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Signaling at neuro/immune synapses

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Review

Immunological and neural synapses share properties such as the synaptic cleft, adhesion molecules, stability, and polarity. However, the mismatch in scale has limited the utility of these comparisons. The discovery of phosphatase micro-exclusion from signaling elements in immunological synapses and innate phagocytic synapses define a common functional unit at a common sub-micron scale across synapse types. Bundling of information from multiple antigen receptor microclusters by an immunological synapse has parallels to bundling of multiple synaptic inputs into a single axonal output by neurons, allowing integration and coincidence detection. Bonafide neuroimmune synapses control the inflammatory reflex. A better understanding of the shared mechanisms between immunological and neural synapses could aid in the development of new therapeutic modalities for immunological, neurological, and neuroimmunological disorders alike.

Signaling at neuro/immune synapses

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Introduction

Cell-cell communication systems in the immune and nervous systems share several features, which has led to the adoption of the common term "synapse" to describe the close cell-cell contacts in each. Chemical synapses in the nervous system can be defined as sites of stability, polarity, and vectoral communication, where two cells may adhere without fusion (1). The concept of the immune synapse was first applied to cells of the adaptive immune system, T and B cells, but has since expanded to include interactions involving innate immune cells such as NK cells and, more recently, phagocytes (2–7). Herein we refer to data from phagocytic, T cell, B cell, and NK cell synapses as specific subtypes of immunological synapses. Among all synapses, phagocytic synapses might serve as an ancestral template. Phagocytosis evolved in early single-cell organisms and allowed them to more efficiently compete for nutrients in the environment; the phagocytosis receptor system utilized by soil amoeba is similar to that employed by innate immune cells of mammals (8). While the term "phagocytic synapse" could be used in a general sense based on early studies of junctions driven by phagocytic receptors (9), the first effort to address how phagocytosis is selectively triggered by particulate, but not polyvalent, soluble ligands engaging the same receptors led to the proposal of a phagocytic synapse (ref. 7 and Figure 1, A and B). The threshold is a diameter of approximately 0.5 μm, which is similar to the size of T cell antigen receptor (TCR) microclusters that drive signaling in T cells (ref. 10 and Figure 1, C and D), and is the characteristic size of the neural synaptic connections (ref. 11 and Figure 1, E and F). In this review I discuss the molecular basis of the convergence on a submicron scale for basic elements, consider signal integration by immune cells and neurons, and discuss central control of inflammation through neuroimmune synapses.

A synaptic relay race with the pathogen

In the nervous system, even simple activities require the serial and parallel function of multiple synaptic connections. Similarly, the immune response is a relay race against the pathogen in which the baton is passed from innate to adaptive immune cells (Figure 2). Recent research suggests that multiple immune cell types employ similar molecular strategies, based on phosphatase exclusion, to target pathogens (7, 10, 12). Immature DCs phagocytose cell fragments greater than 0.5 μm in diameter, an innate immune function (innate leg in Figure 2A and ref. 13). This takes a matter of seconds, and detection of components associated with a live infection, such as microbial RNA, leads to maturation of the DCs and their migration to lymph nodes (14). Partial proteolytic degradation of the phagocytosed material allows for association of component peptides with MHC class II molecules that are routed to the cell's surface for priming of helper T cell precursors, the afferent phase of adaptive immunity (afferent leg in Figure 2A and ref. 15). DCs can also divert peptides to the MHC class I system in the endoplasmic reticulum for priming of cytotoxic T cell precursors (16). T and B cells utilize diverse repertoires of antigen receptors that are generated by somatic gene rearrangement, and the MHC-peptide complex–bearing DCs need to search through this repertoire to find T cells with the appropriate receptors. The DCs form dense networks in secondary lymphoid tissues and contact approximately 5,000 T cells per hour as the T cells move over reticular networks (17–19). Within a day, rare antigen-specific T cells locate these DCs and initiate clonal expansion as well as conditions for an immune response through the formation of provisionally stable T cell–DC interactions lasting on the order of 24 hours (20); by comparison, neural synapses may be stable for years (21). Nonetheless, in the absence of these stable interactions, the generation of long-lived memory T cells fails (22). After clonal expansion, the MHC class I restricted T cells can use a synapse to kill target cells, the efferent phase of adaptive immunity (efferent leg in Figure 2A and ref. 23), whereas the MHC class II restricted cells may use a synapse to help B cells generate neutralizing antibodies (efferent leg in Figure 2A and ref. 24).

B cells use synapses to gather intact viral antigens from macrophages, DCs, or follicular DCs in proportion to the affinity of their antigen receptor and process the antigens to make MHC class II peptide complexes to obtain help from T cells. Obtaining T cell help is a competitive process, and B cells with the highest-affinity receptors switch to producing the IgG isotype and differentiate into antigen-secreting plasma cells with T cell help (25).

NK cells are innate immune cells that work in concert with cytotoxic T cells to defend against viruses by using inhibitory receptors that bind MHC class I antigens and host-derived or virally encoded activating receptors to control the outcome of synapse formation (26). Loss of inhibition when a virus down-

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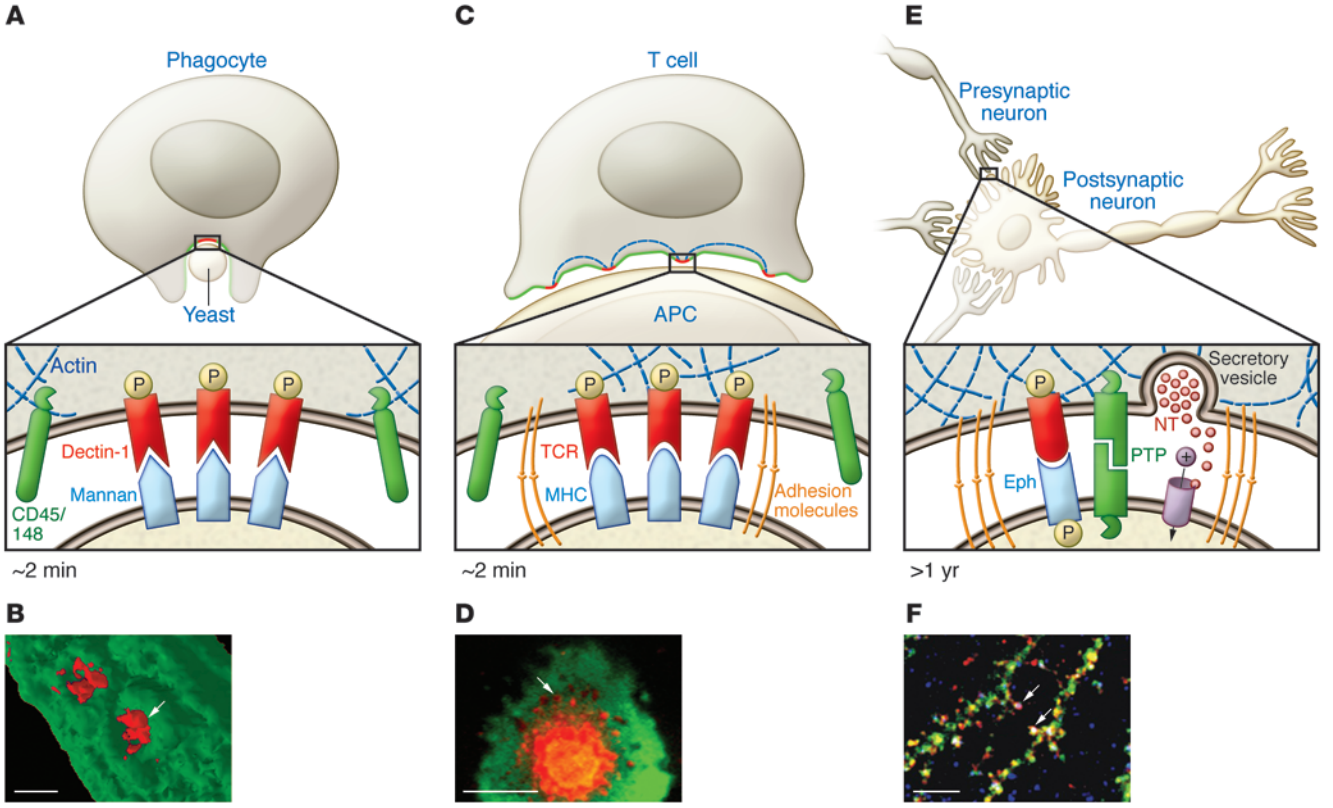

Figure 1
Role of submicron receptor complexes in immunological and neural synapses. (**A**) Phagocytosis is triggered when CD45 and CD148 are excluded from a region more than 0.5 μm in diameter in which Syk is phosphorylated. (**B**) Micrograph of a phagocytic synapse. Scale bar: 4 μm. Arrow indicates the exclusion zone. Reproduced with permission from *Nature* (7). (**C**) T cell synapses are larger interfaces in which TCR microclusters that exclude CD45 are formed. These are linked by mysosin II–based contractility to augment signaling and trigger effector functions. Linkage of microclusters through myosin II ensures that T cells respond to multiple coincident MHC-peptide signals. Inset: TCR/MHC-peptide interactions with the support of adhesion molecules form microclusters that exclude CD45 and trigger robust tyrosine phosphorylation. TCR microclusters are short lived. (**D**) Micrograph of a T cell synapse. Scale bar: 4 μm. Arrow points to an example of a microcluster. Reproduced with permission from *Nature* (10). (**E**) Neural synapses are stabilized by adhesion molecules and can recruit receptor tyrosine kinases. More restrained signaling may promote a longer-lived junction than can then be used to process action potentials into chemical synapse and compute one output from many inputs. Eph, Eph family tyrosine kinase; PTP, protein tyrosine phosphatase; NT, neurotransmitter. (**F**) Micrograph of a neural synapse. Green indicates receptor-type protein tyrosine phosphatase ρ, red indicates neuroligin, blue indicates PSD-95. Scale bar: 4 μm. Arrows indicate examples of RPTPρ colocalization with neuroligin. Reproduced with permission from *EMBO Journal* (43).

regulates MHC class I molecules as an evasion strategy, so called "missing-self" recognition, or increased activation due to expression of virally encoded activating ligands, will trigger the NK cells to kill (27).

The common element in all of these immune synapses is that the key triggering signals are accompanied by phosphatase exclusion from the site of interaction at a submicron scale as a means of enabling activation of kinases by the removal of an inhibitor. The submicron scale is important because it allows triggering to happen fast — in less than a second (28) — whereas large areas would require many seconds or even minutes, which is too slow to win the race with a pathogen.

Phosphatase exclusion from microclusters

Tyrosine phosphatase inhibition with chemical agents such as vanadate rapidly triggers T cell signaling, supporting the notion that tyrosine phosphatase exclusion could be used as a trigger for tyrosine kinase cascades (29, 30). Phosphatase exclusion models for immune cell triggering typically focus on the hematopoietic phosphatase CD45, which is a type I transmembrane protein with a large extracellular domain and a cytoplasmic tyrosine phosphatase domain (31, 32). TCRs and NK cells activating receptors all utilize the Src family tyrosine kinase Lck to mediate early phosphorylation events (33, 34). CD45 maintains Lck in an active state by removing a C-terminal inhibitory phosphate. However, CD45 also deactivates several targets of Lck at antigen receptors, and thus it was proposed, first as speculation by Springer (31) and later with experimental support by van der Merwe and my group (10, 35), that CD45 exclusion is a key initial event in TCR triggering. Addressing this issue at present requires the use of a reductionist model to enable sufficiently high-resolution imaging. Antibodies to the TCR complex and to CD28, a co-stimulatory receptor that is engaged by CD80 or CD86 when DCs are strongly activated by signs of infection, are very effective at activating T cells. Substrates coated with these antibodies completely exclude CD45 (36, 37), but this is not likely to be the physiological situation. Presenta-

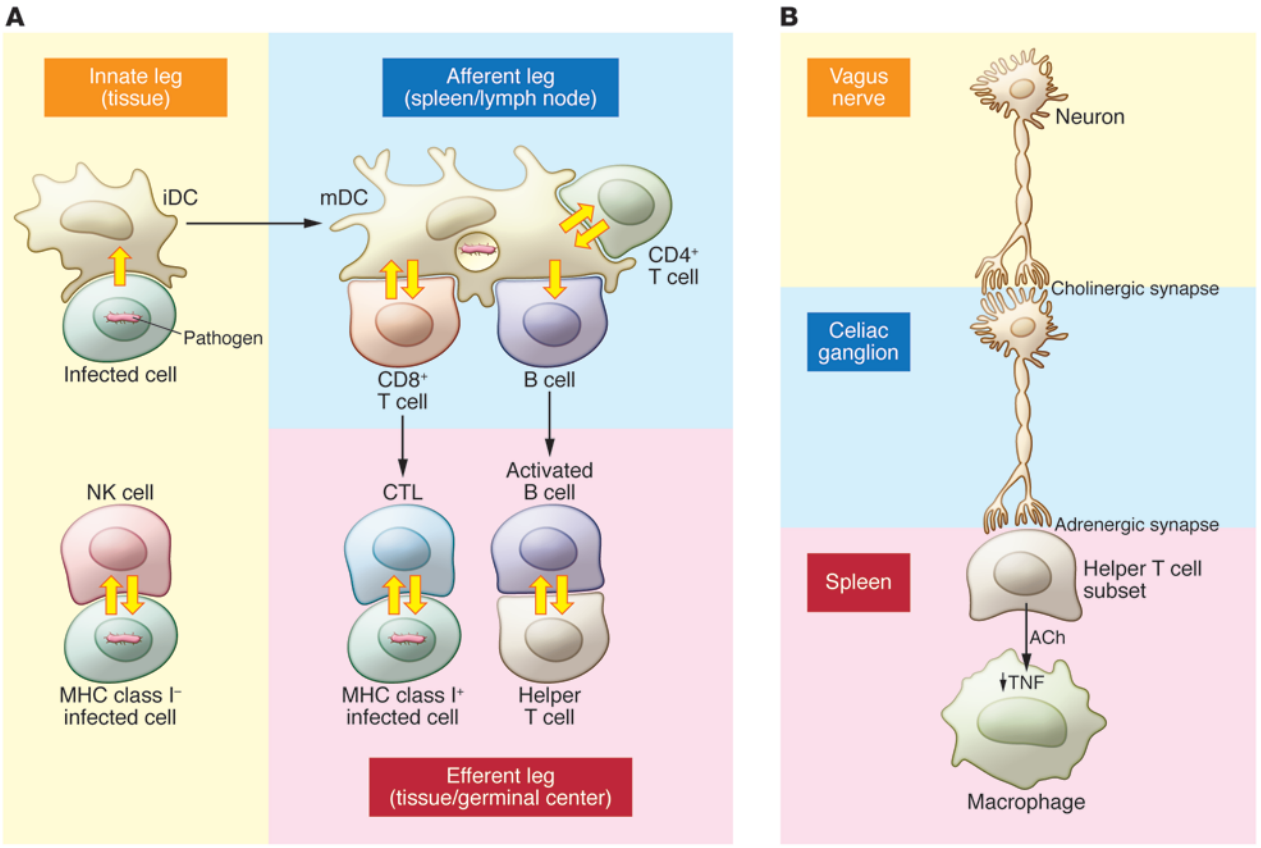

Figure 2
Immunological relay race. (**A**) The immune response is based on a series of immunological synapses with a common mechanism based on phosphatase exclusion. Innate leg: An intracellular pathogen infects cells, activating innate sensing mechanisms and leading to phagocytosis by an immature DC (iDC). This phagocytic synapse contributes to maturation of the DC (mDC). If the pathogen downregulates MHC class I in the infected cell, then the infected cell can be directly recognized by NK cells. Afferent leg: The mDC presents antigens on MHC class I to cytotoxic T cell precursors (CD8), on MHC class II to helper T cell precursors (CD4), and as intact complexes to B cells. Efferent leg: CTLs can directly kill MHC class I–positive infected cells, and the infected target induces cytokine production by the CD8 T cell. Helper T cells allow selection of high-affinity activated B cells and help B cells to generate an appropriate type of antibody. The B cell provides costimulatory molecules that promote cytokine production by the helper T cell. (**B**) The inflammatory reflex is based on innervation of a subset of helper T cells that express choline acetyltransferase. The vagus nerve relays signals to adrenergic neurons in the celiac ganglion that form neuroimmune synapses with the helper T cells. Adrenergic receptors on the T cell trigger production of acetylcholine (ACh), which interacts with cholinergic receptors on macrophages to suppress production of inflammatory cytokines such as TNF.

tion of MHC-peptide ligands and the adhesion ligand ICAM-1 on supported planar bilayers activates T cells (4, 38), but accurate assessment of CD45 exclusion from TCR microclusters requires total internal reflection fluorescence microscopy (TIRFM) (10). With the use of TIRFM, it became evident that TCR microclusters exclude CD45 (10). Clusters of B cell antigen receptors (BCRs) also excluded CD45 in the same spatially restricted fashion (12). TIRFM is required for this observation because when analyzed by confocal and deconvolution microscopy, the two layers of actin-rich protrusions on flat surfaces (lamellipodia) are closely apposed, making it appear as if CD45 levels are 2-fold higher than they actually are (39). The exclusion of CD45 from Dectin-1–rich clusters in the cells phagocytosing yeast cell walls was observed by confocal microscopy (7). Dectin-1, a β-glucan receptor, has kinase recruitment motifs similar to those of the TCR and BCR. The CD45 exclusion zones in this study were defined by contacts with β-glucan–rich yeast cell walls of approximately 5 μm diameter, which generated regions of CD45 exclusion larger than 2 μm. These results are exciting and suggest a unifying mechanism for triggering synapses, but further study of how Dectin-1 forms signaling complexes using systems that enable TIRFM or super-resolution imaging methods would be of great value in more precisely determining the relationship of Dectin-1 to CD45. In contrast, neural synapses are stabilized by receptor tyrosine kinases (40, 41) but actually recruit tyrosine phosphatases into the synaptic adhesion complexes. For example, protein tyrosine phosphatase, receptor type, M (PTPRμ) undergoes homophilic interactions in the context of cadherin-dependent adhesions (42), and other members of this family undergo heterophilic interactions with synaptic adhesion molecules (43, 44). The balance of phosphatase and kinase activity may allow for the much longer lifespan of neural synapses (years) compared with immunoreceptor microclusters (minutes).

The prototypic neural synapse has a scale of approximately 0.2–0.3 μm^2, which means that they have a diameter of only 0.5–0.6 μm,

similar to the scale of the microclusters in immunological synapses (11). The axon-based presynaptic structure includes secretory vesicles, and such structures can be triggered by adhesion to any surface such that restricting the formation of these structures to appropriate locations may be an important process in neural development (45). Presynaptic axonal terminals and postsynaptic dendritic spines transduce action potentials, moving along the axon into a chemical signal that generates a membrane potential change in the dendritic membrane through regulation of neurotransmitter secretion that involves Ca^{2+}-regulated snare proteins (46). In neurons, postsynaptic potentials, which can be activating or inhibitory, are integrated in the dendritic tree to generate (or not) an output action potential — effectively acting as analog-digital converters (47). Thus, in some respects, the T cell synapse, which integrates input from many microclusters, some of which may be activating and others inhibitory, is more akin to the dendritic tree of a neuron than any single neural synapse. Tetanus toxin–sensitive snare proteins deliver vesicles to the T cell synapse in response these signals (48).

Force-dependent coincidence detection in T cell synapses

In neural networks, reliability is ensured, in part, by coincidence detection (49). In the immune synapse, the exclusion of CD45 from activating receptor microclusters is a key process for signaling, but this is not sufficient. In T, B, and NK cell synapses the signaling from the early microclusters rapidly triggers an expansion of the contact area to 50–100 μm^2, even in the absence of other adhesion systems (50). The first evidence that microclusters on their own are insufficient to fully activate T cells came from studies examining activation of T cells by polystyrene beads of different sizes. These studies defined a bead size threshold of over 3 μm in diameter for stimulation of cytotoxic function by purified MHC class I–peptide complexes (51). These studies are the basis for current clinical-grade culture systems for T cell expansion in adoptive immunotherapy (52, 53). One way to interpret this basic result is that CTLs require activation through at least 2 microclusters spaced a few microns apart. T cell receptor signaling is dependent on an intact f-actin cytoskeleton (54). One molecular ruler that operates on this length scale in concert with f-actin is the myosin II thick filament, which requires at least 1 μm of space between sites to generate tension (55). Myosin IIA is the major myosin II isoform in T cells, and its activity is required for full T cell signaling (56). In some contexts, externally applied forces can also be used to trigger T cell signaling (57, 58). It has been unclear why T cells would integrate mechanotransduction modules into the activation process, given that it is not obvious how innate and adaptive signals would be converted into physical forces. One way to avoid errors in activation in a system with single-molecule sensitivity is to require that that same signal be received from physically distinct points on the T cell surface at the same time to trigger a response. Thus, making part of the T cell activation process dependent upon forces exerted by myosin II ensures that at least two MHC-peptide complexes need to trigger signaling events from locations at least 1 μm apart in order to develop force. Even the most sensitive signaling processes in which MHC-peptide counting studies have been performed required at least 3 MHC-peptide complexes to sustain T cell activation (59). Thus, while innate immunity may activate phagocytosis with a single microcluster-based signal, adaptive immunity led by T cells requires multiple, spatially distinct microclusters.

Organizing information in synapses

Both the nervous system and immune system utilize several types of receptors in synapses. In the immune system there are at least 2 types of microclusters into which these receptors are distributed. Kupfer first described the bullseye pattern of the T-B synapses with a ring of LFA-1, an integrin family adhesion molecule, surrounding a central cluster of TCR (60). Parallel studies with MHC-peptide complexes and LFA-1 ligand ICAM-1 presented in a supported planar bilayer with CD2 as an early marker for TCR-rich domains demonstrated that active processes in the T cells generate the pattern (4, 61). Kupfer described the LFA-1–rich ring as a peripheral supramolecular activation cluster (pSMAC) and the central TCR-rich cluster as a central supramolecular activation cluster (cSMAC). The initial contact area is formed by a rapid, f-actin–driven spreading that is mediated by the Rac effector WAVE2 to activate the Arp2/3 complex and formins (62, 63). Cdc42 and Wiscott-Aldrich syndrome protein also play a role in this process but are not needed for this initial spreading phase (64). TIRFM on the bilayer system has revealed that the SMACs are assembled by centripetal transport of LFA-1 and TCR microclusters (10, 65). The LFA-1 microclusters may include other integrin family adhesion molecules, although this has not been extensively studied. The TCR microclusters are well established to incorporate both the CD2-CD58 adhesion system and the CD28-CD80 costimulatory pathway. Negative regulators such as CTLA4 and PD-1 may also be incorporated into these microclusters in a ligand-dependent manner. Although segregated spatially, the LFA-1/ICAM-1 interaction improves the sensitivity of the TCR for ligand by 100-fold and increases the duration of Ca^{2+} signaling (66–68). These two microclusters may thus work as a synergistic functional unit that would be composed of a TCR microcluster surrounded by LFA-1 microclusters. Such a radial organization may exist in neural synapse with different receptors to initiate (neurexin) and limit (polysialated NCAM) the synapse (69, 70). Synaptogenesis has been reconstituted by incorporation of neuroligin into supported planar bilayers (71), but nonspecific adhesive contacts have also been shown to trigger presynaptic structures (45). Since neural synapse survival is dependent upon electrical activity and growth factors, synapse initiation may be less dependent upon specific recognition than the immunological counterpart (72). Furthermore, activation of immunoreceptor-like tyrosine kinase cascades in neurons leads to synapse pruning (73, 74).

In the immunological synapse, the LFA-1 accumulates in a ring associated with the adapter protein talin, whereas TCR microclusters translocate through spaces in this ring to the center of the synapse. This is dependent upon TSG101, an early component in the endosomal sorting complexes required for transport (ESCRTs) (75). TSG101 recognizes receptors with mono-ubiquitin groups. The TCR is ubiquitinated by c-Cbl and Cbl-b ubiquitin ligases that are recruited and activated under stimulation with agonist MHC-peptide complexes (76, 77). In fact, the very robust tyrosine phosphorylation due to CD45 exclusion may paradoxically promote TCR ubiquitination and rapid signal termination. TCR signaling is terminated by the TSG101-dependent step, which also sorts out the CD28-CD80 interactions into a distinct signaling structure rich in PKC-θ (75, 78). Long-term maintenance of neural synapses also depends upon correct function of endosomal sorting complexes required for transports (79, 80). Indeed, mutations in these components are linked to frontotemporal dementia (81).

TCR microclusters are continuously being buffeted by centripetal actin flow and myosin IIA–dependent contractions as discussed above. Theses effects decrease the duration of the TCR–MHC-peptide interaction by 10-fold, and at the same time are required to achieve full signaling activity (56, 82). The stable immunological synapse is dependent upon a continual centripetal actin flow, and the synapse breaks and relocates whenever the symmetry of the pSMAC structure is broken (64, 83). While most of these observations have been made using the supported planar bilayer model system, there is evidence for similar events in T cell–DC synapses in vivo and in vitro (64, 84). DCs add another dimension to the T cell synapse, as the DC cytoskeleton plays an important role in T cell activation (85–87). Each element in the multifocal T cell–DC immunological synapse appears to be a SMAC-like assembly of multiple microclusters, rather than single microclusters (84, 88). The actin cytoskeleton is also critical for pathfinding in axons (89) and in the shape of dendritic spines (90).

Neuroimmune synapses and the inflammatory reflex

The "inflammatory reflex" links vagus nerve activity to inhibition of pro-inflammatory cytokine production by macrophages in the spleen (91). This is important for control of immune homeostasis and to prevent immunopathology during infection. However, such reflexes can also become dysregulated and contribute to infection following injury to the brain (92). The vagus nerve suppresses TNF-α production by spleen through acetylcholine receptors on TNF-producing cells. However, the vagus nerve connection to the spleen is via adrenergic neurons from the celiac ganglion, thus it was unclear what cell produces acetylcholine. Work from Kevin Tracey's group determined that these adrenergic neurons synapse with choline acetyltransferase–expressing T cells in the spleen (91). Adrenergic stimulation of these T cells causes them to release acetylcholine, which then acts on nearby TNF-α–producing cells (Figure 2). These neuroimmune synapses have been documented by electron microscopy (93, 94) and the synaptic cleft is close enough, at 6 nm, to exclude CD45 and potentially induce arrest of motile T cells. In addition, neuroimmune synapses with mast cells that involve N-cadherin expression on the mast cells may be important in allergy (95). It will be interesting to evaluate the status of phosphatases in these neuroimmune synapses. Are phosphatases efficiently excluded, potentially leading to short-lived synapses due to negative feedback, or do T cells that express choline acetyltransferase also express RPTPs to engage in long-lived synapses with adrenergic termini? These are exciting therapeutic targets for inflammatory diseases and allergy.

Conclusions

Advances in the study of neural and immune synapses allow a more refined view of parallels and differences in these systems than was possible a few years ago. Recent studies of different types of immune synapses have emphasized the critical role of submicron structures more similar in scale to neural synapses. The ancestral phagocytic synapse serves as the simplest protoype. Actin-dependent immunoreceptor microclusters operate in part through a principle of receptor tyrosine phosphatase exclusion and coordination of signaling pathways by scaffold proteins. High-order integration through myosin II–dependent mechanisms verifies the presence of multiple agonist MHC-peptide complexes to improve fidelity of T cell signaling. Individual neural synapses are dependent on actin and scaffold proteins. The dendritic tree of a neuron has parallels to the immunological synapse, in that it integrates signaling from multiple submicron elements to generate a unified output. However, the much greater lifetime of neural synapses compared with immunological microclusters may require more sustainable signaling strategies that require recruitment of RPTPs, which can also contribute directly to synaptic adhesion. A better understanding of immunological and neural synapses has clear therapeutic value. The synaptic basis of neuroimmune communication is also coming into focus, and this area is particularly exciting due to the potential to execute rapid changes in immune status.

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