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.

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 vectorial communication, where two cells may adhere without fusion (1). The concept of the innate synapse was first applied to cells of the adaptive immune system, 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-
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-
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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.
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 Ca²⁺-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 to the TCR activation (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, signaling from the early microclusters rapidly triggers an expansion of the contact area to 50–100 μm², 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 apart in TCR-induced activation of T cells (51). These studies are the basis for current clinical-grade work as a synergistic functional unit that would be composed of a CD2-CD58 adhesion system (79, 80). Indeed, mutations in these components are insufficient to fully activate T cells (81). Myosin II is the major myosin II isoform in T cells, and its external application to T cells can also be used to trigger T cell signaling (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 is required 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). 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 the 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 formsins (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 Ca²⁺ 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 II–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. These 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 prototype. 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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10. Varma R, Campi G, Yokosuka T, Saito T, Dustin ML. T cell receptor-proximal signals are sustained through a principle of receptor tyrosine phosphatase exclusion 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.