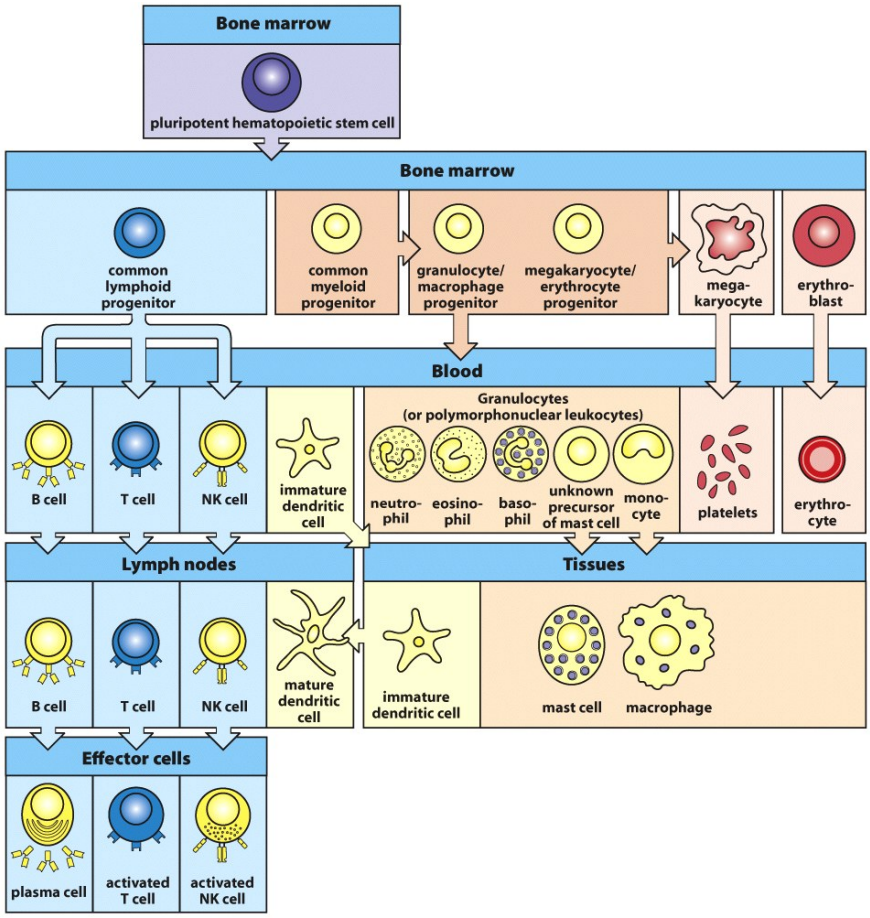

Figure 1.3 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Figure 1. A map of hematopoiesis and its cellular end products [12].

As a continuation, T cells are classified into different groups. Primarily, they are divided in to helper and killer T cells [13]. Together they are both a part of the cell-mediated immune response. The killer cells seek to detect and destroy cells that are damaged or dysfunctional, either due to pathogens or toxins, but also from cancerous

transformations. They are denoted as CD8+ cells, due to a specific surface marker. CD is short for «cluster of differentiation», and the subsequent number is assigned as a running continuation when new surface molecules are discovered and added to the nomenclature [14]. It is worth noting that it tells nothing about the function of the molecule, as two adjacent numbers can have wildly different roles. In this case, CD8 is a transmembrane glycoprotein that serves as a co-receptor for the T cell receptor (TCR), by which it binds to a major histocompatibility molecule (MHC), more specifically MHC type I. Both these molecules are further detailed below.

The helper cells are the master regulators of both the innate and adaptive immune response, and come in a seemingly expanding array of subsets [15]. They do not have the cytotoxic capabilities of the killer cells, but rather reach their means through communication with other immune cells. These cells are called CD4+ cells, for the same reason as the cytotoxic variants, however this TCR co-receptor binds to MHC type II. As previously mentioned, they signal B cells to differentiate into plasma cells, producing large amounts of antibodies. After these molecules bind to an antigen on a target cell, several effects can pursue. One signals NK cells to initiate a cytotoxic mechanism called antibody-dependent cell-mediated cytotoxicity, killing the marked entity through release of cytotoxic granules leading to target cell apoptosis [16]. As a side note, this process is a nowadays a mainstay in modern cancer therapy, through the use of monoclonal antibodies [17]. These are lab made antibodies with a desired specificity against malignant cells. A schematic of the different subsets is shown in figure 2. This text will not go into any more detail explaining the distinction between them, however it should be noted that this division is not set in stone. Rather, current

knowledge points towards this being more of a plastic process, with cells altering their state as a response to environmental stimuli [18].

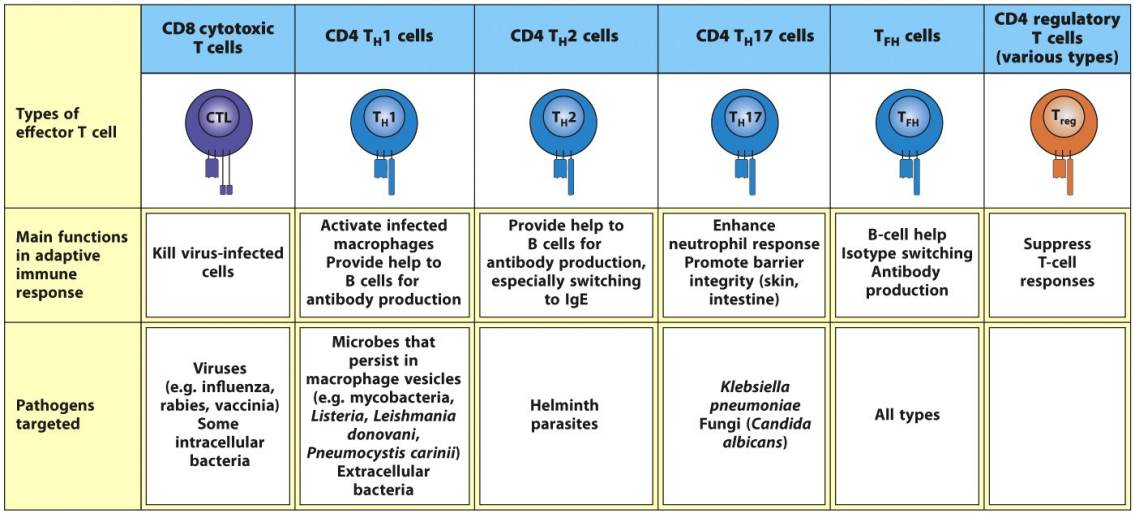

Figure 2. An assortment of various T cell subsets and their function [12].

Antigen Recognition

Dogma dictates that each T cell of any type is specific and can only recognize a limited number of antigens [19]. As a consequence the body needs a diverse repertoire of cells to adequately defend against a manifold of threats. This is achieved through a surface molecule called the T cell receptor (TCR). After the progenitor T cells leave the bone marrow, they travel to the thymus in order to mature [20]. Through a selection process, they are divided in to CD4+ or CD8+ cells, and the TCR go through a series of steps called negative and positive selection. This is to make sure the TCR not only is strong enough to bind antigens, meaning any non functional duds are discarded, but also to guarantee they do not recognize any self-antigens. This central tolerance is significant as it unveils the power of the T cell. Self-recognition is thought to be one of the main reasons for autoimmunity, a group of diseases where the body recognize its own bodily

tissue as foreign, starting an attack through an aberrant immune response. Because of this, it is postulated that the the selection process is imperfect, even though peripheral tolerance exists in order to control self-reactivity [21].

As mentioned earlier, the TCR does not bind directly to an antigen. It does so through the help of another type of molecule, the MHCs [22]. They present the peptides for the TCRs to discover. There are two kinds of MHC molecules, type I and II. The first is the vehicle for cytosolic proteins and is present on almost all cell types in the body. If a cell is infected by a parasite such as a virus, parts of its constituents will be degraded by the proteasome of the host cell and transported to the cell surface, bound to the MHC type I molecule. Then it is finally presented to the CD8+ T cells, which exclusively bind to this type of MHC. By this we understand that T cells also recognize parts of foreign organisms that are hiding inside cells, making it more likely for the body to detect a threat. The type II MHC molecules on the other hand, are mainly expressed on APCs and partner with the TCRs of the CD4+ T cells [23]. They present extracellular proteins, for instance derived from bacteria, captured through endocytosis. After ingestion they are degraded in the lysosomes of the APCs, compartments specialized in breaking down organic matter. The resulting peptide fragments, called epitopes, are then presented on the surface of the APCs.

After a successful link of a TCR and the corresponding peptide loaded MHC molecule, the T cell is activated through a series of downstream intracellular effects [22]. However, for this to happen several other factors play a part. The TCR is a member of a molecular complex, together with three other dimeric signaling molecules. This is due to the cytoplasmic tail of the TCR being very short, the part inside of the T cell, thus

making it unlikely to participate in any signaling events [22]. Therefore other molecules are needed for successful signal propagation. The TCR complex signals together with the TCR co-receptors, either CD4 or CD8, and co-stimulatory molecules, like CD3, CD247 and CD28. While many TCRs recognize the same antigen, and many antigens are recognized by the same TCR, they differ in binding strength, which in turn affects the stability of the connection. Signal propagation in T cell activation is heavily influenced by the longevity of the binding, meaning a low affinity results in weaker activation stimuli, since it disrupts more easily.

Together the T cell and its TCR, co-receptors and co-stimulatory molecules, paired with the APC and its counterpart, the MHC molecule, add up to a structure called the immunological synapse (IS). It lies at the core of the the immune response, and will be discussed in more detail below.

The Immunological Synapse

A synapse is usually thought of as a junction between two nerve cells, more specifically as a small gap where impulses pass by diffusion of neurotransmitters. The word itself is derived from a Greek word comprised of «together» and «to join» (Oxford English Dictionary), first used in immunology in 1984 to describe T cell and APC cross-work. It was then known that upon interaction, receptors and adhesion molecules accumulated at the interface between the two [24]. This was shown in studies using soluble antibodies in order to cross-link TCRs and various receptors on the recipient cell, resulting in the observation of a phenomenon called capping [25]. Here cell-surface receptors and intracellular structures travel to one side of the cell, creating an head-and-tail like state. Further, studies using immunofluorescence on fixed cell conjugates added to the

foundation of the observations [26]. Antibodies combined with fluorescent molecules revealed a marked polarization of the T cell towards the APC. A cytoskeletal rearrangement was shown to occur, where the microtubule organizing center (MTOC) moved to an area beneath the immunological synapse, seemingly required for sustained signaling. The MTOC is a structure present in all eukaryotic cells with microtubules, and has two main functions. Firstly, it organizes flagella and cilia, which briefly can be described as cell protrusions, which take part in cell movement or sensing of the extracellular environment. Secondly, it is the main player in arranging the mitotic and meiotic spindle apparatus, which separates the cell's chromosomes during cell division [27]. In conjunction, a picture emerged detailing a complex system related to T cell activation, with seemingly a multitude of extra- and intracellular changes taking place. However, its constituents were yet poorly described.

To further elucidate on the structural arrangements of the IS, the cells where visualized in three dimensions, using a technique called «optical sectioning». Through this it was shown that the known main players, such as the TCR and an adhesion molecule called leukocyte function associated antigen 1 (LFA-1), where capped at the interface, but also organized in discrete sectors [28]. The name supra-molecular activation complexes (SMACs) was coined. There are three different SMACs, positioned laterally, starting with the central region (cSMAC). This area is enriched with TCRs/CD3, CD28, CD80/CD86, CD152, and a downstream signaling effector called protein kinase C-θ (PKC-θ). Surrounding it is the peripheral SMAC (pSMAC), with a high concentration of the previously mentioned integrin LFA-1, CD2, as well as a cytoskeletal linker called talin. In the outermost distal part, named dSMAC, larger and more sizable molecules linger,

such as the signaling molecules CD43 and CD 45. Together, a picture of a bulls-eye model was painted, as illustrated in figure 3.

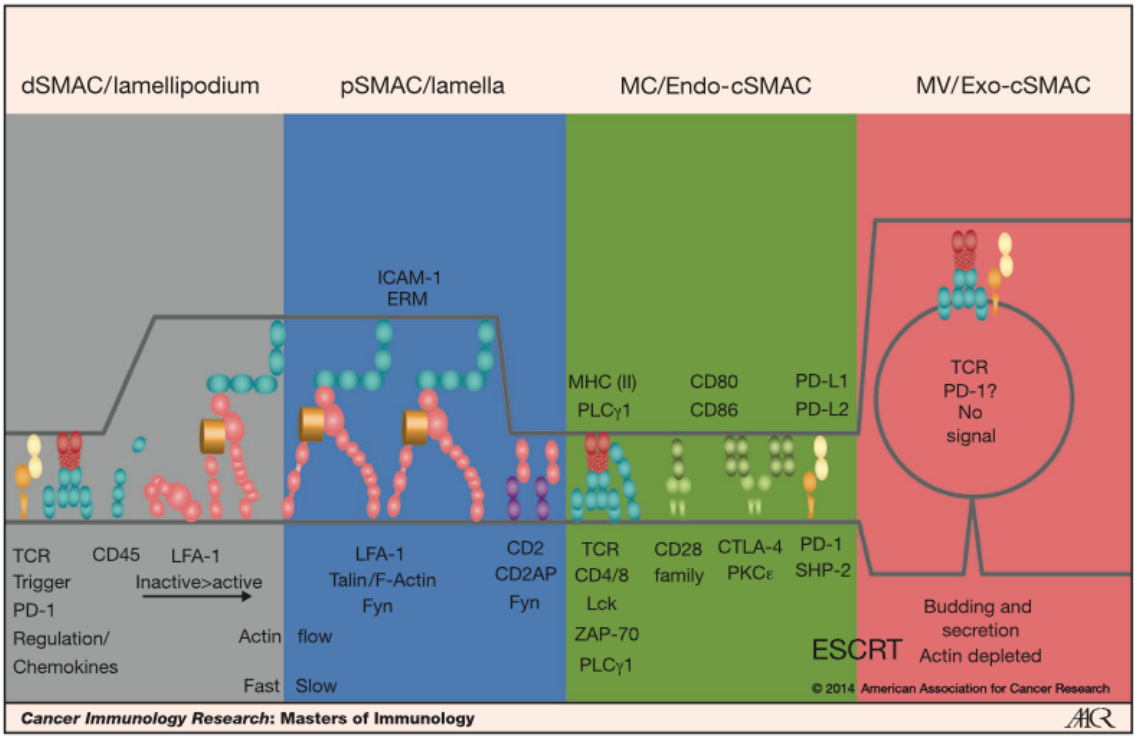

Figure 3. The immunological synapse and its constituents, moving medial from the left. [29].

The Discovery of T Cell Receptor Microclusters

The question was then how such a highly organized structure could trigger and form. In a ground-breaking paper, Dustin and colleagues reacted T helper cells with artificial model membranes, primed with fluorescent molecules, among them MHC [30]. They then studied the resulting interactions using confocal microscopy. By utilizing additional molecular tags targeting important cell-surface molecules, such as intercellular adhesion molecule 1 (ICAM-1) - the ligand of T cell integrin LFA-1 - they eyed the synapse formation. This made it possible to follow a single T cell, and later manipulating the various stages of cell activation. Earlier efforts in live-cell imaging

had been focused on morphological changes occurring after non-specific activation, using calcium-imaging, resulting in less refined data [31].

A T cell going through a successful ligand recognition with an APC stops migrating. As previously mentioned, this causes a reorientation of the MTOC and various cytoskeletal transformations, partly driven by accumulation of the scaffolding protein ADAP, which in turn recruits dynein, a microtubule motor protein [32]. Together with the recruitment of several receptors and signaling molecules, the IS is eventually formed, as it is required for stable cell contact. A proper IS forms in about 10 minutes, but surprisingly, intracellular effects such as calcium mobilization and protein phosphorylation, previously identified in T cell activation, is measurable within 1 minute [30]. In other words, the activation signal is initiated prior to the IS, raising the question what the proper signal transducing structure really is. A second framework, known as the TCR microcluster, was then later discovered [33]. It shows up in-between macroscopic cell contact and IS formation. It is generated in the periphery of the interface between the T cell and APC, and contains the TCR, kinases such as Lck and ZAP-70, adaptor proteins such as Lat and SLP76, and the effector molecules PLC γ and PI3K - all important molecules in T cell activation. By inducing tyrosine phosphorylation of several proteins, the TCR microcluster works as a signalosome, occurring in parallel to intracellular calcium flux. Further, the microclusters travel towards the cSMAC from the outer edge of the IS, leading to the current view that the cSMAC is actually the place where the TCRs are internalized and degraded, not the site for signal transduction. However, costimulation receptors like CD28 and cytotoxic T-lymphocyte antigen 4 (CTLA-4) appears to accumulate in the cSMAC, pointing towards a signaling function for costimulation [34]. Moreover, polarizing the T cell with one area reserved for signaling,

makes it possible to directionally release certain cytokines, like interleukin 2 and 4, towards the interface. Others are usually secreted randomly in to the surroundings, such as tumor necrosis alpha (TNF-α), targeting other cells through paracrine signaling [35]. The TCR microcluster is further described in figure 4.

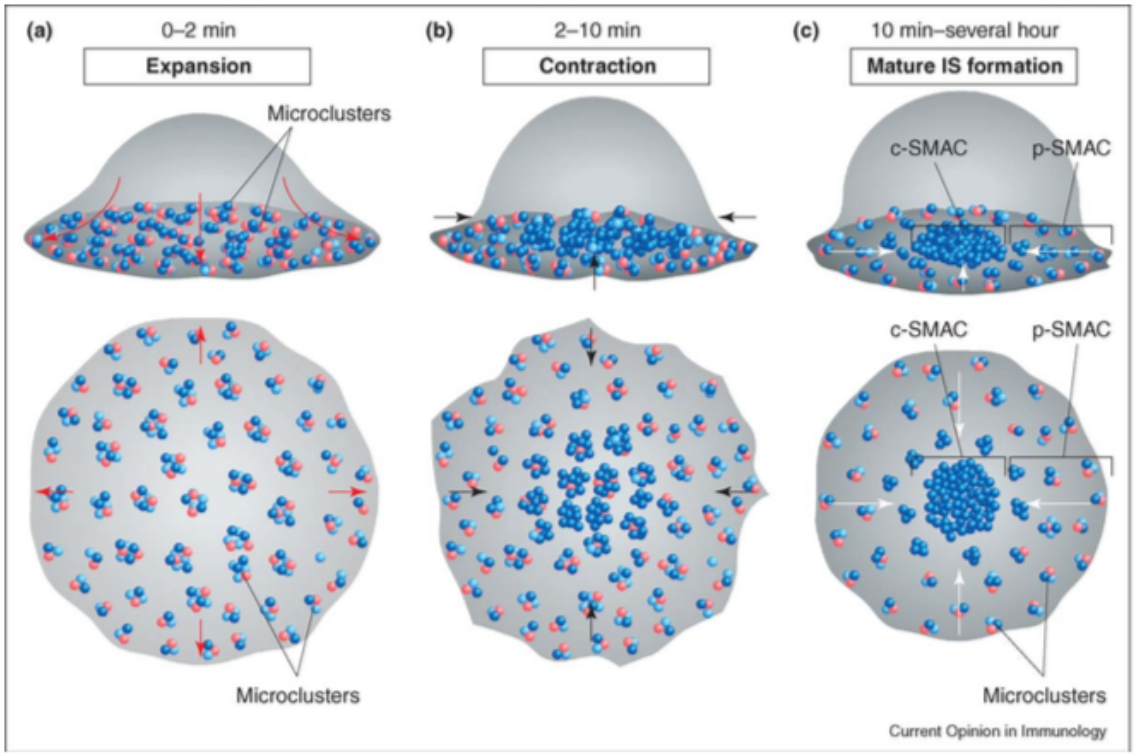

Figure 4. The TCR microcluster and its positioning through IS formation [36].

Lipid Rafts and TCR Nanoclusters

TCR microclusters are lipid rafts. These are cholesterol and sphingolipid rich structures floating in the cell membrane, with the ability to compartmentalize cellular processes, as defined at the Keystone Symposium of Lipid Rafts and Cell Function in 2006. In our case they are loaded with molecules for signal transduction, e.g. the TCR-CD3. Originally it was thought that membrane proteins were randomly distributed on the cell surface, however this changed in 1982, when Klausner & Karnovsky described the concept of lipid domains in membranes [37]. The term lipid rafts was first coined in a

publication from 1996 by Simons & Ikonen, when they proposed a model for the membrane structure. In the paper they describe the organization, and how proteins can be selectively included or excluded from the microdomains, on the cell surface [38]. Nevertheless, the TCR microclusters does not explain how the T cell is initially activated, since they form after the first cell contact with the APC.

In an effort to explain this conundrum, TCR nanoclusters were discovered [39]. Evidence is mounting that there are pre-assembled clusters present in the plasma membrane, too small to be seen with confocal microscopy, before the TCR microcluster is formed. Using electron microscopes after immunogold-labeling T cells with appropriate markers, arrays of gold particles were visualized on the cell surface [40]. This could mirror another previously discovered and more general process, called protein islands. Here evidence show non-random concentrations of various membrane receptors in specific areas of the plasma membrane of a cell [41]. The cell surface is seemingly divided into compartments containing membrane-associated proteins surrounded by protein-free regions. These islands are divided into subregions, due to the localization of lipid raft and non-raft markers to specific areas. Later it was shown using T cells, that both the TCR and the linker for activation of T cells (Lat) - a key adaptor molecule in the TCR signaling pathway - exists in separate membrane domains in quiescent T cells. These only connect after T cell activation, due to an TCR-MHC interaction and subsequent signaling cascade through the CD3 subunit [42]. The conjoined domains formed are identical to the previously mentioned TCR microclusters. This process is outlined in figure 5.

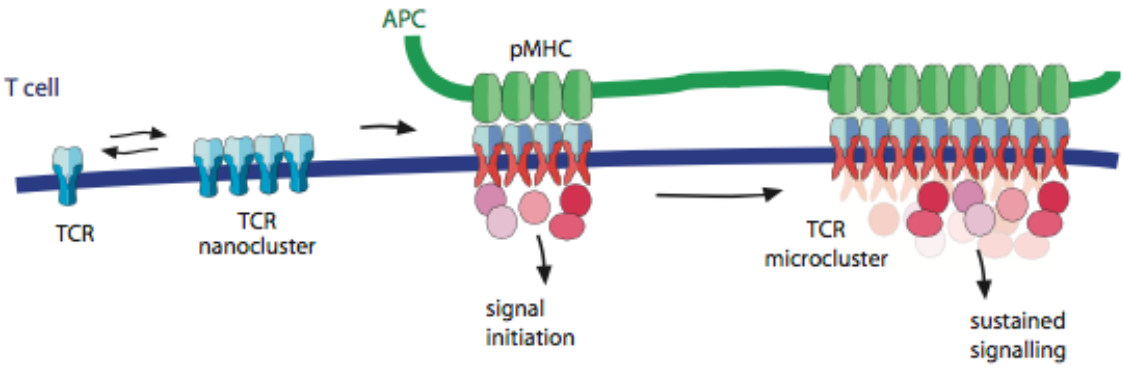

Figure 5. TCR nanocluster and microcluster formation [39].

The question why TCRs clump together is then raised. A monomeric TCR possess low avidity, which is the overall strength of the binding with the MHC molecule. As mentioned before, one TCR-MHC-ligand has low affinity, so in order to increase the avidity a good strategy would seemingly be to gather. Additionally, when in a nanocluster, all TCRs change their confirmation and become competent to signal, even if not all are bound to a peptide loaded MHC [43]. This co-operativity may even be one of the reasons for a T cells high sensitivity to antigen. This process, called antigen discrimination, can explain the paradox that T cells are capable of being activated by a few antigen loaded MHCs, even though the presenting cells are are covered in other MHCs loaded with self-peptides, outnumbering the T cells ligand [44]. The presented self-peptides are of lower affinity, but not to the extent that it explains how the T cell obtains its high specificity [45].

The T cell is activated through the TCR and its various co-receptors [46]. As explained, CD4 or CD8 binds to the MHC molecule. This activates the kinase Lck, which in turn phosphorylates the intracellular parts of the CD3 complex. These phosphorylated structures are called ITAMs, short for immunoreceptor tyrosine-based activation motifs. This creates a docking site for the molecule ZAP-70, in which the aptly named CD3-

zetas doubly phosphorylated ITAMs engage the SH2-domains of said ZAP-70 molecule. This furthers the phosphorylation of the transmembrane protein Lat, which serves as the docking site for various other signaling molecules. Of these SLP-76 is among the more important signaling molecules for promoting T cell growth and activation. In the end T cell stimulation leads to a multitude of gene products needed for further cell function.

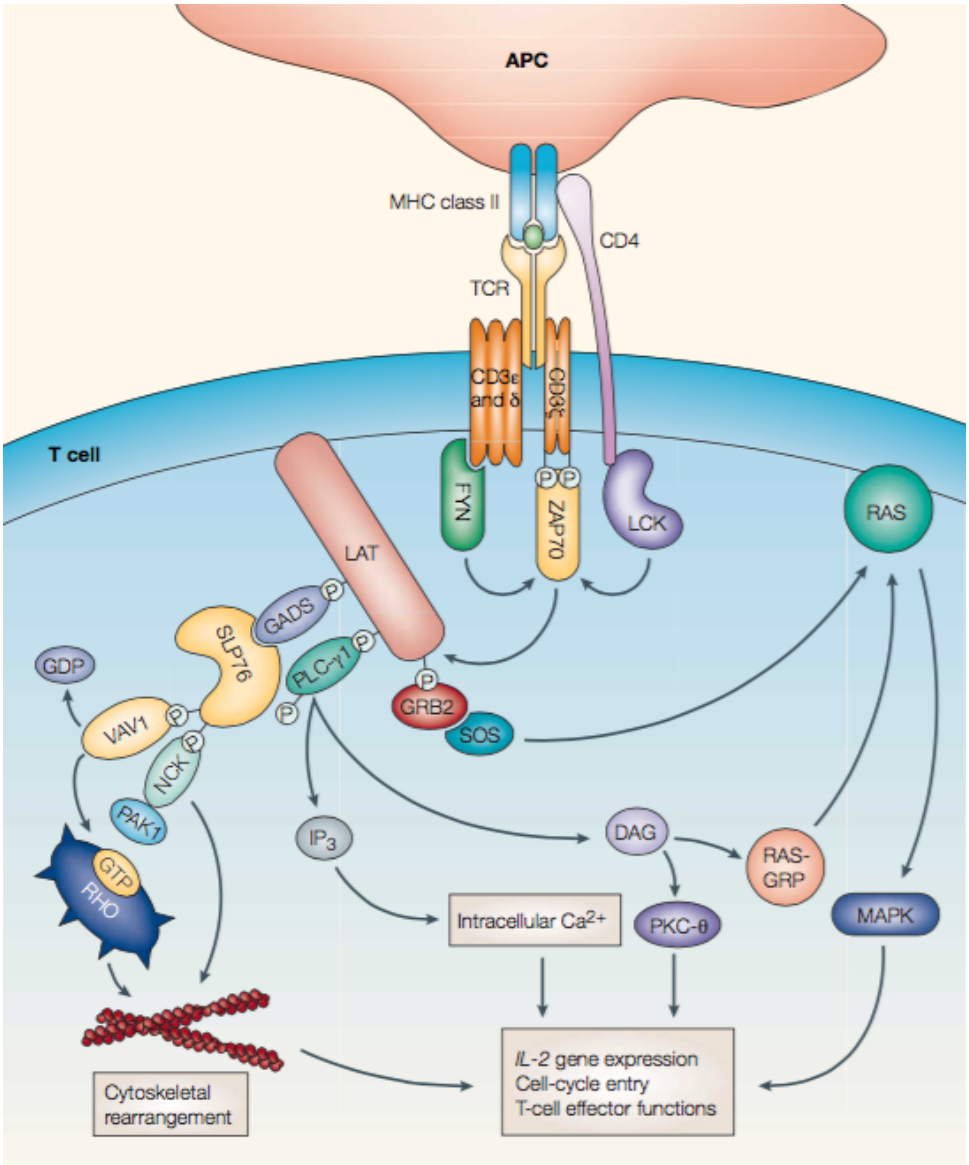

Figure 6. Simplified overview of the intracellular pathways in T cell activation [47].

Morphological Changes Upon Antigenic Challenge

T cells display distinct cytoarchitectural features in order to perform specialized functions, such as polarity, motility, cell–cell contact, exocytosis and proliferation [48]. The TCR clusters and IS described earlier appear linked to the cytoskeleton, due to heavy actin staining properties. Inhibitor studies further show that TCR clusters at least partially depend on the actin cytoskeleton for their formation or maintenance or both [49]. The actin cytoskeleton is the basis for T cell shape, which varies according to its surroundings. The cells travel through various compartments in the body, circulating in the blood and migrating through tissues. Each of these requires special configurations. For instance, in the blood T cells are circular, covered in microvilli. These are small fingerlike protrusions, containing parallel bundles of highly dynamic actin filaments. The tips are covered in low-affinity adhesion molecules, while the high-affinity molecules, such as the aforementioned LFA-1, are distributed elsewhere [50]. This compartmentalization is thought to augment rolling along vessel walls and reduce the amount of nonspecific adhesion. The ultimate goal is to undergo diapedesis, the process in which blood cells squeeze through the vascular endothelium, migrating in to the surrounding tissues [51]. At this point the T cell then exhibit a hand mirror morphology, where a large cell body - together with a leading edge - is trailed by an uropod [52]. The uropod is found in various cell types, yet in leukocytes it contributes to cell motility and chemotaxis towards inflamed tissue, establishing intercellular adhesion, cell-cell communciation and sustaining vesicular trafficking [52]. In the other end, the leading edge of a motile T cell is rich in chemokine receptors [53]. Further, they are a threefold more sensitive to APC contact made at the leading edge than with contact made at the tail [54]. Subsequently, when contact occurs, the T cell again rounds up, withdraws its uropod and extend pseudopodia and lamellipodia toward the APC. At the same time the

previous described MTOC reorientation transpire, with the needed secretary organelles now lying beneath the APC contact site. In regards to the IS, the engagement of the TCR leads to the activation of actin regulatory proteins driving actin polymerization, creating a dense, branched actin network [55]. This facilitates the organization of the various signaling molecules of the TCR microclusters, and the movement of signaling molecules to the cSMAC, where they are internalized and subsequently degraded, terminating signaling.

In the opposite end to the IS, a novel membrane domain have been described, namely the distal pole complex (DPC) [56]. Its function is not fully understood, but its thought to sequester negative regulators of T cell activation and organize overall T cell polarity. The formation of similar structures has been studied in several other cell types, such as apical and basolateral domains in epithelial cells and the polarization of fibroblasts towards the center of a wound. Thus it was not all that surprising that such a bipolar molecular modeling was found in T cells. As with the IS, its constituents seems randomly distributed on the cell surface when in a resting state. Subsequently they are pulled distally, upon T cell activation through the IS, facilitated by a currently unknown motor protein. The DPC appears linked to the aforementioned uropod, and it is speculated that it might be its precursor, as T cells stimulated with APC in culture develops an uropod much later than the 5-10 minutes it takes to organize the DPC.

Discussion

In this text the possibility of a predefined site on a resting T cell for the immunological synapse has been explored. As pictured, the life of a T cell is complex. Starting in the bone marrow as the progeny of the multipotent hematopoietic progenitor stem cell, it develops in to one of the main players in the human immune system. By reaching its effector state it has survived the strict selection process in the thymus, navigated through the blood stream, extravasated into the periphery, in order to finally find its match in an APC, typically in a lymph node. Here the APCs presents a multitude of different peptides on MHC molecules, broken down from larger proteins. Still, through a highly selective process, the T cell can recognize a single peptide. The term finding the needle in a hay stack appears appropriate. Nevertheless, locating the right match is not enough for cell activation. The surrounding milieu of cytokines needs to be finely tuned, stemming from APCs mitigating the correct «danger signals», in response to cells undergoing various forms of stress [57]. Without it the T cell might even die by apoptosis, or become tolerized, a process which effectively silence the cell, deemed important in the defense against autoimmune disease [58].

At the center of this cell-cell interaction lies the immunological synapse. The adhesion facilitated mediates and regulates the activation of T cells. Similar processes also appear in other lymphocytes, such as NK cells preparing lytic activity and during antigen acquisition of B cells [59, 60]. Also known as the stable supramolecular activation structure (SMAC), the synapse is best described using a bulls-eye model. The center is coalesced of TCRs and various co-receptors, with a periphery specialized in cell adhesion. Through improved microscopy techniques, highly complex molecular dynamics were discovered. This has lead to a model where the IS is a thought of as a

three-dimentional structure with signaling networks for lymphocyte activation, where endosomal and cytoskeletal machinery are polarized [61]. Combined, the structure establishes checkpoints for lymphocyte activation, enhance, balance and terminates signaling and effector functions, and direct secretion of various molecules, such as cytokines and lytic granules [62]. TCR micro- and nanoclusters are lipid raft like structures, pre-existing in the plasma membrane in advance of activation, containing molecules necessary for signal transduction [63]. They function as early signaling clusters, and participating vesicles are ferried on sub-synaptic vesicles to the cell membrane by cytoskeletal fibers and motor proteins. However, much is still unknown about the endosomal compartment, the cytoskeleton and its connection to lymphocyte regulation.

Since the discovery of a stationary SMAC in the interface between the T cell and an APC, evidence today points towards a dynamic process. Due to more refined imaging techniques, TCR nanoclusters have been discovered, previously too small to be seen with traditional microscopy [64]. They appear randomly distributed in the plasma membrane, and after the first signaling event is initiated upon contact with a peptide loaded MHC, they fuse in to TCR microclusters. Simultaneously the IS is formed. It is likely to think this can occur anywhere on the cell surface. Even so, there are signs of membrane regions surrounded by barriers, formed by proteins assosciated with the membrane cytoskeleton [65]. These could potentially locate TCRs to specific regions, and there is evidence supporting the idea that the TCR can be preclustered on the cell surface [33]. Further, it has been shown that the leading edge of a motile T cell is the most sensitive part of a polarized T cell in regards to triggering [54]. Thus it is possible that the immunological synapse is most likely to form originating from the leading

edge. However, it is still not possible to draw any definite conclusions based on the current reviewed literature. In part because the question appears not to have been directly addressed.

It is conceivable that the bulk of TCRs are confined to the leading edge of a motile T cell. More specifically in the lamellipodia protruding from an actively seeking cell. This instead of being randomly distributed in even amounts covering the plasma membrane. In concordance to the head-and-tail like state previously described, trafficking the TCRs to the anterior part seems beneficial. Under physiological conditions in vivo, T cells engage APCs in a crowded space. Consequently a large percentage of the surface area will not be in close proximity to peptide-loaded MHCs. Therefore it seems reasonable to postulate that the TCR nanoclusters are in higher density at the leading edge, increasing the odds of a successful link to the appropriate TCR target. Further, T cells actively scan several APCs while in a lymph node, directed by both random and guided migration. The transient contact upon doing so has been termed a kinapse, where the stability and strength of the connection determines whether or not it will transform into a fully fledged immunological synapse [66]. Additionally, it appears that the T cells can accumulate signaling events from a serial of encounters [67]. Thus, when encountering low affinity antigens weaker kinases form, and the cell moves on. However, if the signal is of moderate affinity, the density of relevant APCs presenting the antigen would be of importance, owing to the fact that a higher number could result in T cell activation. In light of the aforementioned circumstances, an apt expectation would be that a T cell attempts to increase the chance of TCR-MHC ligand interactions, by confining the first contact bearing structure - the TCR nanocluster - to a pre-determined site.

Why then is it of import to know whether or not the IS is located randomly, or is restricted to a specific area? Firstly, it can be argued any unknown in science is worth exploring. Secondly, any new information about the cellular workings of our innards are potentially targets for new therapies. The manipulation of the immune system is still in its infancy, and the more we know about the processes involved, the closer we are to treating immune-mediated diseases. In light of this, focusing on the area in which initiates the T cell driven immune response appears valid. The potential is certainly there [68, 69].

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