

Immunological Synapses Within Context: Patterns of Cell–Cell Communication and Their Application in T–T Interactions

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Abstract The cell-biology of intercellular communication between T cells and their partners has been greatly advanced over the past 10 years. The key morphological and motility features of cell contact-based communication between T cells and APCs can now be seen as a collection of patterns for cell–cell interactions amongst immune cells more generally, each serving to contribute to the outcome of the contact both locally and globally. Here we review the conservation of these patterns, amongst which is the emergent “immunological synapse,” and describe a newly defined example, formed between the adjacent activating T cells. We subsequently seek to put these and the pattern more generally into the framework of system-wide behavior of the immune system. We postulate that the patterns are fine-tuned to provide quorum-like decisions by collections of activating and activated cells that interact over time and space.

1 Introduction

The immune system can be conceived as bearing similarities to a community of human beings inhabiting a city or country; immune cells are of varied origin and abilities (T cells, B cells, natural killer (NK) cells, macrophages, dendritic cells (DCs), etc.), inhabit varied physical spaces in tissues (interdigitating or surveilling various organs, peripheral tissues, and secondary and tertiary lymphoid structures), travel over both short and long distances, interact with one another, and, of course, introduce changes in their environment. The behavior of immune cells, like that of individuals, is partially determined by the features of their physical environment. However, at a deeper level, their behavior is also constrained by their limited means of communication and interaction.

In describing the optimum size for a city – based on maintaining a social cohesion – Aristotle concluded that an entire city should be of a sufficiently small size so that all citizens would be able to hear a single herald in peace or a single general in war [Politics VIII]. Such a stipulation has likely been obviated by dramatic changes in the mechanisms for interaction and communication between individuals (e.g., “broadcast” media such as the newspaper, telephone, internet, etc.). Are there equivalent issues of scale for communication in the immune response?

There are indeed clearly equivalent “broadcast” media such as large releases of soluble cytokines that subsequently permeate organs and organisms and influence multiple cell types. Although there are beneficial “bread crumb”-like trails of chemokines which apparently line epithelial layers and address activated cells to particular tissues, are there global soluble signals to communicate for a system requiring careful recruitment of only specific cells? On the whole, the so-called large “cytokine storms,”

particularly those of pro-inflammatory mediators such as γ IFN, are more highly associated with pathogenic states such as “shock” rather than effective and specific surveillance (Rittirsch et al. 2008). As part of the mandate of the immune system to be specific and only destroy invading organism, it is apparently quite necessary to explicitly address messages, even those of “soluble” mediators so that only certain cells are activated. The “immunological synapse,” a recurring pattern of cell–cell junctions for immune-cells represents a portion of the solution for the need for explicit communication. However, as an isolated concept, it does not encompass the total solution for the need for broad communication over a distance.

It is possible to define collections of “solutions” for optimizing human interactions and communication over space. Indeed, such “patterns” are suggested to exist on scales from entire urban design down to considerations of the size of rooms in a house and to be applicable like a “stamp” to treat recurring needs (Alexander et al. 1977). Notably for the analogy to biological systems that arise from defined behaviors of individual players, it is also theorized that design solutions at small scales (quality design of social spaces) are part and parcel of the greater functioning of larger-scales (e.g., entire cities) (Whyte 1988). In a similar manner, the features or patterns that define cell–cell interactions represent the fundamentals toward defining the properties of the immune system as a whole.

In this review, we will address what has emerged as “synapse-based patterns” for cell–cell interactions. We will argue that the “immunological synapse” (IS) as currently described is amongst a collection of a relatively small number of small-scale patterns of motility, morphology, and membrane organization that provide critical features that can permit efficient larger-scale goals to be accomplished: namely self/nonself discrimination, rapid but flexible responses, and group decision making based on the regulated formation of these contacts. We will use an analogous “Pattern” framework as a way to define how the properties of cell–cell contacts provide the adequate specificity, flexibility, and group decision making properties to specific cell types. In particular, we will expand from the T cell–antigen-presenting cell (APC) synapse the synaptic structure that initiated the current intensive study of cell–cell contacts in the immune system, and describe a recently appreciated T–T synaptic contact and the potential quorum sensing that might be facilitated by the application of synapse “patterns” to activating T cells.

2 The Emergent Prototypical Immunological Synapse Dynamics

The contact surface at which T cells recognize and activate in response to peptide fragments in the groove of major histocompatibility complex (MHC) molecules was first proposed to be similar to a neurological synapse by Norcross in 1984 (Norcross 1984). The concept was revived in the late 1990s as a result of the observation of ring-like distributions of integrin lymphocyte function-associated antigen-1 (LFA-1)

and their ligands (peripheral-supramolecular activating complexes; pSMACs (Monks et al. 1998)) that surrounded centralized T-cell receptor (TCR)–MHC complexes (central supramolecular activating clusters; cSMACs (Monks et al. 1998)) at T-APC contact sites. Concurrent observations of CD2 clusters (Dustin et al. 1998) and cytoskeletal movement into the contact region (Wulfiging and Davis 1998) further solidified the comparison. However, the term gained wide acceptance when used to assess the distributions of TCRs and integrins in simplified model lipid bilayers (Dustin and Colman 2002; Grakoui et al. 1999). It was subsequently argued that these distributions at an adhesive contact were definitively “synaptic” (as opposed to focal adhesions, desmosomes etc.) on the basis of being an adhesive contact with a synaptic space, and characterized by polarized secretion and signaling (Dustin and Colman 2002; Grakoui et al. 1999). With this rapid progress, there emerged a frequent but incorrect interchange of terminology “Synapse,” which might best define the cell–cell contact and “cSMAC/pSMAC,” which defined a frequently observed organization and differential exclusion of molecules that could be observed within some of those contacts.

When synapse assembly was analyzed in real-time, concurrent with calcium influx downstream of TCR triggering, it became apparent that cell–cell contact was associated with much earlier and smaller TCR–MHC clusters (Krummel et al. 2000), which only later coalesce to the cSMAC/pSMAC structure. Subsequently, receptor-proximal signaling has been demonstrated to be most active in these and even smaller initial “microclusters” but mostly extinguished in the centralized cSMAC structure (Varma et al. 2006; Lee et al. 2002; Mossman et al. 2005), although some recent data suggests that TCRs in the cSMAC may still support signaling in particular circumstances (Cemerski et al. 2008). Recent use of photo-activation of pMHC ligands for the TCR make it clear that early clusters signal within seconds of ligand engagement (Huse et al. 2007) whereas the formation of the cSMAC/pSMAC architecture may take minutes (Krummel et al. 2000; DeMond et al. 2006). A now-modified understanding of a dynamically rearranging synapse includes active remodeling of the membrane domains giving rise to a dispersed cluster-dominated “immature” and subsequent cSMAC-bearing “mature” form (Krummel et al. 2000; Mossman et al. 2005; Campi et al. 2005).

The characterization of immunological synapse dynamics has also been enriched by other parallel developments. First, it has been revealed that cell–cell communication and TCR stimulation at T-APC contacts is frequently associated with short-lived cell–cell contacts rather than prolonged ones. These have not yet proved tractable to study at the molecular level but were first described for T cells interacting with peptide loaded dendritic cells in collagen matrices (Gunzer et al. 2000) where stable interactions are rarely observed but which nevertheless produced T-cell activation. The functionality of short-lived cell–cell interactions is also suggested by the correlation between expression of early-activation antigens following transient contacts *in vivo* (Mempel et al. 2004) and by the ability of cells to be activated when only given repeatedly interrupted stimuli (Faroudi et al. 2003). While the outcome of these transient interactions may not be complete activation and memory formation (Scholer et al. 2008; Hurez et al. 2003), there is emerging

evidence that such interactions provide ample opportunity for specific and polarized cell–cell signaling. In particular, the functional act of cytotoxic T lymphocyte (CTL) killing at T cell–target interactions is achieved with only short-contacts and does not require the formation of a centralized TCR accumulation (Wiedemann et al. 2006; Purbhoo et al. 2004). It is thus important to see the stable IS model, typically including the coalescence of a cSMAC (Grakoui et al. 1999; Krummel et al. 2000; Varma et al. 2006; Mossman et al. 2005; Campi et al. 2005; Dustin et al. 2006), as one example of signaling and direct cell–cell communication, taken from a broader selection of patterns.

A further enrichment of the cSMAC/pSMAC model of cell–cell signaling at the IS is derived from analyses of the contact face morphology and subsequent consideration of the dynamics of membrane apposition for communication at this junction. In glass-supported lipid bilayers where the apposed system has a flattened topology and cannot deform, membrane–membrane interfaces form a very flat and contiguous contact face with the glass-supported surface (Grakoui et al. 1999; Dustin et al. 2006). In completely juxtaposed settings, aggregation of signaling molecules could only occur by movement along the membrane; such movement is indeed observed and typically involves centripetal flow mediated by actin (Varma et al. 2006; Yokosuka et al. 2005). However, the first live-cell imaging of cell–cell based TCR–signaling clusters noted that the process was highly dynamic with clusters forming, dissociating and reforming (Krummel et al. 2000) rather than smoothly moving only inward. Similar non-radial movement was recently observed for larger clusters within an NK–APC synapse, when the synapse was observed specifically “en face” (Oddos et al. 2008). Is this process the same?

An immunological synapse between immune cells and their ligand-bearing partners appears to contain multiple distinct regions of close membrane–membrane apposition which may dynamically remodel in addition to permitting TCR and integrin movements within that juxtaposed membrane space. In support of this, transmission electron microscopy (TEM) analysis of the physiologically relevant contacts suggests that a contiguous flat contact interface is not, in fact, representative of the physiological case for T–DC interactions (Brossard et al. 2005), CTL–target contacts (Stinchcombe et al. 2001), NK–APC (McCann et al. 2003) and typically even in T–B interactions (Krummel MF, unpublished). Within such contacts, the membranes only touch sporadically, with the non-attached regions separated by distances upwards of 50 nm and for stretches of upwards of 1 μm (Brossard et al. 2005; Stinchcombe et al. 2001; McCann et al. 2003). In synapses formed by CTLs and their targets, lytic granules are aligned with these clefts (Stinchcombe et al. 2001). Thus, the physiologically relevant contacts involve significant synaptic clefts formed between regions of closely apposed membrane (see cartoon in Fig. 3), a result that is even more consistent with analogous synapses in neurons than perhaps was appreciated in early studies. Indeed, the functional significance of the synaptic nature of the contact, namely the formation of synaptic spaces for secretion also appears to be supported by the partitioning of secretory domains (Stinchcombe et al. 2001) and vesicles containing IL-2 and γIFN (Huse et al. 2006; Reichert et al. 2001; Kupfer et al. 1994; Kupfer et al. 1991) as well as receptors for these cytokines (Maldonado et al. 2004) at the IS.

3 Functional Patterns of Cell–Cell Communication

It was then prescient for others (Dustin and Colman 2002) to have previously defined “features” of synaptic contacts when relating them to neuronal synapse; including “discreteness,” “adhesion,” “stability” and “directed” secretion. With our emerging knowledge, it seems timely, however, to look at the current model of an IS as part of a broader pattern that immune cells utilize for cell–cell communication. With less emphasis on the molecular organizations within membrane–membrane junctions of the IS that are to be reviewed by others in this issue, we suggest that the following represent well-established “patterns” of cell–cell communication in the immune system. For each one, we will describe how the pattern appears to provide efficient communication to the system as a whole.

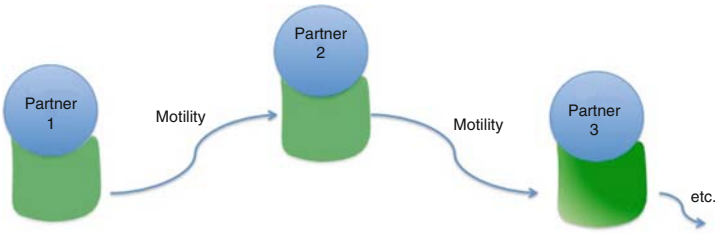
3.1 *Dynamic Cellular Assembly and Disassembly*

T cell–APC interactions are not permanent structures. Rather, the cell–cell contacts last for seconds to hours but all ultimately result in “abscission” of the T cell from the APC and possible reattachment to other partners (Fig. 1a). In vivo, there is considerable variation in the length of contact and the variability appears to be regulated by the strength of antigenic stimulation (Henrickson et al. 2008; Skokos et al. 2007) as well as T-cell intrinsic factors (Sims et al. 2007). The timing of first arrest is also variable: depending on the route of immunization and adjuvant, the “stop” phase can occur between 2 and 18 h after administration of adjuvant. Some of the timing certainly is influenced by the rate of loading and/or trafficking of the antigen to the lymph node. It is clear that, particularly in high-antigen conditions, soluble peptides administered intravenously can induce cell arrest within minutes (Celli et al. 2007), suggesting there is no obligate lag-phase for arrest. Thus, there is variability in the timing of the pattern, but the generation of multiple but transient cell–cell contacts appears conserved.

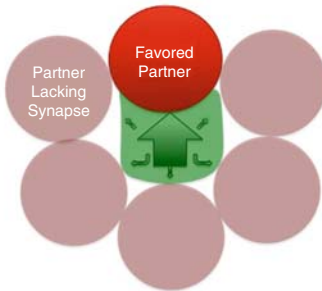
This pattern is repeated in CTL–target and NK–target interactions, in which the effector cells may only stay together with the targets for a few minutes prior to moving on to another target. Perhaps this case exemplifies the utility of transient arrest: the ability to interact serially with multiple partners (Wiedemann et al. 2006), which is clearly a benefit to kill most targets. For activating CD4+ T cells, it likely serves to permit T cells to recognize signals on multiple surfaces, potentially choosing the “best” APC encountered (Depoil et al. 2005). Additionally, it is also possible that it allows T cells to “tag” and thereby mature multiple antigen-presenting cells, providing increased specificity for future T cells. This has been proposed to rely upon the chemokine receptor CCR5 and the locally produced CCL3 and CCL4 (Castellino et al. 2006; Hugues et al. 2007).

As a general rule, the pattern of transience in cell–cell contacts increases the number of cells and the area of sites affected by a single cell. In the case of helper

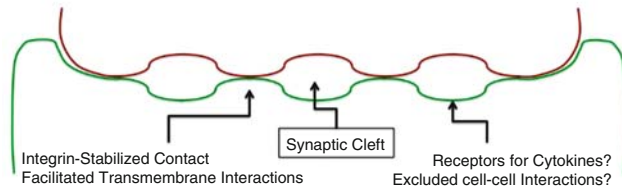
a Pattern 1: Transient Stability and Intermittent Motility.



b Pattern 2: Polarized Secretion Allows Selection of Target Delivery



c Pattern 3: Regulated Adhesion Zone Membrane Morphology Coordinates Numerous Ongoing Activities.



d Pattern 4: Facilitated Clustering of Transmembrane receptors at Juxtamembrane Domains Increase Signalsome Efficacy. Global and Local Mechanisms of Enhancement.

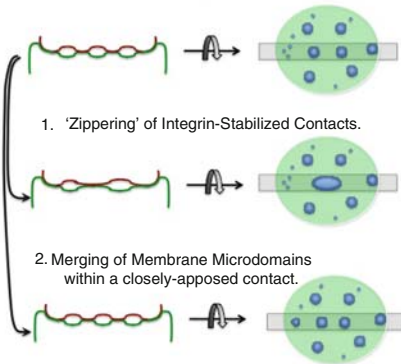


Fig. 1 Basic patterns of immune synapse. **(a)** Dynamic cellular assembly and disassembly. **(b)** Defined but flexible polarity. **(c)** Close membrane–membrane juxtaposition with a synaptic cleft. **(d)** Aggregation and segregation of transmembrane receptors and lipids

T cells, which are limited in numbers but must survey vast regions, it is clear that having multiple contacts may provide clear benefits in expanding the response to include multiple other cells.

3.2 Defined but Flexible Polarity

Synaptic cell–cell contacts allow cells to provide information in the form of signaling or killing events in a specific manner. Polarity of signals generated at cell–cell contacts as well as subsequent secretion into these contacts, then, represents a second highly conserved pattern of immune cell–cell interactions. As shown in Fig. 1b, this pattern permits cells to direct messages to one another while excluding bystanders. As an example, when T cells are engaging a cell presenting peptide–MHC complexes, it has been shown that CD40L is directly accumulated at the IS where it is available to crosslink CD40 (Boisvert et al. 2004). Notably, it has been proposed that this pattern is only true for some signals; vesicles containing γ IFN appear to be more synapse localized while other secreted products such as TNF and chemokines may be more broadly directed (Huse et al. 2006). However, given the strict limitation of vesicle–membrane fusion that occurs, there may ultimately prove to be additional restrictions on these latter molecules. As noted above, this pattern provides exquisite spatial specificity for inter-cellular communication by immune cells.

CTL–target and NK–target interactions provide the simplest and most extreme rationale for highly directional secretion towards a particular cell. Such directionality prevents off-target killing of bystanders and restricts delivery of granules to the IS (Stinchcombe et al. 2001). At present, the full range of molecular players achieving this directional specificity are unknown but SNAREs and other proteins of the microtubule cytoskeleton are likely candidates.

3.3 Close Membrane–Membrane Juxtaposition with a Synaptic Cleft

The T cell–APC “immunological synapse” was first defined as a synapse by virtue of the presence of both adhesion domains and signaling domains but it seems that synaptic clefts are also frequently present. As noted above, TEM analysis of physiologically relevant contacts suggests that T–DC interactions (Brossard et al. 2005), CTL–Target contacts (Stinchcombe et al. 2001) and typically even T–B interactions (Krummel MF, unpublished) contain this architecture. As shown in Fig. 1c, there are frequently spatially restricted areas where cell–cell signaling may occur surrounded by membrane domains which may restrict direct membrane contact. The latter domains, however, sample synaptic spaces and provide a region

for the accumulation of soluble mediators. Notably, the variable spacing of membranes around the closest point of apposition has been suggested to be important for protein organization in the IS (van der Merwe and Davis 2003; Shaw and Dustin 1997) and MHCs with variable length extracellular domains that result in altered capacities to signal (Choudhuri et al. 2005). However, some “large” molecules that are typically excluded, such as CD43, are not excluded on the basis of extracellular size alone, as tail-less forms can enter the central IS but do not interfere with signaling (Delon et al. 2001).

The presence of multiple domains in the membrane with different degrees of junctional “tightness” reflects variations in lipid composition as well as subcortical actin arrays. In this vein, although this exact architecture may be lacking in glass-supported approximations of cell–cell contacts, the generation of unique zones of membrane in the IS of such systems with differing lateral mobility for specific receptors has been observed in at least one such setting (Douglass and Vale 2005) and the presence of “rafts” (Anderson and Jacobson 2002) as well as protein “islands” in distinct regions (Lillemeier et al. 2006), also occurs at T cell–antigen-coated planar substrate junctions.

This architecture provides flexible regions for signaling receptors, but also regions into which vesicles may easily fuse and permits ongoing actin-organized signalosomes to persist in adjacent regions. While the receptors for cytokines are found in the IS (Maldonado et al. 2004) and cytokines are directed there (Huse et al. 2006; Reichert et al. 2001; Kupfer et al. 1994; Kupfer et al. 1991), the organization of these receptors relative to microclusters of TCRs or to the synaptic space has not been resolved. However, it is clear that regions of CTL granule release do not overlap with regions of TCR accumulation (Stinchcombe et al. 2001), suggesting that the TCR in the most tightly apposed regions of membrane are distinct from synaptic clefts.

3.4 Aggregation and Segregation of Transmembrane Receptors and Lipids

A final pattern that is established in all immunological synapses is the aggregation of receptor complexes and lipid domains (Fig. 1d). Based on observations of topology by TEM, there are likely two scales of clusters and at least two methods of cluster coalescence. Small, initial “micro” clusters likely provide for the formation of higher-ordering signaling arrays or “signalosomes.” Clusters of TCRs likely provide a high avidity lattice to capture pMHC complexes on the outside of cells and trap signaling intermediates in their active state on the inside of the membrane. Consistent with this, it has been observed that early microclusters of TCRs are in fact highly enriched for tyrosine-phosphorylation (Varma et al. 2006; Mossman et al. 2005). At the far edges of the synapse, continuous membrane extension and retraction are commonly observed and, at the B–DC synapse, have been observed to be involved in accumulating new ligands for the BCR (Batista et al. 2001).

Distinct from these initial clusters are the centralized clusters, which are most likely, associated with internalization of receptor-complexes (Varma et al. 2006; Mossman et al. 2005). It remains unknown at this point whether coalescence into these larger domains is fundamentally required for internalization of the TCRs or simply occurs most efficiently there. Notably, other participants in signaling intermediates such as CD4 (Krummel et al. 2000) and CD28 (Yokosuka et al. 2008) border centralized TCRs but are not included in the central “cSMAC” (CD4) or segregated from TCR clusters in cSMAC (CD28), consistent with this being an area of less-intense or extinguished signaling.

An unresolved question in the field is the way in which these larger clusters form. As shown for T cells interacting with membranes with reduced lateral protein mobility, it is likely that the formation of these large clusters hastens termination of signaling (Mossman et al. 2005). To this end; the dynamics of coalescence of clusters may involve multiple mechanisms. On the one hand, flat lipid bilayers demonstrate that TCRs can move laterally along the membrane and in a centripetal manner (Varma et al. 2006; Mossman et al. 2005; Yokosuka et al. 2005). In contrast, cluster coalescence in T cell–B cell or NK–APC contacts present a much less concerted effect, although a centralized cSMAC is typically still formed (Krummel et al. 2000; Oddos et al. 2008).

One intriguing possibility, in the confines of a cell–cell interaction, is that multiple mechanisms may act to give the final aggregated structure. While, membrane movement and coalescence of micro clusters in the membrane may drive cluster aggregation within a give domain (Fig. 1d, *middle panel*), the joining of individual membrane–membrane contacts may also be necessary to reorganize contacts in a full synaptic membrane architecture (“zippering,” Fig. 1d, *lower panel*).

Regardless, if signaling is amplified by the formation of initial clusters (Varma et al. 2006) but attenuated (Mossman et al. 2005), or, in other circumstances amplified (Cemerski et al. 2008) by cluster coalescence, the fact that membrane proteins move and membranes remodel provides the scaffold upon which the kinetics of signaling and direct sensing of peptide complexes is regulated. This pattern of clustering of receptors at interfaces is in fact conserved across all types of contacts observed between immune cells, and indeed in most cell–cell signaling contexts generally.

4 Four Fundamental Immunological Synapse Patterns Are Observed in the Interactions of Activating T Cells with One Another

So far, we have described immune synapses formed between two cells in which the *raison d’être* of the synapse is most associated with priming or cytotoxicity in a specialized cell type (e.g., T cell, B cell, NK cell) by an APC or the functional

equivalent. In fact, APC-mediated information transfer plays a central role in the mobilization of multiple arms of immune responses. Thus, it is not surprising that people have primarily focused on the communication between various types of immune cells and APCs. However, immune cell interactions *in vivo* occur in complex microenvironments where multiple cells dynamically migrate and interact on complicated networks of cells or extracellular matrixes (Bajenoff et al. 2006; Lindquist et al. 2004). It seems necessary, then, to begin to consider more complex multicellular interactions in order to fully understand how the immune system works. In this regard, direct observation of dynamics of immune cells under various immunological settings has been instrumental in revealing various modes of immune cell interactions that have not been fully appreciated before (Cahalan and Parker 2008; Germain et al. 2006).

Among activating T cells, our group and many others (Bajenoff et al. 2006; Sabatos et al. 2008; Hommel and Kyewski 2003; Ingulli et al. 1997; Miller et al. 2004; Bousso and Robey 2003; Tang et al. 2006; Garcia et al. 2007) have observed homotypic interactions (clusters) in antigen-specific T cells during priming in lymph nodes. Previously, homotypic clusters of T cells had been extensively observed as features of T-cell activation during *in vitro* culture assay, and indeed were shown to be physiologically mediated by LFA-1 (Rothlein et al. 1986; Rothlein and Springer 1986; van Kooyk et al. 1989). When observing these T-cell clusters by real-time methods *in vitro* and *in vivo*, not only were these clusters facilitated by integrin-based adhesion, but interactions in the clusters were dynamic, like those of initially contacting T–APC couples, with individual cells entering or leaving contacts with dwell times varying from minutes to hours (Sabatos et al. 2008). This provided evidence for the application of Pattern 1, in which individual T cells may visit one another for directed information exchange. As to other comparisons with the exact topological organization of the T–APC IS, it remains unclear at present whether LFA-1 alone is responsible for the contacts or whether other adhesion receptors may contribute and, indeed, dominate in the later phase. On the whole, it is also unclear at present how specificity is maintained beyond the combined effects of affinity upregulation of LFA-1 (Dustin et al. 1997) and increased expression of intercellular adhesion molecule-1 (ICAM-1) (Tohma et al. 1992) induced by TCR signaling. Nonetheless, the transient stability pattern appears to provide specificity, as unactivated T cells did not participate in these multicellular clusters and had short interaction times (typically less than 1 min) during encounters *in vivo* (Sabatos et al. 2008).

APCs are not strictly necessary for the transient nucleation of T-cell clusters; T cells stimulated by anti-CD3/CD28 or phorbol 12-myristate 13-acetate (PMA)/ionomycin formed similarly arrayed and dynamic multicellular clusters. This was apparently borne out by observations of T–T contacts distal to DC cell bodies, giving rise to the model for these interactions shown in Fig. 2. However, given the density of the DC network in lymph nodes (Lindquist et al. 2004), it is impossible to say with certainty that DC contacts were not occurring.

In addition to “transient stability” (Pattern 1), further characterization of cell–cell interfaces in the T-cell aggregates revealed that this emerging cell–cell contact

Fig. 2 A model of homotypic cluster formation of activating T cells during *in vivo* priming. Naïve T cells are activated by antigen presenting dendritic cells after several hours of stable interactions. Then, they regain motility, but swarm around their priming sites rather than migrate away. During this dynamic swarming phase, they form dynamic homotypic clusters

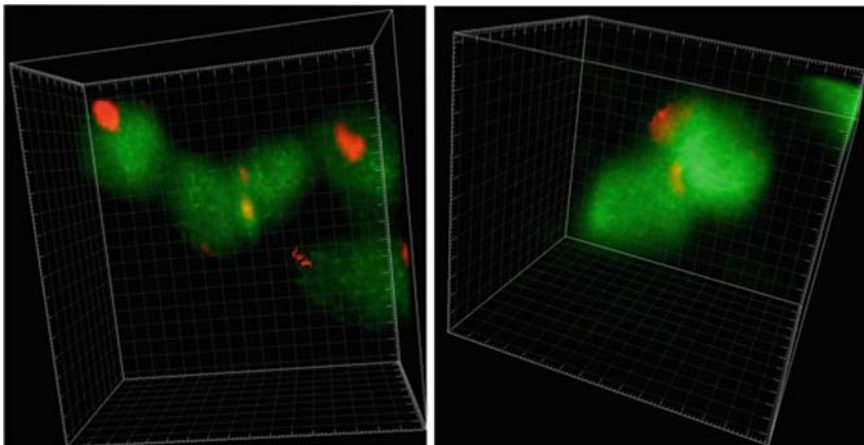
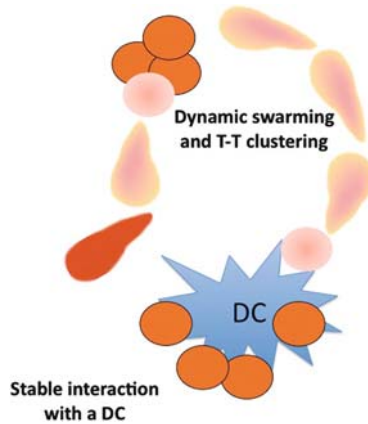


Fig. 3 MTOC polarization toward T-cell synapse *in vivo*. *Green*: OT-II T cell, *red*: pericentrin. CFSE-labeled OT-II T cells were injected to C57BL/6 mice, and subsequently immunized with ovalbumin protein emulsified in complete Freund's adjuvant. Draining lymph nodes were isolated 20 h after immunization, embedded in optimal cutting temperature compound, and frozen under liquid nitrogen. The frozen lymph nodes were sectioned by a cryostat with 80 μm thick and pericentrin was stained fluorophore-conjugated antibodies. Images of pericentrin-stained lymph node sections were acquired using confocal microscope and processed by Imaris

region matches each of the other communication patterns described in Chap. 3. This includes the observation that secretory vesicles of T cells are frequently polarized toward neighboring T cells, indicating directed secretion of soluble factors between two adjacent T cells (Pattern 2). More in-depth assessment of cell polarity demonstrated pronounced polarization of pericentrin, an MTOC associated protein, toward adjacent activating T cells. We have also been able to detect this polarized pericentrin localization in T cells activating directly in the lymph node (Fig. 3) although we've only isolated these with low frequency due to

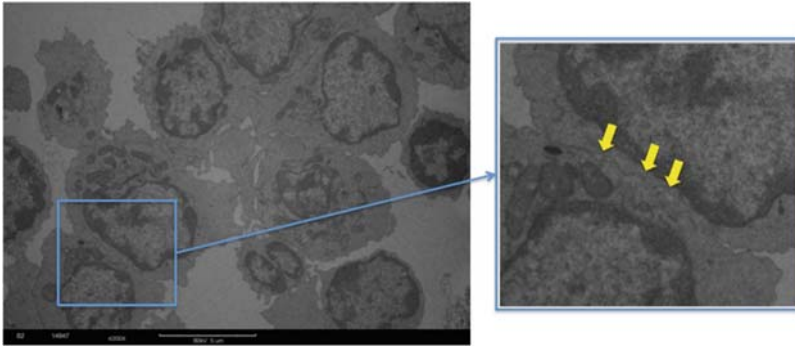


Fig. 4 Ultrastructure of T–T synapses. BALB/c wild type T cells were stimulated by PMA/ionomycin for 18 h, and their clusters were analyzed by transmission electron microscopy

technical limitations of tissue section staining. Along with secretory vesicle polarization shown by TEM, this indicates directional secretion of soluble factors from one cell to another cell through the synaptic space. Extending this to specific cytokines, we demonstrated that polarized vesicles near T–T interfaces contained interleukin-2, a cytokine produced by T cells during the early phase of activation and plays a critical role in T-cell activation, proliferation, differentiation, survival, and even apoptosis (Gaffen and Liu 2004; Kim et al. 2006).

Membrane ultrastructures of interfaces formed between activating T cells analyzed by TEM also exhibited canonical synaptic structure (Pattern 3); tight membrane apposition of two adjacent T cells with multiple clefts, similar to the multifocal synapse structure formed between naïve CD4+ T cells and dendritic cells (Brossard et al. 2005) and that between CTL and targets (Stinchcombe et al. 2001) or NK and their APC (McCann et al. 2003) (Fig. 4). Also, we were able to use “catch” reagents to localize the sites of uptake of T cell secreted IL-2. This demonstrated that IL-2 was indeed directed across and accumulated in these synaptic gaps in the catch assay. Notably, directional secretion of IL-2 is beneficial for T cells in the clusters in IL-2 reception compared with isolated T cells, due to the higher local IL-2 concentration at the synaptic junction – both in terms of the amount of IL-2 accumulated and in terms of the “focusing” of the cytokine into apparent “patches” within the cell–cell contact.

Finally, consistent with the application of Pattern 4, we demonstrated the formation of signaling complexes of IL-2 receptors at the T–T synapses; Intracellular pools of IL-2, IL-2 receptors (IL-2Rs), and signaling components of IL-2R accumulated near the interfaces formed between activating T cells.

Additionally, the synaptic structure appears to alter signaling in a fundamental way for IL-2 signaling. IL-2 binding to IL-2R induces phosphorylation of STAT-5 by Janus kinase 1 (JAK1) and JAK3 which are associated with β and γ subunits of IL-2 receptor, respectively (Lin and Leonard 2000). Phosphorylated STAT-5 (pSTAT-5) is known to dimerize and subsequently translocate to the nucleus for the transcription of target genes. We fluorescently stained pSTAT-5 to measure the

strength of IL-2 signaling, and substantial amounts of T cells in the clusters exhibited higher pSTAT-5 staining than isolated T cells, indicating enhanced IL-2 signaling in the T-cell clusters. Interestingly, pSTAT-5 localized near interfaces of cell–cell contact as well as nuclei, and staining of pSTAT-5 near synaptic junctions revealed bright puncta. When overlaid with intracellular pools of IL-2 by dual staining, the majority of pSTAT-5 puncta were either co-localized or adjacent to IL-2 staining of neighboring cells, suggesting that pSTAT-5 accumulation near synaptic region was a result of directional IL-2 secretion. This observation agrees well with the finding that IL-2 signaling during anti-viral CD4+ priming was mostly paracrine, not autocrine (Long and Adler 2006), and indeed synaptic spaces formed between activated T cells may be the place where IL-2 paracrine delivery occurs.

Together, this provides a newly discovered application of the synapse patterns in activating T cells, following TCR stimulation. Unlike the more prototypical (T-APC, B-DC, NK-Target) examples involved in the initial priming of the cells by antigen–receptor ligand bearing cells, it suggests a specialized platform for cytokine mediated interactions.

5 Signaling Implication of T–T contacts for IL-2 Receptor Structure and Function

What does the discovery of “synaptic T–T IL-2 signaling” in particular contribute to our understanding of this cytokine and its function? It is clear from multiple studies that IL-2 can be added “in solution” and will function this way (Laurence et al. 2007; Liao et al. 2008), suggesting that it is not technically necessary that the secretion starts out being directional. Then, is it possible that there are major differences between cytokine signaling via synaptic junction and cytokine signaling by the binding of cytokines from the bulk? It is straightforward to imagine the enhancement of cytokine signaling via directed secretion of cytokines and polarization of cytokine receptors to the synaptic region by increasing local concentration of cytokines. In fact, that was the case when the local cytokine level was measured by cell-based IL-2 capture assay, and cytokine signaling strength was measured by the level of phosphorylation of STAT-5 (Sabatos et al. 2008). Also, through synaptic secretion and uptake, the majority of cytokines secreted by one cell would be captured by the other cell and little cytokine would be released outside of the synaptic space, resulting in increased specificity/efficiency on a per-molecule basis. However, the functional significance of this array may extend beyond this simple “efficiency” aspect and is indicated, as discussed in the observation of phosphorylated STAT-5 on the membrane in addition to within the nucleus, the latter being the prevailing result from experiments using soluble cytokines. To understand this, it is necessary to review the known mechanisms of IL-2 receptor signaling.

IL-2 receptor is composed of three distinct polypeptide chain subunits; IL-2R α (CD25), IL-2R β (CD122, also IL-15R β), and common γ c (CD132, also a signaling receptor of many other cytokines such as IL-4, IL-7, IL-9, IL-15, and IL-27) (Waldmann 2006). Combinations of three subunits constitute receptors with three different affinities; low affinity receptor IL-2R α ($K_d \sim 10$ nM), intermediate affinity heterodimeric receptor IL-2R $\beta\gamma$ c ($K_d \sim 1$ nM), and high affinity heterotrimeric receptor IL-2R $\alpha\beta\gamma$ c ($K_d \sim 10$ pM) (Gaffen and Liu 2004; Kim et al. 2006). IL-2R α is significantly upregulated upon activation, to at least an order of magnitude higher than the expression level of IL-2R $\beta\gamma$ c (Robb et al. 1987), and has very short cytoplasmic domain. Thus, it is suggested that the main role of IL-2R α is to enhance cytokine binding by forming high affinity heterotrimeric receptors with IL-2R $\beta\gamma$ c, or by first capturing IL-2 from the extracellular environment, due to its high abundance and fast on-rate, and subsequently forming a heterotrimeric receptor with IL-2R $\beta\gamma$ c (Stauber et al. 2006; Wang et al. 2005). IL-2R β and γ c are members of type I cytokine receptor super family and play a central role in IL-2 signaling (Gaffen 2001). Cytokine binding to IL-2R $\beta\gamma$ c triggers phosphorylation of the receptor and JAK1 and JAK3, which are associated with the cytoplasmic tails of IL-2R β and γ c, respectively. Phosphorylation of the receptor induces the association of STAT-5, a key transcription factor of IL-2 signaling, with the phosphorylated receptor and subsequent phosphorylation of STAT-5. Then, pSTAT-5 dissociates from the receptor, dimerizes, and translocates to the nucleus to activate multiple genes. At T–T junctions, when the three polypeptide chains of IL-2 receptors were stained, distinct patterns of receptor distribution were observed; IL-2R α distribution was mostly uniform, while substantial local enrichment of γ c in synaptic regions was frequently observed. (IL-2R β staining was too dim to be detected.) This, a priori, suggests a variable stoichiometry of the three-chains across the cell–cell interface; as mentioned above, expression level of IL-2R α is at least ten-fold higher than that of IL-2R $\beta\gamma$ c.

Under what conditions of receptor–ligand occupancy might this result be explained? It could just reflect the local accumulation of trimeric IL-2R near synaptic interfaces and enhanced paracrine signaling of IL-2 as a result (upper panel of Fig. 5). However, one interesting possibility is that IL-2 captured by one T cell's IL-2R α may interact with IL-2R $\beta\gamma$ c of another T cell through a T–T synapse (middle panel of Fig. 5). This type of cytokine transpresentation has been well documented for IL-15, a cytokine with significant similarities to IL-2; IL-15 bound to the IL-15R α ($K_d \sim 10$ pM) of monocytes or dendritic cells can trigger signaling to NK cells or CD8+ memory T cells, which constitutively express IL-15R $\beta\gamma$ c (Dubois et al. 2002). Given the structural similarity of IL-2R α and IL-15R α , IL-2R α also can present receptor-bound IL-2 to neighboring cells (Chirifu et al. 2007). Indeed there is evidence that IL-2 transpresentation occurs between IL-2R α expressing cells and IL-2R $\beta\gamma$ c expressing cells (Eicher and Waldmann 1998). Since the binding affinity of IL-2 for IL-2R α is about 1,000-fold lower than the binding affinity of IL-15 for IL-15R α , IL-2 transpresentation might require specialized synaptic junctions such as T–T synapses.

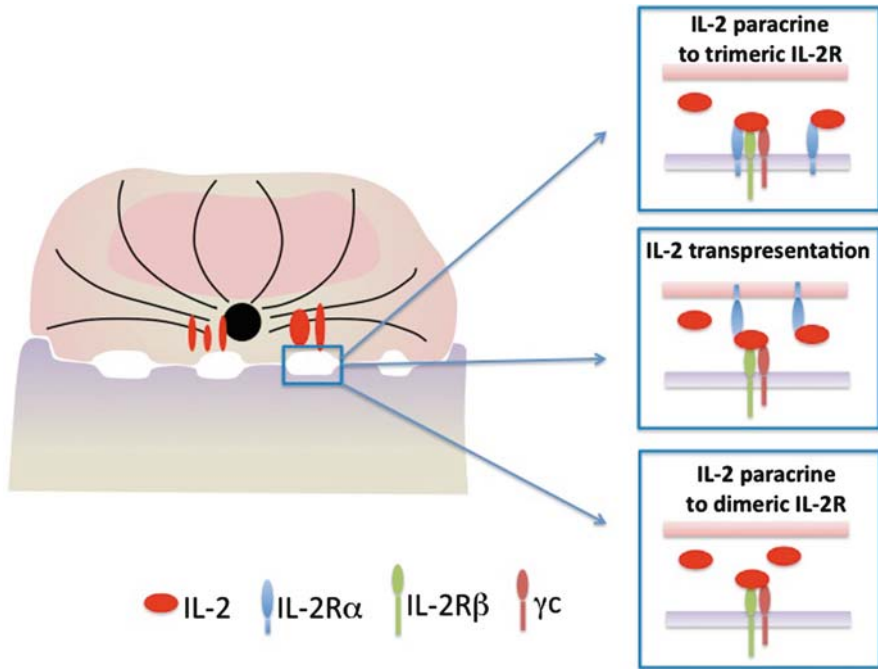


Fig. 5 Three potential configurations of IL-2/IL-2R binding at T-T synaptic junction. IL-2 directionally secreted to the synapses formed between activating T cells can be bound to the heterotrimeric receptor IL-2R $\alpha\beta\gamma$ C (*upper*), or be bound first by IL-2R α of one T cell and subsequently presented to the other T cell (*middle*), or be bound to the heterodimeric receptor IL-2R $\beta\gamma$ C and trigger receptor signaling in the absence of IL-2R α (*lower*)

IL-2 bound to IL-2R $\alpha\beta\gamma$ C was shown to be subsequently internalized and degraded with IL-2R $\beta\gamma$ C, while IL-2R α is dissociated from quaternary complexes in endosomes and recycled to cell surfaces (Hemar et al. 1995). If an IL-2 molecule bound to IL-2R α of one cell can interact with IL-2R $\beta\gamma$ C on the other cell, the duration of IL-2 signaling by the IL-2R $\beta\gamma$ C expressing cell might be substantially extended by the suppression of receptor internalization and degradation. Therefore, local accumulation of pSTAT-5 near the synaptic junction may be an evidence of extended duration of IL-2 signaling by transpresentation of IL-2.

Additionally, IL-2 transpresentation may be beneficial to “less”-activated T cells with lower IL-2R α expression than adjacent “more”-activated T cells that can transpresent IL-2. In this way, successfully activated T cells may assist new clones which arrive at the priming site later, or have weaker TCR affinity, or are specific to less abundant foreign antigens. As a result, diversity of TCR repertoire against foreign pathogens can be increased and immune evasion by mutation or antigen presentation disruption can be minimized.

Transpresentation of IL-2 may not be necessary for cooperation among activating T cells, though. Directionally secreted IL-2 to synaptic spaces could directly

bind intermediate affinity receptor IL-2R $\beta\gamma$ c and signal through IL-2R $\beta\gamma$ c without binding IL-2R α (lower panel of Fig. 5). Again, this is beneficial for T cells with low expression levels of IL-2R α . It is important to note that under model antigen ovalbumin immunization, the activating clusters of T cells were mostly composed of antigen specific transgenic T cells and participation of wild type T cells in the clusters was minimal. This indicates that T-cell synapses would not assist activation of antigen non-specific T cells (Sabatos et al. 2008).

Finally, we note that many receptor–ligand pairs are engaged at the synaptic junction formed between activating T cells. One example is the interaction of LFA-1 with ICAM-1 and ICAM-2, which plays a critical role in the formation and maintenance of the synapse, but may also trigger some signaling to T cells. It is possible that T–T synapses may mediate crosstalk between IL-2R signaling and other receptor signaling pathways by promoting various receptor–ligand interactions.

6 Additional Roles of T–T Synaptic Contact

In the previous chapters, we described a novel immune synapse formed between activating CD4+ T cells, mostly at the molecular and cellular levels. In this chapter, we will discuss further the potential roles of T–T synapses in modulating immune responses under various physiological circumstances. Also, we will extend the discussion of T–T synapses from homotypic clusters of activating CD4+ T cells to multicellular clusters composed of multiple subsets of T cells.

6.1 *Physiological Circumstances of T-Cell Cluster Formation and Its Role in Secondary Responses*

Only tiny fractions of T cells recognize antigens from a specific pathogen. Therefore, if all the T cells in the lymph node are randomly migrating in search of antigens, the possibility of multiple activating T cells intermingling within the same lymph node would be extremely low. However, it has been recently shown that in inflamed lymph nodes, when T cells (either CD4+ or CD8+) recognize DCs presenting their target antigens, both T cells and DCs secrete chemokines CCL3 and CCL4 to recruit CCR5 expressing T cells (Castellino et al. 2006; Hugues et al. 2007). This chemokine driven migration of activating T cells might enable multicellular cluster formation of activating T cells, even low physiological precursor frequencies.

CCL3/CCL4 secreted by activating T cells can recruit both CD4+ and CD8+ T cells. Thus, activating CD4+ T cells and CD8+ T cells may intermingle via synaptic interactions. Synaptic delivery of IL-2 during priming of CD8+ T cells could drive then IL-2 paracrine signaling critical for the expansion of CD8+ memory T cell upon secondary challenge (Williams et al. 2006).

Increases in precursor frequencies would also increase the probability of synaptic T–T interactions; memory responses and alloreactive T-cell activation leading to transplantation rejection are two examples of physiological high precursor frequencies. In fact, substantial clustering of memory T cells in lymph nodes was observed during the secondary challenge of lymphocytic choriomeningitis virus (R.S. Friedman, J. Hu, M.F.K., and M. Mattloubian). The clusters we observed may ultimately play a more prominent role once precursor levels are higher or in response to pathogens that stimulate a large fraction of T cells in the primary activation.

6.2 “Quorum Sensing” by the Immune System for Activation and Differentiation of the Effectors

Can information sharing across a synaptic junction confer the capacity for quorum decision making in populations of T cells, such that the response ultimately focuses on the correct response? Even under identical stimulation conditions, cytokine secretion profiles at the single cell level are quite diverse, and typically only a subpopulation of T cells produces certain cytokines. A detailed mechanism or exact reason for this heterogeneity is not clear yet, but the heterogeneity of activating T cells may require their cooperation for optimal activation and differentiation by sharing resources. IL-2 is indeed a critical factor for survival, proliferation, and differentiation of T cells, whose mRNA transcription occurs in only subpopulation of activating T cells (Saparov et al. 1999). If collaboration is necessary, there might be a “critical number” of T cells for full activation and differentiation, something akin to bacterial “quorum sensing.” There are evidences that increases in precursor frequencies may inhibit full activation and differentiation of T cells due to the internal competition among T cells for the acquisition of limited amount of resources in vivo (Bar et al. 2008; Hataye et al. 2006). These results appear to contradict our “quorum sensing” hypothesis, but it can be reconciled if there are “optimal” ranges of initial precursor frequencies – below which T cells are poorly activated due to lack of cooperation, and above which T cells are poorly activated due to internal competition. Alternatively, clonal competition may occur among identical clones or clones specific against identical epitopes, while cooperativity among T cells may take place among activating T cells specific for different epitopes from identical pathogens.

6.3 Polarization of Helper T-Cell Differentiation via Synaptic Cytokine Sharing

CD4+ helper T cells differentiate into various subsets of effectors depending on the cytokine milieu they are exposed to for the effective clearance of diverse pathogens

(Constant and Bottomly 1997; Bettelli et al. 2008). Key cytokines for Th differentiation and their genetic regulation have been extensively studied, but how those cytokines coordinate the differentiation of T cells *in vivo* has still remained elusive. We propose that immune synapses formed between T cells serve as platforms to spread differentiated phenotypes of effector T cells by directional secretion of key cytokines. It has been shown that some cytokines critical for Th skewing such as IL-2, γ IFN, and IL-10 are directionally secreted (Huse et al. 2006), and some of their receptors are also polarized toward the immune synapses (Maldonado et al. 2004), suggesting that synaptic secretion of those cytokines via T–T synapse may happen, and indeed may play a critical role in the propagation of phenotypes of already polarized T cells participating in the synapses. However, IL-4, a critical cytokine for Th2 differentiation and also for differentiation of T cells to a newly discovered IL-9- and IL-10-producing subset (Dardalhon et al. 2008; Veldhoen et al. 2008), is secreted multidirectionally. Additionally, many pro- and anti-inflammatory cytokines critical for Th differentiation and reprogramming at the periphery are not, or may not be delivered directionally. Therefore, it is likely that combinations of synaptic and non-synaptic secretion of cytokines would guide proper differentiation of activating and activated T cells depending on the circumstances.

6.4 T–T Interactions during the Cessation of the Immune Response: The Facilitation of Fas/TNF Interactions Leading to Apoptosis?

Immune synapses formed, even transiently, between T cells may also down-modulate the response by facilitating engagements of TNF-receptor family members, inducing apoptosis to each other. It is well-established that cell–cell contacts by activating T cells can lead to activation-induced cell death (AICD) (Lenardo 1991), frequently via Fas/FasL or TNF receptor engagements (Sytwu et al. 1996). It is thus tempting to speculate that the pattern we have observed for T–T engagements both *in vitro* and *in vivo* will play regulatory roles in permitting or facilitating the down regulation of the response by this mechanism. To this end, it is worth noting that synaptic contacts have been shown to recruit other TNF-family-member transmembrane proteins to the T–APC synapse (Boisvert et al. 2004).

6.5 Treg Exclusion in T–T Contacts

It was recently reported that regulatory T cells take up IL-2 more rapidly than activating T cells even though their IL-2R α expression levels are comparable (Pandiyani et al. 2007). Since IL-2 is a critical survival factor for activating T cells, IL-2 deprivation in activating CD4⁺ T cells due to rapid IL-2 uptake by

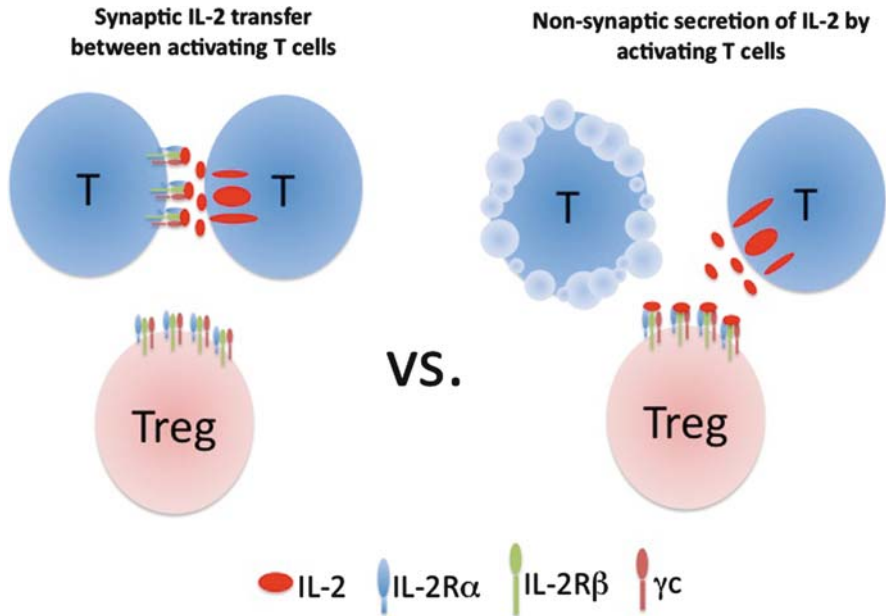


Fig. 6 Effect of synaptic secretion of IL-2 on IL-2 dependent survival of activating T cells. If IL-2 is secreted via synaptic interface, it will be successfully transferred to the neighboring activating T cells (*left*). In contrast, if IL-2 is secreted in non-synaptic manner, most of IL-2 secreted to the bulk will be taken up by adjacent Tregs, and neighboring T cells may undergo apoptosis due to the starvation of IL-2 (*right*)

adjacent Treg may cause the death of activating T cells. Synaptic secretion of IL-2 among activating CD4⁺ T cells may be important in situations where activating T cells and regulatory T cells compete for limited amount of IL-2 (Fig. 6). If combined with the recently reported negative feedback regulation of IL-2 secretion (Villarino et al. 2007), synaptic secretion of IL-2 in multicellular clusters of activating T cells may allow for optimal secretion of IL-2 “just enough” for T cells in the clusters, so that IL-2 uptake/signaling by neighboring Treg may be minimal. According to our observation, regulatory T cells make only transient contact with CD4⁺ T cells during priming in lymph nodes, indicating that regulatory T cells will not take part in multicellular clusters of activating T cells (Tang et al. 2006).

7 Creating System-Wide Decisions Through Collective and Spatiotemporal Information Sharing

It should be clear from the discussion of the application of synapse patterns at T–T junctions that such contacts are not neutral for the functioning of the immune response. In the case of the T–T junction, we have discussed numerous implications

of the pattern for the outcome of the T-cell response. These can be summarized by the following criteria that would seem critical for the integrity of the immune system.

7.1 Cell-Based Vectorial Spreading of Information

High motility between contact formations allows the immune system to use individual immune cells as “vectors” to carry information from one contact to another. Modifications to the cells’ signaling potential from one contact is thus purveyed, possibly over a great distance, and transmitted as a secretory or transmembrane signal, at the next.

7.2 Selection of a System of Appropriate Cell Types

Implicit in Pattern 2 is the idea that a given cell may choose to secrete only into a single cell at any one time. This also implies that it is capable of choosing the “type” of cell (APC, T, NK, Macrophage, etc.) into which it will secrete. Thus, a wide variety of synapse opportunities permits the activated cell to discriminate and activate specific cell types.

7.3 A Very Steep Gradient of Cues at Each Encounter Point

Pattern 3 dictates a very steep gradient of the most important cues surrounding activated cells. While some secreted molecules might “spill” from the synaptic space, the concentration would be dramatically higher in the inter-cellular clefts. In this light it is interesting that chemokines, which attract cells to a region, appear not to follow Pattern 2 (i.e., may be non-directionally secreted). However, the ultimate ability of each attracted cell to reap signaling benefits from following a chemokine cue is still maintained, possibly by selection for the dwell-time in contacts once contact is achieved. By analogy to the single Herald of Aristotle, it is as if non-citizens (cells) can still hear the herald but only citizens have the right to vote (to make a substantial synapse bearing the features of Patterns 2–4).

7.4 Repeated Selection for Specificity and Mutual Enhancement

Since each T, NK, or B cell that moves and re-engages has already been selected for its recognition of foreign antigen, it transmits to each APC or target a signal that corresponds to the strength of the signal. Thus, T cells are not only first activated based on their ability to recognize the pMHC complex alongside the arsenal of

costimulatory signals that a local APC may provide – it also carries along the ability to retransmit the degree of stimulation to APCs based on their ability to interact. In the case of secondary T–APC contacts, this may again rely on the presence of pMHC complexes on the second APC to influence the synapse duration and therefore the accumulation of focal signaling clusters (Pattern 4). In the case of T–T contacts, more primordial “activation” may serve as the basis of mutual adhesion and lead to specific transmission of information to cells bearing appropriate cell-surface signatures. This permits enhancement of the response over a period of time while selecting against cells whose specificity for the insult is not as significant.

8 Concluding Remarks

Two final notes bear stating. Firstly, these patterns are malleable with regard to microenvironment. Some parts of the patterns may be inhibited by prevailing conditions – for example the tumor microenvironment could allow contact surfaces while preventing polarization of secretion (Allows patterns 1, 3, and 4 while inhibiting 2). Secondly, to understand the building blocks and their implications is to be able to consider therapeutics. It may be that successful particle-based therapeutics may be designed to “seed” synapses of various kinds and for various stages of cell activation.

Therefore, it is the quality of these interactions that actually defines the efficacy of the system as a whole. In the case of human beings, the quality of interactions defines the growth and prosperity of the human community; destructive interactions lead to erosion of structure and the social fabric whereas positive interactions lead to cooperative growth and prosperity. In the case of the immune system, the quality of cell–cell interactions defines the successful survival of the larger organism in a world crowded with parasites and pathogens.

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References

- Alexander C, Ishikawa S, Silverstein M (1977) A pattern language: towns, buildings, construction. Oxford University Press, New York
- Anderson RGW, Jacobson K (2002) Cell biology – a role for lipid shells in targeting proteins to caveolae, rafts, and other lipid domains. *Science* 296(5574):1821–1825

- Bajenoff M et al (2006) Stromal cell networks regulate lymphocyte entry, migration, and territoriality in lymph nodes. *Immunity* 25(6):989–1001
- Bar JJ, Khanna KM, Lefrancois L (2008) Endogenous naive CD8(+) T cell precursor frequency regulates primary and memory responses to infection. *Immunity* 28(6):859–869
- Batista FD, Iber D, Neuberger MS (2001) B cells acquire antigen from target cells after synapse formation. *Nature* 411(6836):489–494
- Bettelli E et al (2008) Induction and effector functions of T(H)17 cells. *Nature* 453(7198):1051–1057
- Boisvert J, Edmondson S, Krummel MF (2004) Immunological synapse formation licenses CD40-CD40L accumulations at T-APC contact sites. *J Immunol* 173(6):3647–3652
- Bouso P, Robey E (2003) Dynamics of CD8(+) T cell priming by dendritic cells in intact lymph nodes. *Nat Immunol* 4(6):579–585
- Brossard C et al (2005) Multifocal structure of the T cell – dendritic cell synapse. *Eur J Immunol* 35(6):1741–1753
- Cahalan MD, Parker I (2008) Choreography of cell motility and interaction dynamics imaged by two-photon microscopy in lymphoid organs. *Annu Rev Immunol* 26:585–626
- Campi G, Varma R, Dustin ML (2005) Actin and agonist MHC-peptide complex-dependent T cell receptor microclusters as scaffolds for signaling. *J Exp Med* 202(8):1031–1036
- Castellino F et al (2006) Chemokines enhance immunity by guiding naive CD8(+) T cells to sites of CD4 T cell-dendritic cell interaction. *Nature* 440(7086):890–895
- Celli S, Lemaitre F, Bouso P (2007) Real-time manipulation of T cell-dendritic cell interactions in vivo reveals the importance of prolonged contacts for CD4(+) T cell activation. *Immunity* 27(4):625–634
- Cemerski S et al (2008) The balance between T cell receptor signaling and degradation at the center of the immunological synapse is determined by antigen quality. *Immunity* 29(3):414–422
- Chirifu M et al (2007) Crystal structure of the IL-15-IL-15R alpha complex, a cytokine-receptor unit presented in trans. *Nat Immunol* 8(9):1001–1007
- Choudhuri K et al (2005) T-cell receptor triggering is critically dependent on the dimensions of its peptide-MHC ligand. *Nature* 436(7050):578–582
- Constant SL, Bottomly K (1997) Induction of TH1 and TH2 CD4+ T cell responses: the alternative approaches. *Annu Rev Immunol* 15:297–322
- Dardalhon V et al (2008) IL-4 inhibits TGF-beta-induced Foxp3(+) T cells and, together with TGF-beta, generates IL-9(+) IL-10(+) Foxp3(-) effector T cells. *Nat Immunol* 9(12):1347–1355
- Delon J, Kaibuchi K, Germain RN (2001) Exclusion of CD43 from the immunological synapse is mediated by phosphorylation-regulated relocation of the cytoskeletal adaptor moesin. *Immunity* 15(5):691–701
- DeMond AL et al (2006) Control of antigen presentation with a photoreleasable agonist peptide. *J Am Chem Soc* 128(48):15354–15355
- Depoil D et al (2005) Immunological synapses are versatile structures enabling selective T cell polarization. *Immunity* 22(2):185–194
- Dougllass AD, Vale RD (2005) Single-molecule microscopy reveals plasma membrane microdomains created by protein-protein networks that exclude or trap signaling molecules in T cells. *Cell* 121(6):937–950
- Dubois S et al (2002) IL-15R alpha recycles and presents IL-15 in trans to neighboring cells. *Immunity* 17(5):537–547
- Dustin ML, Colman DR (2002) Neural and immunological synaptic relations. *Science* 298(5594):785–789
- Dustin ML et al (1997) Antigen receptor engagement delivers a stop signal to migrating T lymphocytes. *Proc Natl Acad Sci USA* 94(8):3909–3913
- Dustin ML et al (1998) A novel adaptor protein orchestrates receptor patterning and cytoskeletal polarity in T-cell contacts. *Cell* 94(5):667–677

- Dustin ML et al (2006) T cell-dendritic cell immunological synapses. *Curr Opin Immunol* 18 (4):512–516
- Eicher DM, Waldmann TA (1998) IL-2R alpha on one cell can present IL-2 to IL-2R beta/gamma (c) on another cell to augment IL-2 signaling. *J Immunol* 161(10):5430–5437
- Faroudi M et al (2003) Cutting edge: T lymphocyte activation by repeated immunological synapse formation and intermittent signaling. *J Immunol* 171(3):1128–1132
- Gaffen SL (2001) Signaling domains of the interleukin 2 receptor. *Cytokine* 14(2):63–77
- Gaffen SL, Liu KD (2004) Overview of interleukin-2 function, production and clinical applications. *Cytokine* 28(3):109–123
- Garcia Z et al (2007) Competition for antigen determines the stability of T cell-dendritic cell interactions during clonal expansion. *Proc Natl Acad Sci USA* 104(11):4553–4558
- Germain RN et al (2006) Dynamic imaging of the immune system: progress, pitfalls and promise. *Nat Rev Immunol* 6(7):497–507
- Grakoui A et al (1999) The immunological synapse: a molecular machine controlling T cell activation. *Science* 285(5425):221–227
- Gunzer M et al (2000) Antigen presentation in extracellular matrix: interactions of T cells with dendritic cells are dynamic, short lived, and sequential. *Immunity* 13(3):323–332
- Hataye J et al (2006) Naive and memory CD4(+) T cell survival controlled by clonal abundance. *Science* 312(5770):114–116
- Hemar A et al (1995) Endocytosis of interleukin-2 receptors in human T-lymphocytes – distinct intracellular-localization and fate of the receptor alpha-chain, beta-chain, and gamma-chain. *J Cell Biol* 129(1):55–64
- Henrickson SE et al (2008) T cell sensing of antigen dose governs interactive behavior with dendritic cells and sets a threshold for T cell activation. *Nat Immunol* 9(3):282–291
- Hommel M, Kyewski B (2003) Dynamic changes during the immune response in T cell-antigen-presenting cell clusters isolated from lymph nodes. *J Exp Med* 197(3):269–280
- Hugues S et al (2007) Dynamic imaging of chemokine-dependent CD8(+) T cell help for CD8(+) T cell responses. *Nat Immunol* 8(9):921–930
- Hurez V et al (2003) Restricted clonal expression of IL-2 by naive T cells reflects differential dynamic interactions with dendritic cells. *J Exp Med* 198(1):123–132
- Huse M et al (2006) T cells use two directionally distinct pathways for cytokine secretion. *Nat Immunol* 7(3):247–255
- Huse M et al (2007) Spatial and temporal dynamics of T cell receptor signaling with a photo-activatable agonist. *Immunity* 27(1):76–88
- Ingulli E et al (1997) In vivo detection of dendritic cell antigen presentation to CD4(+) T cells. *J Exp Med* 185(12):2133–2141
- Kim HP, Imbert J, Leonard WJ (2006) Both integrated and differential regulation of components of the IL-2/IL-2 receptor system. *Cytokine Growth Factor Rev* 17(5):349–366
- Krummel MF et al (2000) Differential clustering of CD4 and CD3 zeta during T cell recognition. *Science* 289(5483):1349–1352
- Kupfer A, Mosmann TR, Kupfer H (1991) Polarized expression of cytokines in cell conjugates of helper T cells and splenic B cells. *Proc Natl Acad Sci USA* 88(3):775–779
- Kupfer H, Monks CR, Kupfer A (1994) Small splenic B cells that bind to antigen-specific T helper (Th) cells and face the site of cytokine production in the Th cells selectively proliferate: immunofluorescence microscopic studies of Th-B antigen-presenting cell interactions. *J Exp Med* 179(5):1507–1515
- Laurence A et al (2007) Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. *Immunity* 26(3):371–381
- Lee KH et al (2002) T cell receptor signaling precedes immunological synapse formation. *Science* 295(5559):1539–1542
- Lenardo MJ (1991) Interleukin-2 programs mouse alpha-beta-lymphocytes-T for apoptosis. *Nature* 353(6347):858–861

- Liao W et al (2008) Priming for T helper type 2 differentiation by interleukin 2-mediated induction of interleukin 4 receptor alpha-chain expression. *Nat Immunol* 9(11):1288–1296
- Lillemeier BF et al (2006) Plasma membrane-associated proteins are clustered into islands attached to the cytoskeleton. *Proc Natl Acad Sci USA* 103(50):18992–18997
- Lin JX, Leonard WJ (2000) The role of Stat5a and Stat5b in signaling by IL-2 family cytokines. *Oncogene* 19(21):2566–2576
- Lindquist RL et al (2004) Visualizing dendritic cell networks in vivo. *Nat Immunol* 5(12):1243–1250
- Long MX, Adler AJ (2006) Cutting edge: paracrine, but not autocrine, IL-2 signaling is sustained during early antiviral CD4 T cell response. *J Immunol* 177(7):4257–4261
- Maldonado RA et al (2004) A role for the immunological synapse in lineage commitment of CD4 lymphocytes. *Nature* 431(7008):527–532
- McCann FE et al (2003) The size of the synaptic cleft and distinct distributions of filamentous actin, ezrin, CD43, and CD45 at activating and inhibitory human NK cell immune synapses. *J Immunol* 170(6):2862–2870
- Mempel TR, Henrickson SE, von Andrian UH (2004) T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. *Nature* 427(6970):154–159
- Miller MJ et al (2004) Imaging the single cell dynamics of CD4(+) T cell activation by dendritic cells in lymph nodes. *J Exp Med* 200(7):847–856
- Monks CRF et al (1998) Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* 395(6697):82–86
- Mossman KD et al (2005) Altered TCR signaling from geometrically repatterned immunological synapses. *Science* 310(5751):1191–1193
- Norcross MA (1984) A synaptic basis for T-lymphocyte activation. *Ann Immunol (Paris)* 135D:113–134
- Oddos S et al (2008) High-speed high-resolution imaging of intercellular immune synapses using optical tweezers. *Biophys J* 95:L66–L68
- Pandiyani P et al (2007) CD4(+) CD25(+) Foxp3(+) regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4(+) T cells. *Nat Immunol* 8(12):1353–1362
- Purbhoo MA et al (2004) T cell killing does not require the formation of a stable mature immunological synapse. *Nat Immunol* 5(5):524–530
- Reichert P et al (2001) Cutting edge: in vivo identification of TCR redistribution and polarized IL-2 production by naive CD4 T cells. *J Immunol* 166(7):4278–4281
- Rittirsch D, Flierl MA, Ward PA (2008) Harmful molecular mechanisms in sepsis. *Nat Rev Immunol* 8(10):776–787
- Robb RJ et al (1987) Interleukin 2 binding molecule distinct from the Tac protein: analysis of its role in formation of high-affinity receptors. *Proc Natl Acad Sci USA* 84:2002–2006
- Rothlein R, Springer TA (1986) The requirement for lymphocyte function-associated antigen 1 in homotypic leukocyte adhesion stimulated by phorbol ester. *J Immunol* 136(5):1132–1149
- Rothlein R et al (1986) A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. *J Immunol* 137(4):1270–1274
- Sabatos CA et al (2008) A synaptic basis for paracrine interleukin-2 signaling during homotypic T cell interaction. *Immunity* 29(2):238–248
- Saparov A et al (1999) Interleukin-2 expression by a subpopulation of primary T cells is linked to enhanced memory/effector function. *Immunity* 11(3):271–280
- Scholer A et al (2008) Intercellular adhesion molecule-1-dependent stable interactions between T cells and dendritic cells determine CD8(+) T cell memory. *Immunity* 28(2):258–270
- Shaw AS, Dustin ML (1997) Making the T cell receptor go the distance: a topological view of T cell activation. *Immunity* 6(4):361–369
- Sims TN et al (2007) Opposing effects of PKC theta and WASp on symmetry breaking and relocation of the immunological synapse. *Cell* 129(4):773–785
- Skokos D et al (2007) Peptide-MHC potency governs dynamic interactions between T cells and dendritic cells in lymph nodes. *Nat Immunol* 8(8):835–844

- Stauber DJ et al (2006) Crystal structure of the IL-2 signaling complex: paradigm for a heterotrimeric cytokine receptor. *Proc Natl Acad Sci USA* 103(8):2788–2793
- Stinchcombe JC et al (2001) The immunological synapse of CTL contains a secretory domain and membrane bridges. *Immunity* 15(5):751–761
- Sytwu HK, Liblau RS, McDevitt HO (1996) The roles of Fas/APO-1 (CD95) and TNF in antigen-induced programmed cell death in T cell receptor transgenic mice. *Immunity* 5(1):17–30
- Tang QZ et al (2006) Visualizing regulatory T cell control of autoimmune responses in nonobese diabetic mice. *Nat Immunol* 7(1):83–92
- Tohma S, Ramberg JE, Lipsky PE (1992) Expression and distribution of CD11a/CD18 and CD54 during human T cell-B cell interactions. *J Leukoc Biol* 52:97–103
- van der Merwe PA, Davis SJ (2003) Molecular interactions mediating T cell antigen recognition. *Annu Rev Immunol* 21:659–684
- van Kooyk Y et al (1989) Enhancement of LFA-1-mediated cell adhesion by triggering through CD2 or CD3 on T lymphocytes. *Nature* 342(6251):811–813
- Varma R et al (2006) T cell receptor-proximal signals are sustained in peripheral microclusters and terminated in the central supramolecular activation cluster. *Immunity* 25(1):117–127
- Veldhoen M et al (2008) Transforming growth factor-beta “reprograms” the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. *Nat Immunol* 9(12):1341–1346
- Villarino AV et al (2007) Helper T cell IL-2 production is limited by negative feedback and STAT-dependent cytokine signals. *J Exp Med* 204(1):65–71
- Waldmann TA (2006) The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design. *Nat Rev Immunol* 6(8):595–601
- Wang XQ, Rickert M, Garcia KC (2005) Structure of the quaternary complex of interleukin-2 with its alpha, beta, and gamma(c) receptors. *Science* 310(5751):1159–1163
- Whyte WH (1988) *City: rediscovering the center*. Doubleday, New York
- Wiedemann A et al (2006) Cytotoxic T lymphocytes kill multiple targets simultaneously via spatiotemporal uncoupling of lytic and stimulatory synapses. *Proc Natl Acad Sci USA* 103(29):10985–10990
- Williams MA, Tyznik AJ, Bevan MJ (2006) Interleukin-2 signals during priming are required for secondary expansion of CD8(+) memory T cells. *Nature* 441(7095):890–893
- Wulfiging C, Davis MM (1998) A receptor/cytoskeletal movement triggered by costimulation during T cell activation. *Science* 282(5397):2266–2269
- Yokosuka T et al (2005) Newly generated T cell receptor microclusters initiate and sustain T cell activation by recruitment of Zap70 and SLP-76. *Nat Immunol* 6(12):1253–1262
- Yokosuka T et al (2008) Spatiotemporal regulation of T cell costimulation by TCR-CD28 microclusters and protein kinase C theta translocation. *Immunity* 29(4):589–601