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Immunological Synapse Molecules

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Abstract

Immunological Synapse (IS) is a multi-molecular assembly functional structure formed at the interface of T lymphocyte and antigen presenting cell. These molecules include antigen presenting molecules, adhesion molecules, co-stimulatory molecules, and inhibitors or checkpoint molecules, etc. The spatial and temporal changes of these molecules determine the structure type and the function of the IS, which further affect the fate of T cells. To date, some molecules involved in the IS formation have been suggested as the targets of immunotherapy. Here, we reviewed the current investigations in the structure and function of the IS, and the molecules participated in the IS formation.

Keywords: Immunological synapse; Adhesion molecules; Co-stimulatory molecules; Checkpoint molecules

Introduction

T lymphocyte activation plays a vital role in the adaptive immune response, and relies upon molecular signalling and cellular communication initiated by direct cell-cell contact. The signalling and adhesion molecules accumulated at the interface of T lymphocytes and antigen presenting cells (APCs) and formed a multi-molecular assembly platform, called immunological synapse (IS) [1], which is critical in the activation, effective function and development of T lymphocytes. These molecules include signalling molecules, adhesion molecules, and co-stimulatory/checkpoint, etc. The spatial and temporal changes of these molecules at the interface of T lymphocyte and APC regulate the structure of the IS and T lymphocyte immune response. Blocking adhesion molecules inhibits T lymphocyte activation and the contacts of T lymphocytes and APCs [2]. Co-stimulatory and checkpoint receptors alter the functional outcome of the immunological synapse formation substantially and can also influence the structure of synapse [3,4]. Understanding the structure and function of the IS and the mechanisms of the IS formation might be conducive to seek the target for immunotherapy. Immunotherapy targeting checkpoint receptors have provided the most promise [5,6]. This review summaries the development of the structure of the immunological synapse, the function of IS, and the molecular factors those participated the formation and the function of the IS.

Diverse Structures of the Immunological Synapse

The immunological synapse is a multi-molecular assembly of receptors and adhesion molecules formed at the interface of T cell and APC during the antigen recognition. The formation of the IS is a dynamic process, which involved the movement and spatial location of surface molecules, cytoskeleton proteins, and signal transduction molecules (Table 1) in the synapse. The molecules participated in the synapse formation play different role in T-cell activation and synapse formation (Table 1).

Molecules	T cell	APC	Function
Surface molecules	TCR, CD3, CD4, CD8, CD2, CD27, CD28, CD43, CD45, IFr-R, CCR5, CXCR4, PrPC, agrin, Ca^{2+} microdomain correlated proteins, Integrins (VLA-4, LFA-1)	MHC, ICAM-1/3, CD40, B7-1/2, CD70, CD81, Notch pathway, PrPc	adhesion, presenting antigen, intracellular signal transduction and induce calcium releasing, promote cytoskeletal movement and T cell differentiation
Cytoskeletal protein	F-actin, tubulin, cytoskeleton associated protein, ERM, MTOC	F-actin, tubulin, MTOC	cytoskeletal movement, signal transduction
Signal transduction molecules	tyrosine kinase family, LAT, SLP-76, WASP, GTPases	Akt, WASP, Small GTPase Rho	Signal transduction, cytoskeleton movement, regulate Ca^{2+} releasing

Table 1: Molecules involved in the formation and regulation of the immunological synapse.

According to the different molecular localization, the IS structure is distinguished into three types (Figure 1) [7]. One is the bullseye IS, which was first observed by Kupfer [1] and co-authors as a classical mature synapse formed between T cells and the artificial planar lipid bilayer containing fluorescence labelled peptide major histocompatibility complex (pMHC) and intercellular adhesion molecule [1] (ICAM-1) embedded. In the centre of the bullseye IS, T cell receptors (TCRs) and the other signaling molecules assembled a central supramolecular activation clusters (cSMAC) [1]. The cSMAC is dominated by the interaction of TCR and pMHC. Adhesion molecule interactions, such as the interaction of lymphocyte function-associated antigen [1] (LFA-1) and ICAM-1, occur surrounding the cSMAC and form the peripheral SMAC (pSMAC) [1,7]. Another type of structure is the multifocal IS, which is characterised as accumulated LFA-1-

ICAM-1 molecules at the T-cell–APC interface, which is interspersed by multiple small clusters of TCR-pMHC complexes and phosphorylated signalling molecules [8-10]. In addition, an immunological kinapse (IK), which has a polarized shape with a well-defined lamella and uropod, was observed when cells interacted with planar lipid bilayers. In the IK, TCRs clusters are polarized to the uropod, while adhesion molecules are accumulated to the lamella [11].

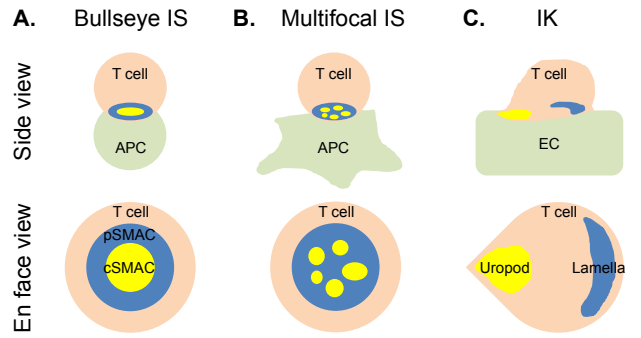

Figure 1: Different structures of immunological synapse. (A) Bullseye immunological synapse; (B) Multifocal immunological synapse; (C) Immunological kinapse. The TCR micro-domains were shown as yellow, whereas the LFA-1 micro-domains were shown as blue. APC: Antigen Presenting Cells; EC: Endothelial Cells; cSMAC: central Supramolecular Activation Clusters; pSMAC: peripheral Supramolecular Activation Clusters.

The multifocal IS was commonly observed between T cells and DCs [8,9]. The bullseye IS was observed between T cells and planar lipid bilayers, or between CD8+ cytotoxic T lymphocytes (CTLs) or natural killers (NKs) and target cells [1,12,13]. Recently, the bullseye IS was found to form at the T-DC contact, and its formation was correlated with the staphylococcal enterotoxin B (SEB) stimulation and cytotoxic T-lymphocyte antigen-4 (CTLA-4, CD152) translocation into IS [14].

The IS formation is a dynamic process, including both the dynamic molecular movement at the interface and the dynamic movement of T cells and DCs. During the IS formation, the TCR and pMHC molecules are bound with each other and form some micro-clusters, then these clusters are fused to the center of the synapse and form cSMAC. Meanwhile, the LFA-1 and ICAM-1 molecules are interacted with each other and are accumulated in the T-DC contact, followed by the rearrangement from the central to the peripheral of the synapse [15]. A large-scale, actin-dependent rearrangement of receptors, downstream signaling molecules and adhesion molecules accumulate into synapse and a relatively stable IS is formed. The newly generated microclusters move centripetally from the periphery to the cSMAC, where signaling is extinguished and the TCR is down-modulated [16,17]. In addition, the morphological shape of the T cell changes during the contact of T cell and DC. The T cell firstly moves fast and "pecks" with the APC repeatedly, contacts for a short time and detaches. This first stage will sustain for about 30 minutes. Then the motility of the T cell is decreased, and T cell undergoes a relatively long-lived contact with the APC (about several hours). Finally, the T cell detaches from the DC, restores the rapid motility and the "peaking", and starts proliferation [18,19]. The dynamic movement of synapse molecules and the cells are suggested correlates with the T cell activation.

Functions of the Immunological Synapse

The different structures of the IS are proposed leading to the different IS function and regulating T-cell activation. Although the function is controversial [20], the IS is still believed to be a platform for enriching signaling molecules and controlling T-cell activation. *In vitro* and *in silico* experiments showed that the IS was an adaptive controller to boost TCR triggering and attenuated strong signals [21]. Especially cSMAC formation is believed to be associated with TCR down modulation [21]. cSMAC formation without ZAP70 (Zeta-chain-associated protein kinase 70), LAT (linker for T-cell activation), PLCγ1 (phospholipase Cγ1) location into the IS induce CD4+ T cells tolerance [22-25]. Our previous report showed that the bullseye IS formed between a naïve T cell and a DC pulsed with SEB or OVA peptide (323-339) was correlated with a low level of calcium response in the T cell and the loss of molecules involved in the TCR signaling pathway, such as ZAP-70, PLCγ1 and PKC-θ, in the IS. Such IS accumulated CTLA-4 to maintain the structure of synapse and played a suppressive role in the early T cell activation [14]. However, in the same cell-cell contact model, the multifocal IS showed a significantly higher level of calcium response in the T cell. The multifocal IS accumulated more ZAP-70, PLCγ1 and PKC-θ molecules than the bullseye IS did [14]. The results suggested the multifocal IS as a positive regulatory synapse for T-cell activation.

Another important function of the IS is the directed secretion of soluble components into the synaptic cleft [26]. This function usually occurs in CTL or NK cell-mediated killing of infected cells or tumor cells. Cytolytic granules, perforin or cytokines were directly delivered to a secretary domain near the endo-cSMAC compartment [27]. The bullseye IS was believed to be the main synapse type for this function. IK was also reported to have such function in the NK cell mediated tumor cell. Converting IKs into ISs by the tumor-specific antibodies increased the killing efficiency of the tumor cells [28]. Comparison of synaptic versus kinaptic killing in the context of CD8 versus CD4 cytotoxic T cells *in vitro* showed that a stable IS had about 3-fold killing efficiency than IK [29,30]. The potential advantage of the bullseye IS was concentrating the cytolytic components on the contact surface to kill the target cells. Thus, increasing the proportion of the stable IS formation might contribute to the immune therapy in clearing tumor cells or infected cells. Additionally, T help cells were shown capable of secreting some cytokines, including IFN-γ and IL-10, directly to the APC through the bullseye IS [31].

Besides the cytokines and granules secretion, cyclic adenosine 3',5'-monophosphate (cAMP) was reported to influx into conventional T cells or antigen-presenting cells through cell-cell contact to control immune activation [32]. cAMP was generated after the initial binding of hormones, neurotransmitters, and ligands to cell-surface receptors [33]. Then, cAMP activated the canonical protein kinase A (PKA) pathway and the exchange protein activated by cyclic AMP (EPAC) non-canonical pathway [34,35]. cAMP suppressed actin polymerization at the interface of DCs and conventional T cells. Regulatory T cells (Tregs) generated and accumulated high levels of cAMP and transfer it into the target cells through cell-cell gap junctions to play a suppressive role [36,37]. Additionally, MHC clusters was reported to be transferred from cell-cell contact [38]. MHC class II can be directly transferred to the CD4+ T cell from the APC through the IS upon cellular dissociation [38].

Overall, the multifocal IS is important for signal integration, and the bullseye IS tends to deliver the effector molecules and regulate of T-cell activation. T-cell priming necessitates the formation of an

immunological kinapse. Interacting naïve CD4+ T cells with planar bilayers showed that these cells alternated between forming a bullseye IS or a migratory immunological kinapse [11]. The bullseye IS can be transitioned form multifocal IS in T-DC pulsed SEB model [14]. The changed structure from the multifocal IS to the bullseye IS to the kinapse might reflect the initiation of T-cell activation, the quenching of T-cell activation and T-cell migration, respectively. This suggests the IS as a modulator platform for the function of early T-cell activation.

There are three molecular functions of the IS suggested in medicating the early immune response. One is moving some activated signaling molecules away from the IS. After the stable mature IS formed, signaling molecules, such as ZAP-70 and PLC-γ, are extinguished and the TCR is down-modulated. Another function is recruiting some molecules from the periphery to the cSMAC to regulate T cell functions and fates. For example, CTLA-4, a check pointed protein was generated and trans-located into cSMAC and pSMAC to maintain the suppressive synapse and stop T-cell activation [14]. The third function is secreting soluble molecules from one cell to another cell. The secreted molecules may affect the function of T-cell through binding with probability receptors [32]. The detail mechanism still needs further investigated.

Factors Affect the Formation and Function of the Immunological Synapse

The factors regulating the IS formation and T-cell activation were well studied but insufficient. The molecules known participating in the IS formation includes adhesion molecules, co-stimulated molecules, checkpoint molecules, and cytoskeletons (Table 2). During cell recognition, the localization of these molecules in the synapse indicates how they regulate in T-cell functions. Eliminating or promoting some molecules into the IS may provide new insights for understanding the IS biology and be benefit to immune therapy.

Surface molecules

Molecules expressed on the T cell surface are uniformly distributed before the T cell activation. When T cells contacting with other cells or being stimulated by antigens, some molecules are clustered to increase the affinity for their binding ligands and provide amplified signals for the intracellular cascade reaction of the T-cell activation. These surface molecules include adhesion molecules, co-stimulated molecules, and checkpoint molecules (Table 2). Adhesion molecules are critical for sensitive antigen recognition. On T cells, LFA-1 is the main adhesion molecule involved in the IS. LFA-1, also known as αLβ2 integrin or CD11a/CD18, is a member of the integrin family. It is a heterodimer with an unique α subunit that shares the β subunit with three other cell-surface heterodimers, each of which has an α subunit with a distinct expression pattern [39,40]. ICAM-1, which is a member of the Ig superfamily, is one of the main ligands for LFA-1. LFA-1 binding with ICAM-1 was reported to be accumulated at the interface of T cell and APC at the first stage of the IS formation. The activation state of LFA-1 (extension) on T cells is critical to induce targeted movements of both ICAM-1 and MHC class II to the IS on APCs [41]. And the LFA-1 cluster size determined transport and spatial distributions of LFA-1 in the IS [42]. In the absence of antigen stimulation or TCR-pMHC recognition, LFA-1 could interact with ICAM-1 to form a transmitted cell-cell contact and induce calcium signaling in T cells [43]. The LFA-1 outside-in signaling (binding with ICAM-1) may activate T-cell through the Src family kinase Fyn, which might be distinguished from the TCR-pMHC signaling. Thus, LFA-1-ICAM-1 interaction might be an early stage factor that initiated the IS formation and T-cell activation. Additionally, CD2 and CD58 were defined as a heterophilic adhesion receptor pair [44]. CD2 and CD58 are both the members of the immunoglobulin (Ig) superfamily. The complex of CD2 and CD58 interaction is similar in length to the TCR and pMHC complex, suggesting that CD2 may closely cooperate with the TCR [45]. However, same as LFA-1, CD2 engages the ligands in the pSMAC, even though the CD2-CD58 interactions are the correct length to be co-localized with TCR-pMHC complex in the cSMAC (Table 2). Integrins are the drug targets in many diseases [46], which indicating LFA-1 as a potential target for the IS related immunotherapy.

In the absence of co-stimulation, T cells lead to a second round of colonial deletion that protects the host against immune responses to the harmless environmental antigens. Costimulatory receptors can enhance adhesion and signaling transduction and coordinate the activation of TCR signaling pathway. These molecules include CD28, ICOS, the TNF receptor (TNFR) superfamily including CD27, GITR (CD357), 4-1BB (CD137), and OX40 (CD134), and so on (Table 2). CD28 is an Ig superfamily member with a homodimeric structure and a cytoplasmic domain. It recruits and activates Lck and indirectly, protein kinase C (PKC)-θ, an important PKC isoform in T cells to contribute to the activation of NF-κB transcription factors and promotes IL-2 production [47]. The activity of CD28 is dependent upon the up-regulation of B7-1 (CD80) and B7-2 (CD86) on APCs [48]. CD28-CD80/CD86 interaction promotes T-cell activation and migration [47]. A single point mutation in the CD28 cytosolic tail (tyrosine 188) interferes with the ability of CD28 to preferentially accumulate at the cSMAC, which lead to CD28-mediated localization of PKC-θ to the cSMAC disrupted and the efficient signal transduction interfered.

Type	T cell	APC	Localization in IS
Adhesion molecules	LFA-1 (CD11a/CD18)	ICAM-1 (CD54)	pSMAC
		ICAM-2 (CD102)	
		ICAM-3 (CD50)	
	VLA-4 (CD49d/CD29)	VCAM-1 (CD106)	Unknown
Co-stimulated molecules	CD28	B7-1 (CD80)	cSMAC
		B7-2 (CD86)	
	ICOS	B7-H2 (B7RP-1)	Colocalized with PI3K in IS
	Unknown	B7-H3	Unknown
	Unknown	B7-H4	Unknown
	Unknown	B7-H5	Unknown
	4-1BBCD137	4-1BBL	localized in synapse but separately from pMHC I/II
	CD2	LFA-3 (CD58)	pSMAC
	CD9	Unknown	Unknown
	CD44	Unknown	Unknown
	CD45L	CD45	dSAMC, it can enter the cSMAC at later stages

	OX40	OX40L	localized in synapse but separately from pMHC I/II
	CD70/CD27	CD70L (CD27)/ CD70	cSMAC
	CD30 (Ki-1)	CD30L (CD153)	Unknown
	CD81	Unknown	Unknown
	CD82	Unknown	Unknown
	CD53	Unknown	Unknown
	CD63	Unknown	Unknown
	Tim-1 (Th2 cell)	Tim-4	Unknown
Inhibitor / check point	Tim-3 (Th1 cell)	Galectin-9	pSMAC or dSMAC
	CTLA-4 (CD152)	B7-1 (CD80)	pSMAC and cSMAC
		B7-2 (CD86)	
	CD5	Unknown	Unknown
	PD-1	B7-H1 (PD-L1, PD-L2)	pSMAC and cSMAC
	BTLA	HVEM	Unknown

Table 2: Localizations of the surface molecules involved in the immunological synapse.

Cytotoxic T-lymphocyte antigen-4 (CTLA-4, CD152) as a checkpoint protein competes with CD28 for binding to CD80 more potently than for CD86 [49]. CTLA-4 is constitutively expressed in the regulatory T cells and is coupled to the endocytic pathway, which leads to the removal of CD80 and CD86 from APCs and impairs CD28-dependent responses of other T cells [50]. CTLA-4 localized at the pSMAC and cSMAC. The transportation of CTLA-4 into synapse mediated the stable bullseye IS formation. Up-regulating the LFA-1 movement and interaction of LFA-1-ICAM-1 might be a possible reason for CTLA-4 to promote the formation of a stable synapse [51]. Another checkpoint receptor is the programmed cell death-1 (PD-1, CD279). PD-1 binds PD-1 ligand 1 and 2 (PD-L1, CD274 and PD-L2, CD273) [52] and is recruited to the IS in a manner related to MHC-peptide strength and abundance [53]. An interesting aspect of PD-1 is that it is expressed on and suppresses the activity of Tregs. Thus, blockade of PD-1 may increase Treg function, suggesting a rationale for combining anti-PD-1 with anti-CTLA-4 [54], the latter suppresses Treg function. Other checkpoint inhibitors include Vista and SIRPα (CD172A) [55,56]. CTLA-4, PD-1 and PD-1 legends have been served as the target for cancer therapy to enhance the immunological cellular toxicity for tumor cells [57,58].

Cytoskeleton

Cytoskeleton is a complex network of the interlinking filaments and tubules that distributed throughout the cytoplasm, from the nucleus to the plasma membrane. Among them, the filamentous actin (F-actin) network has been carefully studied. It plays a critical role in the IS formation, the morphological change of T cells and the TCR signalling [59,60]. Forming the IS by organising distinct supramolecular activation clusters through actin cytoskeleton rearrangements [1,15]. The centripetal movement of TCR and LFA-1 microclusters are actin dependent and parallels the retrograde actin flow that occurs during cell spreading and migration [16]. TCR signaling initiates in numerous microclusters at the periphery of the synapse [61], which migrate toward the center where they coalesce to form the cSMAC. Actin retrograde flow has been shown to promote these molecules to move into the synapse. As treatment of T cells with latrunculin, an inhibitor of actin polymerization, halts the transport of TCR microclusters to the cSMAC and abrogates formation of new signaling assemblies [16,61]. However, the mechanism of this actin retrograde flow is not well understood.

TCRs are accumulated into the F-actin excluded cSMAC, indicating that the TCR trafficking to the cSMAC is F-actin independent. The actin nucleation promotion factors Wiskott–Aldrich syndrome protein (WASp), WASp family verprolin homologous protein [2], and HS1 are thought to cooperate with Arp2/3 to polymerize F-actin from the plasma membrane triggering centripetal inward movement toward the F-actin-poor cSMAC, where subsequent depolymerization is thought to occur [62,63]. These proteins might promote TCR transportation. Myosin IIA has also been implicated in TCR microcluster translocation to the cSMAC and maintenance of synapse architecture [64].

Microtubule organizing center (MTOC) also plays a key role in the engagement of molecular motors, directional transport of granules, and polarization of subcellular structures and molecules. MOTC is localized in the uropod [65] in migrating T cells. The position of MOTC changes dramatically upon target cell recognition. It translocates from the rear of the cell to the leading edge where the synapse forms [66], to accumulate in the distal SMAC (dSMAC). The same organization is found not only in the synapses formed between cytotoxic T lymphocytes (CTLs) and target cells but also in other cytolytic cells, including natural killer (NK) and invariant NKT cells [67], as well as in CD4+ T cells where the MOTC also docks within the center of the synapse [68] and actin accumulates toward the edge of the cell.

Besides mediating the IS formation, F-actin modulates T-cell activation. The amount of F-actin accumulated in the IS modulate the calcium releasing in T cells by controlling the localization of calcium micro domain in the synapse. Treatment of T cells with actin depolymerising agents leads to loss of Ca^{2+} mobilization and downstream transcriptional activation [69]. Not only does T-cell receptor (TCR) ligation initiate a robust actin polymerization response, but actin dynamics are also required for effective TCR signaling as inhibitors of actin polymerization disrupt T-cell activation [70].

Synapse and antigen

Antigen stimulation is important for the aggregation of TCR, especially for TCR localization into cSMAC. The TCR microclusters are translocated into the actin poor cSMAC by a specific-antigen dependent mechanism [17]. With antigen stimulation, only antigen-specific CD4+ T cells formed the bullseye and the multifocal IS [14]. Both of them are effective synapses to form TCR clusters to participate in the specific immune response. Non-antigen-specific CD4+ T cells interacted with DCs and formed synapses with CD28 accumulation, but not TCR accumulation [14]. Without antigen stimulation, none IS was formed between T cell and DC. The different type and specificity of the antigen determined the different type of the IS formation and the activation of T cells. SEB could induce more percentage of the bullseye IS formation at the T-DC contact than OVA peptide (323-339) did [14]. That might be different type of antigens influent the amount

of TCR localization or aggregation in the IS, which may lead to the formation of the IS and the T-cells activation. Antigens from virus or tumour cells escape T cell recognition might be these antigen are a low affinity antigen for TCR recognition and could not induce efficient TCR clusters accumulate to form synapse. Additionally, antigen from virus is more likely to recognize the adhesion molecules or integrin in immune cells, or penetrates into the cell to bind with cytoskeleton to disrupt the movement of molecules to form synapse of T-APC synapse formation.

Conclusion

The structure of the IS was affected by the localization of molecules in IS, which were regulated by the affinity of receptor-ligand and cytoskeleton, and the antigens. The researches of the IS have provided the molecular target for immune therapy. Regulating the localization of specific molecules or cytoskeleton on the IS might provide a better idea for immune therapy.

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