Supplement Review

The immunological synapse

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Chapter summary

T-cell activation requires interaction of T-cell antigen receptors with proteins of the major histocompatibility complex (antigen). This interaction takes place in a specialized cell–cell junction referred to as an immunological synapse. The immunological synapse contains at least two functional domains: a central cluster of engaged antigen receptors and a surrounding ring of adhesion molecules. The segregation of the T-cell antigen receptor (TCR) and adhesion molecules is based on size, with the TCR interaction spanning 15 nm and the lymphocyte-function-associated antigen-1 (LFA-1) interaction spanning 30–40 nm between the two cells. Therefore, the synapse is not an empty gap, but a space populated by both adhesion and signaling molecules. This chapter considers four aspects of the immunological synapse: the role of migration and stop signals, the role of the cytoskeleton, the role of self-antigenic complexes, and the role of second signals.

Keywords: activation, adhesion, immunological synapse, inhibition, signaling

Introduction

The immunological synapse (IS) is a specialized cell–cell junction between a thymus-derived lymphocyte (T cell) and an antigen-presenting cell (APC) [1,2]. Initiation of an antigen-specific immune response is based on the interaction between T-cell receptors (TCRs) and major histocompatibility complex proteins that have bound antigenic peptides (MHCps) [3,4]. Because the TCRs and MHCps are attached to the surface of the T cell and the APC, respectively, the initiation of an immune response requires a molecular grasp between the T cell and the APC – a synapse. A current focus of research on the IS is to determine how this supramolecular structure contributes to T-cell sensitivity and to the fidelity of the T-cell response. Four areas in which the concept of the IS is contributing to our understanding of T-cell activation are the coordination of antigen recognition and T-cell migration; the role of the cytoskeleton in T-cell activation; the mechanism of sensitive antigen recognition by T cells; and the integration of the adaptive and innate immune responses.

Historical background

The formation of the IS has been followed over time in live T cells interacting with planar bilayers [2] and studied at specific time points in fixed cell–cell conjugates [5]. The T cell forms an adhesion zone with the antigen-presenting bilayer; this zone is then surrounded by areas of close contact where TCR can reach the MHCp. If the TCR engagement exceeds a threshold rate and level, the T cell stops migrating and forms a ring of engaged TCRs at the periphery of the nascent IS (Fig. 1a). This pattern takes ~30 seconds to form and corresponds to the peak of TCR-associated tyrosine phosphorylation and Ca^{2+} mobilization. Within a few more seconds, the sites of TCR

A glossary of specialist terms used in this chapter appears at the end of the text section.

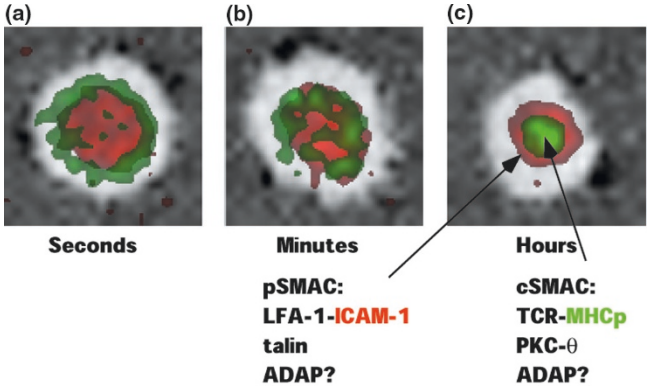
engagement move from the periphery of the contact area to the center of the contact area to form the mature IS (Fig. 1b). During this time, the disk-like region of LFA-1–ICAM-1 (intercellular adhesion molecule-1) interaction appears to give way to the centrally moving TCR, but the LFA-1–ICAM-1 interactions maintain the contact area and evolve into a ring of ~5 μm outer diameter (Fig. 1c). It is not clear if the same TCRs move from the outside to the center or if new TCRs are continually recruited. The interaction of the TCRs with agonist MHCp complexes has a short half-life (~5 seconds) [6], and it is known that TCRs are degraded after effective engagement [7]. However, at some point in IS formation, the interaction of the TCRs and the major histocompatibility complex protein (MHC)–peptide complexes change so that they no longer dissociate. Thus, while serial engagement may dominate in the nascent IS, parallel engagement of at least 50 TCRs is characteristic of the center of the mature IS. These observations have emphasized the concept that biochemical reactions are highly compartmentalized in the IS, in such a way that the location of receptor and signaling molecules must be considered if we are to to understand the biochemical basis of T-cell activation [8].

Migration and the immunological synapse

T-cell activation requires a sustained signal. The duration of signaling required to initiate proliferation of T cells is at least 2 hours [9–11] but may be much longer to achieve appropriate differentiation of helper T cells [12]. T cells migrate continually between the blood and the secondary lymphoid tissues where they encounter APCs. In the absence of an immune response, the T cell completes this cycle about twice a day [13]. During the initiation of an immune response, the T cells are held in the antigen-exposed lymph nodes or the spleen for 2–3 days and then effector cells are released after the third day [14]. *In vitro* T-cell recognition of agonist MHCp in the context of the adhesion molecule ICAM-1 delivers a stop signal to migrating T cells [15]. This stop signal is the first stage in the formation of an IS [2].

The mechanism of the stop signal is not known, but it appears to involve the polarization of the T-cell toward the source of antigen, as indicated by the position of the microtubule organizing center (MTOC) and the associated Golgi apparatus [16]. The environment of the T-cell–APC interaction regulates the stop signal. One example of this is that APCs with agonist MHCp do not stop T cells in three-dimensional collagen gels *in vitro* [17]. The mechanism of this effect is not known, but it may involve chemokine gradients [18] or interactions with extracellular matrix that prevent T-cell polarization toward the APCs. In lymph nodes, however, T cells are not exposed to collagen fibers, which are sequestered in reticular fibers [19]. Reticular fibers may provide a weakly adhesive reticular scaffold decorated with APCs that define corridors through which the T cells migrate [19]. Based on the lymph node environment and *in vitro* data, it is most likely that the IS coordinates T-cell migration and the antigen recognition process to allow full activation of T cells by small numbers of APCs that express the appropriate MHCp. When there are only a few APCs with agonist MHCp, it seems more efficient to have the T cell stop upon interaction with agonist MHCp-bearing APCs rather than having the T cells interact transiently with both agonist-MHCp-bearing and -deficient APCs equally, because the former procedure maximizes the interaction with the agonist MHCp and favors activation early in an immune response. This view is supported by *in vivo* data demonstrating clustering of polarized T cells around dendritic cells [20,21].

Figure 1



The development of the immunological synapse. Images adapted from [2] based on fluorescence microscope images of T-cell interaction with agonist MHC–peptide complexes (green) and ICAM-1 (red) in a supported planar bilayer with a T cell. The accumulation of fluorescence represents interactions in different time frames. **(a)** Within seconds, the T cell attaches to the substrate using LFA-1/ICAM-1 interactions in the center based on TCR signaling triggered at the periphery of the contact area. **(b)** Over a period of minutes, the engaged TCRs are translocated to the center of the contact area. **(c)** The final pattern, with a central cluster of engaged TCRs surrounded by a ring of engaged LFA-1, is stable for hours. Molecular markers for the cSMAC and pSMAC are indicated. For scale, the pSMAC is ~5 μm across. ADAP, adhesion and degranulation adapter protein; cSMAC, central supramolecular activation cluster; ICAM, intercellular adhesion molecule; LFA, lymphocyte-function-associated antigen; MHCp, major histocompatibility complex protein complexed to a foreign or self-peptide; PKC-θ, a protein kinase C isoform that is activated by DAG but not Ca^{2+}; pSMAC, peripheral supramolecular activation cluster – the ring of LFA-1 and talin on the T cell and ICAM-1 on the antigen-presenting cell in the mature immunological synapse; TCR, T-cell antigen receptor.

The cytoskeleton and the immunological synapse

Our expectations about molecular interaction in the IS have been shaped by early molecular definition of the molecules involved in this process [22]. The complex of the LFA-1 with ICAM-1 (~48 nm) is more than three times as

large as the complex of the TCRs with MHCp (~15 nm) [23–25]. Therefore, the LFA-1/ICAM-1 and TCR/MHC interactions segregate into different compartments within the contact area [26]. This receptor segregation forms receptor aggregates whose size and organization are determined by the rigidity of the membrane, the kinetics of the interactions, and the degree of differences in molecular size of the participating receptor–ligand pairs [27]. This immediate segregation may be the initial trigger of receptor clustering and signaling in the nascent IS [28]. These events take happen in seconds and set the stage for the formation of the mature synapse.

The formation of the synapse is highly active and depends on an intact actin cytoskeleton. The formation of the central cluster of TCR has a superficial similarity to antibody-mediated capping, in that it requires an intact actinomyosin cytoskeleton. A plausible model based on this similarity has been proposed and initial results support some aspects of the model [29]. However, the IS has many elements that are completely absent in capping of cross-linked antigen receptors. For example, most capping is based on a network of interactions on a membrane surface that lead to cross-linking, whereas receptor aggregation is a cell–cell contact is more likely to result from membrane fluctuations, receptor–ligand size differences, and interaction kinetics. These components have been incorporated in a physical model by Chakraborty and colleagues [27]. The predictions of this model are remarkably concordant with the observations on the formation of the IS. This more physical view is compatible with an active role for the cortical cytoskeleton, because signaling-induced changes in cytoskeletal dynamics in activated T cells will profoundly regulate the Brownian bending movements of the membrane that are required for movement of the receptor interactions. This model could be described as a physical and mathematical elaboration on the kinetic-segregation model [28]. Thus, the early signals from the TCRs that trigger increased actin polymerization may induce the membrane fluctuations that drive the maturation of the IS. Both the capping and the kinetic-segregation models predict that cytoskeletal dynamics are critical for IS formation.

Signaling pathways activated during synapse formation

The TCR activates three major transcription-factor families. In addition to regulation of gene expression, intermediates and side branches of these signaling pathways also appear to be partly responsible for formation of the immuological synapse. The importance of this concept is that these signaling pathways play an important role in creating the physical environment for sustained signaling.

The initial events at the engaged TCR activate a tyrosine kinase cascade that requires the participation of three families of tyrosine kinases: the src family, the syk family, and the tec family. The src family kinase $p56^{lck}$ and the syk family kinase ZAP-70 are sufficient for phosphorylation of the immunotyrosine activation motifs (ITAMs) in the cytoplasmic domains of the TCR complex [30]. These phosporylation events then enable tyrosine phosphorylation of the transmembrane adapter protein called linker of activated T cells (LAT), and phosphoLAT recruits enzymes including phospholipase Cγ (PLCγ), the Grb2/SOS complex, phosphatidylinositol-3-kinase and the GADS/SLP-76/NCK/VAV complex [31], which links LAT to activation of the small G protein Rac and recruitment of PKC-θ. The recruitment of PLCγ leads to cleavage of phospatidylinositol-4,5-bisphosphate (PI-4,5-P_2) to diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3). IP_3 activates Ca^{2+} channels in the endoplasmic reticulum to release Ca^{2+} into the cytoplasm, and depletion of this store opens plasma-membrane capacitative Ca^{2+} channels that enable sustained cytoplasmic Ca^{2+} elevation [32]. Full activation of PLCγ for sustained Ca^{2+} signals requires activation of members of the Tec family protein kinase, Itk, which is recruited to the membrane PI-3,4,5-P_3, a product of phosphatidylinositol-3-kinase activation. Sustained Ca^{2+} elevation activates calcineurin, leading to dephosphorylation and nuclear translocation of NFAT (nuclear factor of activated T cells) [33]. The activation of RAS by Grb2/SOS and also by Ras-GRP leads to activation of AP-1 (activation protein 1). Activation of AP-1 is also promoted by the Jun kinase, which is regulated by p21rac downstream of VAV and other guanine nucleotide exchange factors. The third major family of transcription factors activated by the TCR is the PKCθ/IκB/inhibitor of κB kinase pathway leading to phosphorylation and degradation of IκB, the regulatory subunit of the nuclear factor-κB (NF-κB) transcription factor [34]. All three of these pathways also feed back on the synapse through effects on the cytoskeleton and regulation of adhesion. For example, the adhesion and degranulation protein (ADAP) identifies a signaling pathway linking the TCR to LFA-1 regulation [35,36].

The mechanisms that turn off signaling in the synapse are poorly understood. The broad regulatory mechanisms that have been identified are phosphatases and ubiquitin-pathway-mediated degradation [37–39]. It is apparent that activation of tyrosine kinase signaling pathways lead to recruitment of tyrosine phosphatases that may be involved in turning off signals. The most notable is SHP-1, an SH2 domain containing tyrosine phosphatase-1 that is deficient in motheaten mice. The SH2 domains of SHP-1 interact with immunotyrosine-based inhibitory motifs in the cytoplasmic domains of inhibitory receptors such as the killer inhibitory receptors and receptors expressed in T cells such as LAIR [40,41]. When an immunotyrosine-based inhibitory motif is phosphorylated by an active Src family kinase it recruits SHP-1, which can extinguish downstream signaling by ITAM-containing receptors [42]. It has also

been shown that SHP-1 can be recruited to partially phosphorylated ITAMs and therefore may play an important role in negative signaling by antagonist MHCp [39].

The second process that appears to downregulate signaling is TCR degradation through a ubiquitin-mediated pathway [43]. Ubiquitin is added to substrates by an enzyme (E1) that is linked to the substrate by an adapter (E3). The adapter for addition of ubiquitin to the TCR complex is not known for certain, but may include members of the cbl family of ring finger domain containing E3s [44]. Cbl-b has also been implicated in regulating phosphatidylinositol-3-kinase (PI-3-K) [45]. TCR degradation takes place both in lysosomes and via the proteosome. How the TCR is delivered to the proteosome is not clear, but some TCR is internalized via clathrin-coated pits. The relatively fast off-rate for TCR-MHCp interaction suggests that it should be easy for TCR to dissociate from MHCp prior to internalization. However, studies in planar bilayers suggest that the kinetics of the TCR–MHCp interaction is substantially slower in the central cluster of the IS than in solution. Photobleaching experiments suggest, in fact, that the interactions become irreversible [2]. These irreversible complexes may require a more radical strategy for their destruction that would involve removal of an entire large membrane fragment from either the T cell or APC to accommodate internalization by one cell or the other. Consistent with this, fragments containing MHCp, CD80, and ICAM-1 are transferred from the APC to the T cell [46,47]. This process results in significant loss of molecules from the APC but does not reduce the cell's viability. It is not clear if the internalized TCR continues to signal before it is degraded or if all signaling takes place at the cell surface. When TCR internalization is suppressed by presenting MHCp on supported planar bilayers, from which the TCR cannot pull the MHC molecules, it is clear that endosomal structures collect near the central cluster [48]. These endosomes contain CD45 and the glycolipid GM1, a marker for glycolipid- and cholesterol-enriched membrane domains known as rafts. T cell–APC contacts lack these synaptic endosomes, perhaps because TCR can extract MHCp from the APC to achieve internalization of 'locked in' TCR-MHCp interactions. Once the TCR–MHCp interactions are internalized, they may meet CD45- and GM1-containing endosomes at other sites inside the cell. The purpose of these endomembrane structures for T-cell activation is not known. TCR downregulation clearly resets the threshold of the T cell for subsequent interactions and thus TCR downregulation can certainly be seen as a desensitizing mechanism [49].

The role of self MHCp in T-cell sensitivity to foreign MHCp

Any single TCR interacts with a degenerate spectrum of MHCp: null MHCps alone do not activate T cells, and agonist MHCp, the model foreign MHCp, induces full T-cell activation. Weak agonists induce a subset of T-cell responses, and antagonists interfere with T-cell responses to agonists. Approximately half of the TCR–MHCp binding energy comes from the TCR contacts with the MHC molecule [50]. Thus, the remaining peptides can be further divided. Some null peptides actively interfere with the TCR interactions and thus allow no interaction of the TCR with MHC, while other null peptides are neutral and allow the TCR to interact with MHC. The kinetics of this latter group of null MHCp are too fast to induce a response in mature T cells [51]. Self-peptides that form MHCp that are agonists, weak agonists or antagonists all induce apoptosis of immature T cells *in vivo* [51]. In contrast, a subset of null MHCps enhances positive selection. Thus, most mature T cells face APCs that are loaded with a mixture of null peptides (self). These mature T cells are triggered by APC bearing a few agonist/weak agonist MHCp mixed with diverse null MHCp. Naïve T cells respond to approximately 300 agonist MHCps on the APC, while memory T cells require only 50 agonist MHCps [52]. A single agonist MHCp is sufficient to trigger cytotoxic T-cell killing [53].

How is the high sensitivity of immune recognition achieved? Can a single agonist MHCp achieve T-cell activation, or do other MHCps promote this process? Wülfing *et al.* tested the hypothesis that some null MHCps contribute to T-cell activation through analysis of proliferation and formation of the IS [54]. They found that an interacting null MHCp contributes to IS formation and T-cell activation triggered by subthreshold amounts of agonist MHCp. It was demonstrated that fluorescently labeled null MHCps were accumulated in the center of the IS and synergized with trace levels of agonist MHCp for T-cell actvation. In contrast, the subset of null MHCp that does not interact with the TCR did not synergize with agonist MHC for T-cell activation and did not cluster in the center of synapses. Thus, agonist MHCps do not have to do it alone: they are substantially helped by this subset of null MHCps. We propose that this functional subset of MHCps be termed co-agonists. These experiments provide proof in principle that very weak TCR–co-agonist MHCp interactions can contribute to T-cell activation by the stronger TCR–agonist MHCp interactions. It will now be important to establish how prominent these synergizing null MHCps are in the self-peptide repertoire. The density of these complexes may vary with the specific APC types and between different TCR and MHC molecules. While contributing to sensitive recognition of MHCp by T cells, it is also possible that these null MHCps may contribute to autoimmune disease.

Integration of adaptive and innate responses

The IS is not limited to adhesion molecules and MHC–peptide complexes. The activation of naïve T cells involves a system of checks and balances that are integrated to make activation decisions. An important aspect of this

integration is that T cells test both the MHC–peptide complex and the status of the innate immune response in the APC. In response to evolutionarily conserved microbial products such as lipopolysaccharide, the APC can be become activated. This increases expression of a number of molecules including the MHCp, adhesion molecules, and ligands for costimulatory receptors. Ligands for co-stimulatory receptors include CD80 and B7-DC (also know at PDL2) [55]. CD28 is the receptor for CD80 and by binding CD80 it indirectly transduces an innate immune system signal that can be integrated with the TCR signal. CD28–CD80 interactions are very inefficient due to the low density of CD28 and its low lateral mobility on naïve T cells [56]. Upon immunological synapse formation, CD28–CD80 interactions are facilitated and focused in the central region of the immunological synapse, very close to the site of TCR engagement. However, CD28–CD80 interaction does not help the TCR–MHCp interaction, which sets it apart from adhesion molecules such as LFA-1 and CD2[56]. This suggests a sequential model for T-cell response to TCR and innate signals. The formation of the IS corresponds to the antigen signal. Once this signal is received, the T-cell becomes competent to receive the signal through CD28. While this is an attractive hypothesis, there are a number of results that still must be reconciled. First, Sprent and colleagues have shown that the CD28–CD80 interaction is very effective at mediating the transfer of APC membrane proteins to the T-cell in a process that appears to require adhesion but does not require antigen [47]. This implies an interaction of CD28 and CD80 in the absence of TCR signals. The basis of this interaction is yet to be determined but might involve preclustering of CD80 on the APC membrane, as recently reported by Mellman and colleagues [57]. Such preclustering might partially overcome the low expression and mobility of CD28 on the T cell. It has also been shown that the MHCp and CD80 do not necessarily have to be presented on the same APC, *in vivo* or *in vitro* [58]. Thus, IS formation with one APC may facilitate the CD28–CD80 interaction in a contact with a second APC. Additional work will be required to understand the full implications of the low mobility of CD28 in diverse interactions *in vivo*. It will be important to determine if other secondary signals are dependent on IS formation.

Future prospects

Studies on the IS are still in their early days. The relation between IS formation and migration must be explored *in vivo*. This is important because it is difficult to simulate the *in vivo* environment *in vitro*. T cells and APCs can be labeled *in vitro* and transferred into recipient mice or endogenously labeled through GFP transgenes or knock-in mutant mice. The likely variables are the local extracellular matrix and chemokine gradients. This may be approached either in organ culture or by true *in vivo* imaging on live animals with natural perfusion of the lymphoid organs. The latter will be most important, since afferent lymph is essential to maintain lymph node architecture and interactions. Blood perfusion is also likely to be critical to maintain the viability of cells deep in the lymph node. The depth of imaging needed can be achieved with two-photon excitation. The relationship between IS formation and the cytoskeleton needs to be experimentally tested using very specific genetic, biochemical, and imaging approaches. The entire question of self-MHCp and the prevalence of co-agonist MHCp in this group now takes on great importance. If the proportion of co-agonist MHCp differs between APCs, then these self-peptides could play an important role in autoimmunity as well as in normal responses to pathogens. Finally, the field of co-stimulation is exploding. There are additional recent members of the CD28/B7 family of receptors and ligands and there are also new molecular families that are implicated in providing co-stimulatory signals. The relation of these signals to the IS will need to be addressed through genetic, biochemical, and imaging experiments in the future.

Concluding remarks

In summary, the IS concept provides a number of insights into the process of T-cell activation. First, it provides a stop signal that coordinates antigen recognition and T-cell migration. Second, the essential role of the actin cytoskeleton in T-cell activation is related to the role of actin in IS formation. Third, the sensitivity of T-cell to agonist MHCp is related to the role of weakly interacting, but probably more abundant, self MHCp in promoting IS formation. Finally, the IS provides a framework for orderly integration of the TCR and innate immune signals such as CD28–CD80 interaction.

Glossary of terms

AP-1 = activation protein 1 – a transcription factor composed of Jun and Fos oncogene products; APC = antigen-presenting cell – generally a cell dedicated to the process of generating MHCps from intact antigens and then interacting with T cells to allow possible TCR-MHCp interactions and T-cell activation; cSMAC = central supramolecular activation cluster – the cluster of molecules including TCR and PKC-θ on the T cell and MHC-peptide complexes on the antigen-presenting cell in the mature immunological synapse; Grb2/SOS = a complex that links to phosphorylated LAT and activates guanine nucleotide exchange and activation of RAS; ICAM-1 = intercellular adhesion molecule-1 – type I transmembrane glycoprotein of the immunoglobulin superfamily that interacts with the integrins LFA-1 and Mac-1; IS = immunological synapse – the junction between a T cell and an APC bearing antigenic MHC–peptide complexes; ITAM = immunotyrosine activation motifs; LFA-1 = lymphocyte-function-associated antigen-1 – a member of the integrin family of adhesion molecules that interacts with ICAMs 1, 2, and 3; MHC = major histocompatibility complex

protein – class I and class II proteins from this gene locus encode type I transmembrane glycoproteins with peptide-binding grooves to hold foreign or self-peptides; MHCp = major histocompatibility complex protein complexed to a foreign or self-peptide; NFAT = nuclear factor of activated T cells; PKC-θ = a protein kinase C isoform that is activated by DAG but not Ca^{2+}; PLCg = phospholipase Cg; pSMAC = peripheral supramolecular activation cluster – the ring of LFA-1 and talin on the T cell and ICAM-1 on the antigen-presenting cell in the mature immunological synapse; RAS = a small G protein that regulates the mitogen-activated protein kinase pathway; Ras-GRP = a protein that mediates an alternative pathway to activation of Ras that is stimulated by DAG; TCR = T-cell antigen receptor – type I transmembrane protein generated by somatic recombination of gene segments to generate millions of possible MHCp-binding receptors; Two-photon microscopy = a technique based on using mode-locked titanium-sapphire lasers to excite fluorescence of visible light fluorophores with two photons of infrared light. The use of infrared light provides excellent penetration. The two-photon excitation is achieved only at the focal point of the laser beam, so all fluorescence can be collected to generate the image – even highly scattered photons. Effective penetration is of the order of 500 mm; VAV = an oncogene product that acts as a guanine nucleotide exchange factor for Rac and may also contribute to recruitment of PKC-θ.

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