Immune Netw. 2023 Feb;23(1):e3
https://doi.org/10.4110/in.2023.23.e3
pISSN 1598-2629·eISSN 2092-6685

IMMUNE NETWORK

Review Article

Check for updates

T Cell Microvilli: Finger-Shaped External Structures Linked to the Fate of T Cells

Hye-Ran Kim [1,2,3], **Jeong-Su Park** [1,2], **Won-Chang Soh** [1,2], **Na-Young Kim** [1,2], **Hyun-Yoong Moon** [1,2], **Ji-Su Lee**[1,2], **Chang-Duk Jun** [1,2,*]

[1]School of Life Sciences, Gwangju Institute of Science and Technology (GIST), Gwangju 61005, Korea
[2]Immune Synapse and Cell Therapy Research Center, Gwangju Institute of Science and Technology (GIST), Gwangju 61005, Korea
[3]Division of Rare and Refractory Cancer, Tumor Immunology, Research Institute, National Cancer Center, Goyang 10408, Korea

Received: Dec 25, 2022
Revised: Feb 11, 2023
Accepted: Feb 11, 2023
Published online: Feb 21, 2023

***Correspondence to**
Chang-Duk Jun
School of Life Sciences, Gwangju Institute of Science and Technology (GIST), 123 Cheomdangwagi-ro, Buk-gu, Gwangju 61005, Korea.
Email: cdjun@gist.ac.kr

ORCID iDs
Hye-Ran Kim
https://orcid.org/0000-0001-6854-4774
Jeong-Su Park
https://orcid.org/0000-0002-8710-0455
Won-Chang Soh
https://orcid.org/0000-0002-8134-466X
Na-Young Kim
https://orcid.org/0000-0003-2650-3653
Hyun-Yoong Moon
https://orcid.org/0000-0002-8842-0880
Chang-Duk Jun
https://orcid.org/0000-0002-1372-9277

Conflict of Interest
The authors declare no potential conflicts of interest.

ABSTRACT

Microvilli are outer membrane organelles that contain cross-linked filamentous actin. Unlike well-characterized epithelial microvilli, T-cell microvilli are dynamic similar to those of filopodia, which grow and shrink intermittently via the alternate actin-assembly and -disassembly. T-cell microvilli are specialized for sensing Ags on the surface of Ag-presenting cells (APCs). Thus, these finger-shaped microprotrusions contain many signaling-related proteins and can serve as a signaling platforms that induce intracellular signals. However, they are not limited to sensing external information but can provide sites for parts of the cell-body to tear away from the cell. Cells are known to produce many types of extracellular vesicles (EVs), such as exosomes, microvesicles, and membrane particles. T cells also produce EVs, but little is known about under what conditions T cells generate EVs and which types of EVs are released. We discovered that T cells produce few exosomes but release large amounsts of microvilli-derived particles during physical interaction with APCs. Although much is unanswered as to why T cells use the same organelles to sense Ags or to produce EVs, these events can significantly affect T cell fate, including clonal expansion and death. Since TCRs are localized at microvilli tips, this membrane event also raises a new question regarding long-standing paradigm in T cell biology; i.e., surface TCR downmodulation following T cell activation. Since T-cell microvilli particles carry T-cell message to their cognate partner, these particles are termed T-cell immunological synaptosomes (TISs). We discuss the potential physiological role of TISs and their application to immunotherapies.

Keywords: T cell; Microvilli; Extracellular vesicle; Molting

IMMUNOLOGICAL SYNAPSE AND TCR NANO AND MICROCLUSTERS

The immunological synapse (IS) is a dynamic structure formed at the contact site between T cells and Ag-presenting cells (APCs) or target cells (1-7). The IS is a spatiotemporal signaling platform through which T cells are activated to initiate adaptive immunity. The first step in IS formation is the engagement of the TCR with specific Ag peptide-MHC molecules, leading to actin cytoskeletal rearrangement (8). Next, signaling molecules, including TCRs, form

IMMUNE NETWORK

Abbreviations

3D, 3-dimensional; APC, Ag-presenting cell; ARRDC1, arrestin domain containing 1; c-SMAC, central supra-activation cluster; DC, dendritic cell; ESCRT, endosomal sorting complex required for transport; EV, extracellular vesicle; FLT3L, fms-like tyrosine kinase 3 ligand; hsPALM, high-speed photo-activated localization microscopy; ICAM-1, intercellular adhesion molecule-1; IS, immunological synapse; LFA-1, lymphocyte function-associated Ag-1; MVB, multivesicular body; MβCD, methyl-β-cyclodextrin; OXPHOS, oxidative phosphorylation; pMHC, peptide MHC; TIS, T-cell immunological synaptosome; TMP, T cell microvilli-derived particle; VLA-4, very late Ag-4; Vstm5, V-set and transmembrane domain containing 5

Author Contributions

Conceptualization: Jun CD. Supervision: Jun CD. Writing - original draft: Kim HR, Park JS, Soh WC, Kim NY, Moon HY, Lee JS. Writing - review & editing: Kim HR, Jun CD.

microscale clusters and migrate along retrograde actin flow toward central supra-activation clusters (c-SMACs), leading to the maturation of IS. lymphocyte function-associated Ag-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1) interactions are critical for IS stabilization, and they are positioned at the peripheral SMAC surrounding the c-SMAC (5,9). Until recently, when, how, and where TCRs microclusters on the T cell membrane are formed before and during IS formation has remained controversial.

A previous report suggested that the TCR is organized as pre-assembled nanoclusters in the plasma membrane (10). These TCR nanoclusters require cholesterol for stabilization as they are disrupted if cells are exposed to methyl-β-cyclodextrin (MβCD) (11-14). TCR nanoclusters were visualized with high-speed photo-activated localization microscopy (hsPALM), which has a higher resolution than conventional fluorescence microscopy. The hsPALM analysis showed that the adapter protein linker for T cell activation also forms nanoclusters in resting T cells (15). Following T cell stimulation, TCR nanoclusters may allow cooperativity to develop microclusters. This cooperativity may explain T cells' exquisite sensitivity and signal amplification in response to Ags (10). Like αβTCR nanoclusters, γδTCR nanoclusters also exist (16). Other multi-chains, such as FcεRIs and B cell receptors, are also found as preclusters in the plasma membrane of mast cells and B cells, respectively (17). However, whether TCR nanoclusters are located in a specific plasma membrane structure is unclear.

A study based on 2 complementary super-resolution microscopy methods demonstrated that TCRs are highly clustered at microvilli tips in resting T cells (18), highlighting these membrane projections as effective sensors for antigenic moieties on APCs or target cells. We corroborated that the TCR ζ chain is localized at microvilli tips (**Fig. 1**). Pre-clustering of TCRs on microvilli could add an important structural element to the kinetic segregation model of IS formation, which predicts that activated surface receptors segregate into distinct, concentric regions based on the relative sizes of their extracellular domains (19). Consistent with this model, we tracked the centripetal movement of both a microvilli-specific protein (V-set and transmembrane domain containing 5; Vstm5) and TCR clusters with total internal reflection fluorescence microscopy (20). The mechanism of how microvilli on T cells search the surface of opposing cells before and after Ag recognition has also been reported (19).

Microvilli may serve as a structural scaffold for TCR pre-clustering and to deliver microclusters upon TCR engagement. This could lead to a major shift in the paradigm of IS research. Previously, microclusters were thought to arise from stochastic interactions between freely exchanged membrane proteins (10). Conversely, current evidence suggests

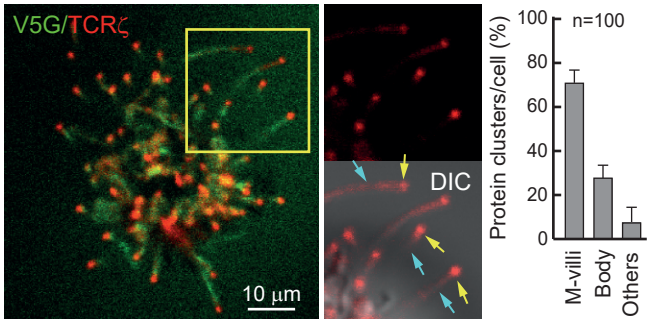

Figure 1. The TCR localizes to the microvilli tip. Jurkat T cells expressing Vstm5_GFP, a microvilli-specific protein, and TCRζ_tdTomato were imaged at the terminal stage of T cell activation. Reproduced from Kim et al. (20), licensed under Creative Commons (CC BY 4.0).

https://doi.org/10.4110/in.2023.23.e3

that microcluster formation may be limited to areas of the membrane that initiate mutual microvillar contact between T cells and APCs. Moreover, microcluster size may be restricted to the physical dimensions of the microvilli tips. Additionally, this fact may mirror the selective localization of specific molecules involved in the sensing role of the ends of membrane protrusions in dendritic cells (DCs), B cells, and mast cells (21).

THE SURFACE OF T CELLS

Typically, T cells express as many as 20,000 molecules on their membrane surface. [INSERT FIGURE 001]The sizes and dimensions of receptor molecules on the membrane surface can differ vastly. For example, one of the largest membrane glycoproteins is a CD45 with a rigid core of 15.2 nm and a variable mucin-like extracellular domain that extends the height to 10 (CD45R0), 20 (CD45RA), or 40 (CD45RABC) nm. On the contrary, TCR chains of the αβ or γδ type consist of a small extracellular domain. They create a ligand-binding heterodimer (TCRαβ or γδ) that senses or recognizes a peptide engaged with an MHC on the APC. However, how these molecules efficiently participate in sensing external signals to trigger TCR signaling quickly remains unclear. The organization of proteins into distinct membrane compartments such as lipid rafts may facilitate the initial signaling events (22,23). The surface of T cells is densely covered with finger-like membrane protrusions called microvilli (20). Lipids and lipid domains are important for T cell microvillus morphogenesis (24). Although a comprehensive analysis of lipids in microvilli has not been performed, sphingolipids and phosphatidylinositol 4,5-bisphosphate molecules, which occasionally form dimpling domains in the membrane, may be essential for microvillus formation (25).

Conversely, these outer membrane layers may allow for a more complex 3-dimensional (3D) receptor organization than a flat membrane. A recent super-resolution microscopy study of microvilli revealed that T cell activation receptors, such as TCR, CD2, CD4, and CD28, accumulate exclusively at the microvilli tips (18,26). Thus, the non-random 3D distribution of receptors is likely important to optimize signaling and cellular responses (24,26,27).

T CELL MICROVILLI

Microvilli are composed of cross-linked actin filaments laterally connected by bundling proteins. The most well-characterized are the microvilli in intestinal epithelial cells, which have a constant length and are specialized for nutrient absorption (**Fig. 2A**) (25,28). Although T cell microvilli have characteristics of epithelial microvilli, they are also similar to filopodia (27,29). Filopodia are dynamic structures that vary in length (up to 100 μm) (**Fig. 2B**) (30-32). Filopodia play pivotal roles in cellular processes such as cell adhesion to the extracellular matrix, cell migration toward chemokine gradients, wound healing, development, and cell signaling. Key proteins involved in cell adhesion or probing environmental changes localize at the tips of filopodia, mediating their functions (30). These activities of filopodia are similar to those of microvilli in T cells but not in epithelial cells. Microvilli in epithelial cells are strikingly uniform in length, exhibiting only around 5% variation in mean length (up to 1.1 μm) in the mouse small intestine (30).

T cell microvilli are important structures that determine the external morphology of T cells, but their importance has only recently been recognized. Two lymphocyte receptors,

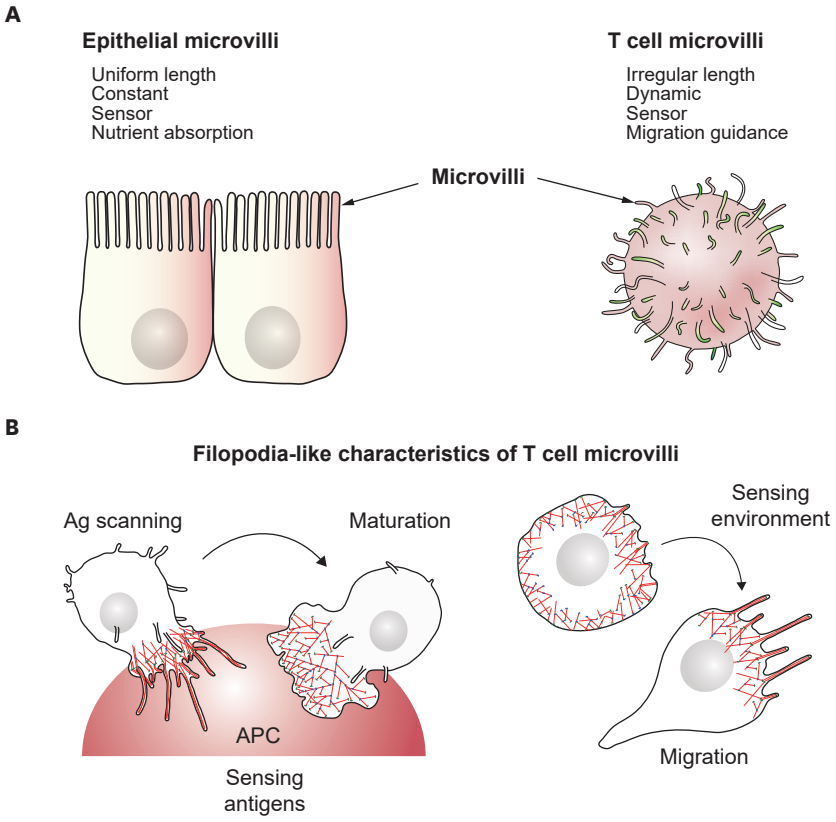

Figure 2. Morphological comparison of microvilli between epithelial cells and T cells and characteristics of T cell microvilli. (A) Epithelial cell microvilli are constant in length and specialized for nutrient absorption; however, T cell microvilli are irregular and more like filopodia, which extend and shrink intermittently via the alternate assembly and disassembly of their actin filaments. (B) T cells use microvilli to sense Ags on the surface of APCs. Filopodia are actin-rich surface projections specialized for sensing the external environment and mediate cell adhesion and migration.

L-selectin and α4β7 integrin, are segregated on the microvillus tip and play critical roles in lymphocyte attachment and rolling under physiologic flow (33,34), suggesting an important role for extravasation. Nevertheless, the structural and biophysical features of these abundant outer layers imply important functional capabilities. For instance, microvilli tips are a structural scaffold for TCR and functionally important T cell protein clustering, enabling highly effective, rapid, and spatially defined signal processing (35). Moreover, EVs are released through microvillus fragmentation in activated T cells (**Fig. 3A**), suggesting that microvilli are a source of Evs associated with activated T cells (20,27). Protrusive membrane architectures can provide sites for producing microvesicles from the cell surface. Primary and motile cilia/flagella, which function as cellular antennae, can produce microvesicles from their tips (36). Unlike the cross-linked F-actin bundles in microvilli, the internal cilium structure is mainly composed of a microtubule-based core architecture, implying that membrane protrusions provide sites for vesicle shedding (27,36-38). Furthermore, cilia formation is similar to IS formation, and ciliary microvesicles contain the endosomal sorting complex required for transport (ESCRT) (6).

https://doi.org/10.4110/in.2023.23.e3

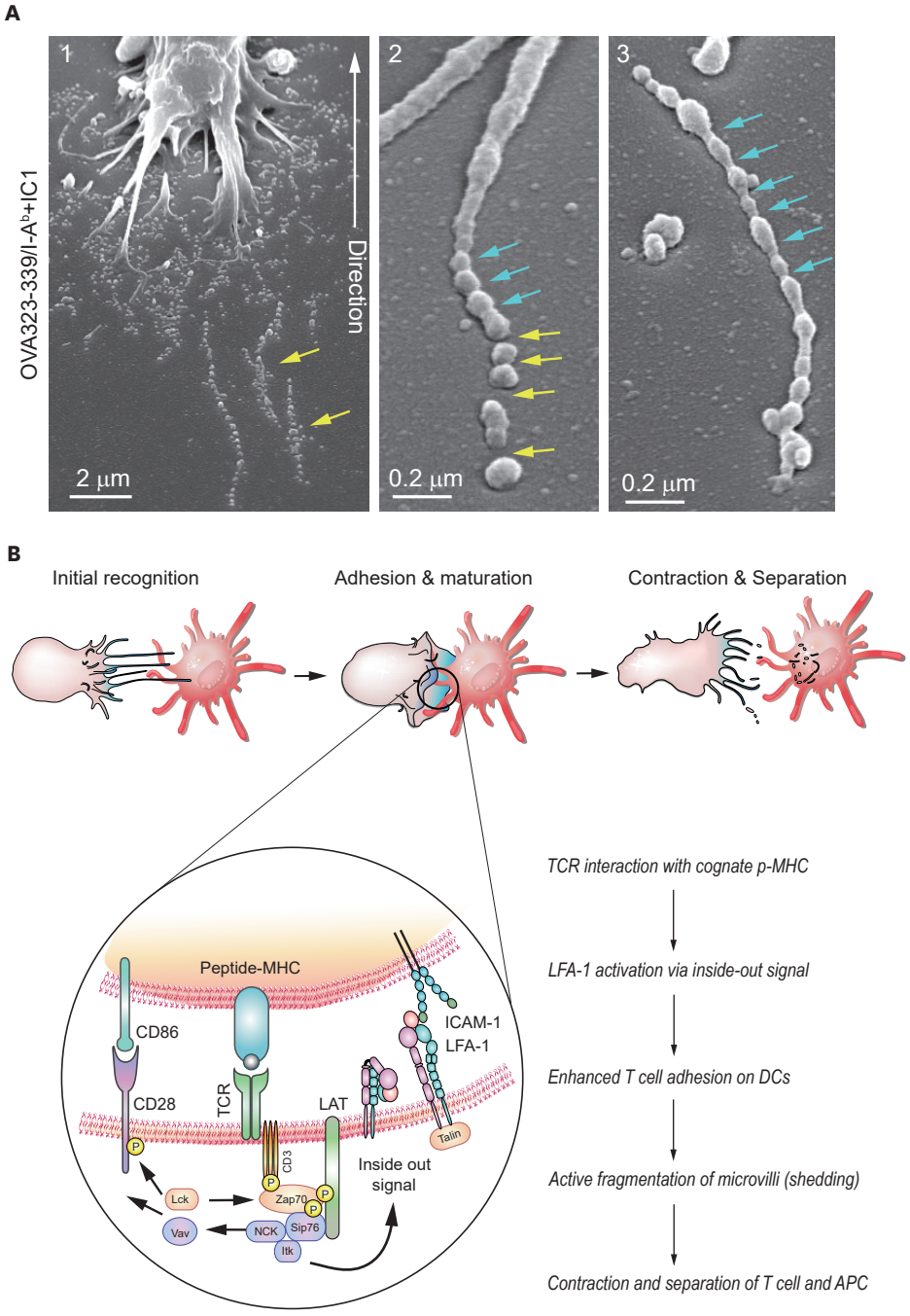

Figure 3. Generation of TMPs from T cells necessarily requires TCR signaling and adhesion on APCs via integrin activation. (A) Fragmentation of TMPs during and after disconnection from T cells in the kinapse on a lipid bilayer presenting cognate peptide-MHC and ICAM-1 (yellow arrows=fragmented sites; blue arrows=sites yet to be fragmented). Reproduced from Kim et al. (20), licensed under Creative Commons (CC BY 4.0). (B) Schematic illustration of the proposed mechanism of TMP generation. When T cells recognize a cognate Ag presented on APCs via elongated microvilli, T-cell-receptor-induced inside-out activation of integrins, such as LFA-1 and VLA-4, leads to enhanced cell adhesion and spreading on dendritic cells. When the immune synapse terminates and switches back to the kinapse phase, the T cells leave abundant TMPs on the surface of the APCs and surface – anchored large microvilli particles are further fragmented into nano-sized microparticles of approximately 50–100 nm by plasma membrane budding complexes, such as Arrdc1, TSG101, and Vps4.

https://doi.org/10.4110/in.2023.23.e3

EXTRACELLULAR VESICLES: COMPARISON OF EXOSOMES AND MICROVESICLES

EVs are small, nano-sized vesicles surrounded by a lipid membrane that are released from cells into the extracellular space (39). They have been found in the blood, cerebrospinal fluid, bronchial fluid, amniotic fluid, ascites fluid, urine, semen, saliva, tears, bile, gastric acid, milk, and most laboratory cell lines (39-42). There are 2 types of EVs according to their subcellular origins: exosomes and microvesicles. Exosomes are derived from endosomal compartments. The cell plasma membrane is internalized into the cell in the initial step of the endosomal pathway and is called an early endosome. During this process, intraluminal vesicles are formed by inward budding of the membrane in late endosomes to develop multivesicular bodies (MVBs). These are transformed into lysosomes or auto-phagosomes under specific conditions or are secreted into the extracellular space by exocytosis. Secreted intraluminal vesicles are defined as exosomes. Exosomes range from 30 to 150 nm in size (43-45). Unlike exosomes, microvesicles are directly derived from the external plasma membrane (40). Microvesicles are initially formed by various factors (physical and enzymatic pathways) and are known as microparticles, microvesicles, ectosomes, and shedding vesicles (46). Compared with exosomes, microvesicle size is heterogeneous, ranging from 30 to 1,000 nm (40,43-46).

The molecules in exosomes and microvesicles may be different as they originate from different cellular processes. Tetraspanin (CD63) modulates the formation of exosome and intraluminal vesicles (43,45,47-49). Studies have shown that molecules such as cholesterol, lysobisphosphatidic acid, and epidermal growth factor can determine the fate of MVBs into liposomes or exosomes (40,45,50). In contrast to exosome generation, the molecules involved in the generation and release of microvesicles are unclear. Wilson et al. reported that activation of purinergic receptors with ATP stimulates microvesicle release (51). Kuo et al. demonstrated that arrestin domain containing 1 (ARRDC1) is a mediator of microvesicle budding (52). Several reports indicated that the ESCRT machinery regulates microvesicle and exosome secretion (52-54). Thus, although exosomes and microvesicles are derived from different origins, they share secretion mechanisms in cellular systems.

T CELL MICROVILLI-DERIVED PARTICLES (TMPs)

T cells generate EVs (9,55-59). Choudhuri et al. reported that TCR-enriched microvesicles are released in the IS formed between T and B cells (9). Saliba et al. demonstrated synaptic ectosomes enriched with tetraspanins, TCR signaling, and ESCRT proteins (55). Blanchard et al. (59) showed that TCR activation of human T cells induces the production of exosomes bearing the TCR/CD3/ζ complex. However, no reports have demonstrated that T cells generate EVs by shedding the outer layer membrane, particularly of microvilli, during T cell interactions with APCs. Moreover, whether TCR activation itself is enough to produce exosomes or microvesicles is unclear. Our recent work suggests that T cell microvilli, which serve as a structural platform to segregate TCR and TCR signaling complexes, are the most easily separable structures during T cell adhesion to APCs. Additionally, since microvilli are enriched with adhesion molecules at their tips, microvilli on the surface of T cells allow them to form multiple bridges with APCs (20,27). Activation of TCR signaling at the microvilli tips leads to the activation of adjacent adhesion molecules, such as LFA-1 and very late Ag-4 (VLA-4), which may separate microvilli due to increased receptor affinity and avidity (**Fig. 3B**) (60,61). Thus, microparticle or microvesicle generation from T cells requires T cell adhesion to the

APC matrix. Additional experiments are necessary to examine whether TCR stimulation using soluble CD3/28 Abs can also release EVs.

However, since TMPs are derived from the cellular outer layers, overlapping origins with TCR-enriched microvesicles (9), synaptic ectosomes (55), and exosomes (59) cannot be completely excluded. Therefore, we termed all membrane particles released from activated T cells on adhesion substrates TISs.

PHYSIOLOGICAL FUNCTIONS OF TISs

T cell-derived EVs, including microvesicles, exosomes, or synaptic ectosomes, interact with other cells to form EV networks and modulate target cell functions (62,63). Previously, we showed that TISs contain proteins, cytokines, miRNA, lipids, and other functional substances. Indeed, treatment of TISs from $CD4^+$ T cells *in vitro* immediately triggered calcium influx into DCs and induced their maturation and activation by upregulating costimulatory molecules such as CD40, MHCII, CD80, and CD86 (20). Cytokine arrays provided clues to explain the underlying mechanisms of how TISs directly regulate DC functions. We found that various functional cytokines and chemokines, such as IL-33, TNF-α, and CCL5, which regulate DC activation or maturation, are highly enriched in TISs compared to the cell lysate. In particular, IL-33 activates myeloid DCs to produce IL-6, IL-1β, TNF-α, and CCL17 and to express high levels of CD40, CD80, OX40L, and CCR7 (64). Importantly, IL-33-activated DCs prime naive lymphocytes to produce the Th2 cytokines IL-5 and IL-13 (64). Additionally, TNF-α is critical for activating the adaptive immune response to viral infection by the maturation of local DCs (65). CCL5 is a potent chemoattractant for eosinophils, basophils, monocytes, effector memory T cells ($CD4^+$/$CD45RO^+$), B cells, natural killer cells, and immature DCs (66).

Evidence suggests that the "licensing" of DCs by $CD4^+$ T cells is required for cytotoxic T cell responses (67). In particular, IFN-γ produced by $CD4^+$ T cells is necessary for classical DC licensing by upregulation of costimulatory molecules such as CD80 and CD86, subsequently inducing the cross-priming of cytotoxic T cells. Interestingly, TISs contain a high amount of IFN-γ and may facilitate DC licensing by sustaining IFN-γ mediated signaling by membrane fusion on the DC surface.

How are TISs transferred and fused to other cells? Proteomic analysis revealed that molecules involved in regulating TCR signaling, including TCR subunits, CD2, or CD28, and in proximal signaling cascades, including Lck, zap70, or Syk, are exclusively enriched in TISs. DC activation by TISs is inhibited by treatment with blocking Abs against CD2, suggesting that TISs can be transferred and fused to the membrane on target cells via CD2–CD58, broadly expressed on APCs including DCs. Indeed, the interaction of T lymphocytes with APCs and target cells is critically dependent on *CD2–CD58*-mediated adhesion (68).

APPLICATION OF TISs

Many immunotherapeutic treatments have unpredictable or uncontrollable side effects (69-77). Since TIS is a product naturally produced by activated T cells, unlike current artificial therapeutics, the application of TIS to immunotherapy may be safe with fewer side effects.

In a previous report, we demonstrated that TISs directly activate DCs independent of Ags (20), suggesting that TIS could be useful as an immune adjuvant to induce DC recruitment and activation. An important adjuvant mechanism is to induce DC maturation and prolong Ag exposure (78). Various adjuvants have been studied in association with aluminum-based nanoparticles, saponin-based adjuvants, and TLR ligands or pathogen-associated molecular patterns (78). However, most adjuvants strongly trigger adaptive immunity by inducing DC activation but cause side effects such as redness, edema, pain, fever, and chills (46,78-81). Since TISs are naturally generated in the early stages of adaptive immunity, TIS could be utilized in cancer vaccinations based on tumor Ag-selective high immunogenicity. Although TISs derived from a patient's T cells may be the safest immune adjuvant, TISs from allogeneic healthy individuals will not induce immune rejection because exosomes do not induce a severe immune reaction, as shown in mice subjected to repeated administration of allogenic mouse or human cell-derived EVs (82). Despite the potential benefits of TIS application, however, there may still be potential unintended immune responses to auto-Ags carried by TISs. Therefore, it will be important to systematically investigate and understand the various aspects possibly that can occur in the body after TIS treatment.

Among TISs, TMPs are mainly derived from microvilli, so molecules can be engineered to be localized and expressed on the microvilli tips. For example, Vstm5 is specifically distributed throughout the entire stem region of microvilli with little localization in the intracellular region (20). Therefore, engineering fms-like tyrosine kinase 3 ligand (FLT3L) or GM-CSF, known to induce DC proliferation and development, to localize at the microvilli using the Vstm5 transmembrane domain, we can generate FLT3L or GM-CSF-enriched TISs. TISs generated in this way will induce a more robust immune response.

FATE OF T CELLS AFTER THE RELEASE OF TISs

Although the functions of TISs on APCs are somewhat understood, what happens to T cells themselves is largely unknown. This section will address the physiological implications of TIS release in T cells. TCR is thought to be downregulated from the cell surface due to increased TCR internalization, decreased recycling, and increased degradation (83-86). If T cells are activated by cross-linking TCR by soluble anti-CD3/28 Abs, surface TCR and TCR complex are significantly internalized (86). However, we identified that a large portion of TCR^+ particles is released from T cells if the cells are stimulated on plate-bound (immobilized) anti-CD3/28 Abs or on planar lipid bilayers presenting cognate peptide-MHC and the recombinant ICAM-1 in a density-dependent manner (87). These findings demonstrate that T cells lose surface TCRs during T cell-APC interactions, but not by internalization.

In molting or shedding in invertebrates, ecdysis occurs when an animal routinely casts off a portion of its outer layer at specific points in its lifecycle, typically to facilitate the growth of the organism. Molting involves releasing the skin, hair, feathers, or other external layers, including the entire exoskeleton in arthropods. However, it remains a mystery whether mammalian cells, including immune cells, also have a molting process related to their growth or proliferation. TIS release from the T cell surface may be similar to molting. Strikingly, this event, in contrast to TCR internalization, leads to the rapid upregulation of surface TCRs and metabolic reprogramming to meet the demands of clonal expansion that drives multiple rounds of division and cell survival (87).

How this program works in T cells can be understood by examining the component molecules in TISs. First, membrane-essential lipids, such as cholesterols, glycerolipids, and fatty acids, indispensable for T cell metabolism (activation and proliferation), are concentrated in TISs. According to our lipidomics analysis, a molting process induces a substantial shift in essential lipids, such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin—4 major phospholipid species present in the membrane. Physiologically, the major loss of essential lipids through the molting process can trigger de novo synthesis of gene clusters for lipid metabolism. Indeed, microarray analysis revealed that gene expression related to fatty acid synthetic metabolism—a critical pathway for cell survival and proliferation—is dramatically increased during T cell surface molting.

Second, TISs contain significant amounts of mitochondrial proteins. Although mitochondrial proteins are considered preferentially localized in mitochondria, plasma membrane lipid rafts are also enriched for mitochondrial oxidative phosphorylation (OXPHOS) proteins, a major source of ATP production for cell survival (88). Thus, the loss of energy-related mitochondrial proteins may trigger de novo synthesis of genes related to the OXPHOS pathway to compensate for and control homeostasis. T cells may release molecules essential for Ag recognition (TCR and its complex), energy metabolism (glycolysis, the TCA cycle, and ATP-binding proteins), and membrane components (fatty acids and cholesterols), which paradoxically promote T cell proliferation for clonal expansion.

FUTURE DIRECTIONS AND CONCLUDING REMARKS

Microvilli are a current research hotspot. Studies in recent years have shown that microvilli on T cells act as sensory organelles to survey APCs (19). Our group recently reported that microvilli not only provide a structural platform for TCR clustering to initiate TCR-mediated signaling but are also a source of nano-sized EVs, called TISs, delivering cytosolic proteins, lipids, and nucleic acids, which are potentially functional, to neighboring cells (20). Furthermore, these drastically change T cells after breaking off large portions of the cell membrane. Two recent findings challenge the long-held paradigm in T cell biology. First, immunologists have believed that TCR internalization is a major mechanism of surface TCR disappearance after T cell activation. Therefore, many studies have examined the internalization process of TCR and the TCR complex. However, our data suggests that large numbers of TCRs and membrane proteins are released as TMPs or synaptic ectosomes. Indeed, sufficient data support that the major cause of surface TCR loss is TCR^+ TIS release, not TCR internalization. Second, TCR^+ TIS release may have an important impact on T cell proliferation. If the TCR signal is activated, T cells can generate TISs even when they are not explicitly adhered; since T cells already acquired specificity from the peptide MHC (pMHC)-TCR signal, we believe another specificity signal may not be required in the molting process. If the pMHC-TCR signal is correct, molting occurs naturally by the adhesion process, which is thought to play a decisive role in the proliferation of T cells.

The transfer of TISs from T cells to the DCs induces a robust immune response by priming interacting DCs, which recruit polyclonal $CD8^+$ T cells, natural killer cells, and other immune cells. Thus, TISs are a good candidate for cancer and antiviral vaccines that naturally boost immune functions. Moreover, as a delivery system for exogenous substances, TISs have an advantage compared with artificial vesicles such as liposomes, microspheres, micro-emulsions, and other synthetic drug-delivering systems. Since TISs are mainly derived from cell membrane microvilli,

https://doi.org/10.4110/in.2023.23.e3

TISs may be useful as a carrier capable of inducing a more robust immune response by microvilli-specific engineering. Thus, TIS could be a promising tool as a novel, biologically compatible nano-based platform. Hence, additional systematic *in vivo* studies will provide potential clues regarding the potency and broad application of TISs a step closer to clinical usage.

ACKNOWLEDGEMENTS

This work was supported by the Creative Research Initiative Program (2015R1A3A2066253) through National Research Foundation (NRF) grants funded by the Ministry of Science and ICT (MSIT), the Basic Science Program (2022R1A2C4002627) through National Research Foundation (NRF) grants funded by the Ministry of Education (MOE), and supported by Global University Project (GUP), GIST Research Institute (GRI) IBBR grant funded by the GIST (in 2021-2022), and the Joint Research Project of Institutes of Science and Technology (2021–2022), Korea.

REFERENCES

1. Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, Allen PM, Dustin ML. The immunological synapse: a molecular machine controlling T cell activation. *Science* 1999;285:221-227.
PUBMED | CROSSREF
2. Monks CR, Freiberg BA, Kupfer H, Sciaky N, Kupfer A. Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* 1998;395:82-86.
PUBMED | CROSSREF
3. Stinchcombe JC, Bossi G, Booth S, Griffiths GM. The immunological synapse of CTL contains a secretory domain and membrane bridges. *Immunity* 2001;15:751-761.
PUBMED | CROSSREF
4. Carlin LM, Eleme K, McCann FE, Davis DM. Intercellular transfer and supramolecular organization of human leukocyte antigen C at inhibitory natural killer cell immune synapses. *J Exp Med* 2001;194:1507-1517.
PUBMED | CROSSREF
5. Dustin ML, Cooper JA. The immunological synapse and the actin cytoskeleton: molecular hardware for T cell signaling. *Nat Immunol* 2000;1:23-29.
PUBMED | CROSSREF
6. Griffiths GM, Tsun A, Stinchcombe JC. The immunological synapse: a focal point for endocytosis and exocytosis. *J Cell Biol* 2010;189:399-406.
PUBMED | CROSSREF
7. Dustin ML. T-cell activation through immunological synapses and kinapses. *Immunol Rev* 2008;221:77-89.
PUBMED | CROSSREF
8. Piragyte I, Jun CD. Actin engine in immunological synapse. *Immune Netw* 2012;12:71-83.
PUBMED | CROSSREF
9. Choudhuri K, Llodrá J, Roth EW, Tsai J, Gordo S, Wucherpfennig KW, Kam LC, Stokes DL, Dustin ML. Polarized release of T-cell-receptor-enriched microvesicles at the immunological synapse. *Nature* 2014;507:118-123.
PUBMED | CROSSREF
10. Schamel WW, Alarcón B. Organization of the resting TCR in nanoscale oligomers. *Immunol Rev* 2013;251:13-20.
PUBMED | CROSSREF
11. Schamel WW, Arechaga I, Risueño RM, van Santen HM, Cabezas P, Risco C, Valpuesta JM, Alarcón B. Coexistence of multivalent and monovalent TCRs explains high sensitivity and wide range of response. *J Exp Med* 2005;202:493-503.
PUBMED | CROSSREF
12. Molnár E, Swamy M, Holzer M, Beck-García K, Worch R, Thiele C, Guigas G, Boye K, Luescher IF, Schwille P, et al. Cholesterol and sphingomyelin drive ligand-independent T-cell antigen receptor nanoclustering. *J Biol Chem* 2012;287:42664-42674.
PUBMED | CROSSREF

13. Beck-García K, Beck-García E, Bohler S, Zorzin C, Sezgin E, Levental I, Alarcón B, Schamel WW. Nanoclusters of the resting T cell antigen receptor (TCR) localize to non-raft domains. *Biochim Biophys Acta* 2015;1853:802-809.
PUBMED | CROSSREF
14. Crites TJ, Padhan K, Muller J, Krogsgaard M, Gudla PR, Lockett SJ, Varma R. TCR Microclusters pre-exist and contain molecules necessary for TCR signal transduction. *J Immunol* 2014;193:56-67.
PUBMED | CROSSREF
15. Lillemeier BF, Mörtelmaier MA, Forstner MB, Huppa JB, Groves JT, Davis MM. TCR and Lat are expressed on separate protein islands on T cell membranes and concatenate during activation. *Nat Immunol* 2010;11:90-96.
PUBMED | CROSSREF
16. Siegers GM, Swamy M, Fernández-Malavé E, Minguet S, Rathmann S, Guardo AC, Pérez-Flores V, Regueiro JR, Alarcón B, Fisch P, et al. Different composition of the human and the mouse γδ T cell receptor explains different phenotypes of CD3γ and CD3δ immunodeficiencies. *J Exp Med* 2007;204:2537-2544.
PUBMED | CROSSREF
17. Wilson BS, Pfeiffer JR, Surviladze Z, Gaudet EA, Oliver JM. High resolution mapping of mast cell membranes reveals primary and secondary domains of Fc(ε)RI and LAT. *J Cell Biol* 2001;154:645-658.
PUBMED | CROSSREF
18. Jung Y, Riven I, Feigelson SW, Kartvelishvily E, Tohya K, Miyasaka M, Alon R, Haran G. Three-dimensional localization of T-cell receptors in relation to microvilli using a combination of superresolution microscopies. *Proc Natl Acad Sci U S A* 2016;113:E5916-E5924.
PUBMED | CROSSREF
19. Cai E, Marchuk K, Beemiller P, Beppler C, Rubashkin MG, Weaver VM, Gérard A, Liu TL, Chen BC, Betzig E, et al. Visualizing dynamic microvillar search and stabilization during ligand detection by T cells. *Science* 2017;356:eaal3118.
PUBMED | CROSSREF
20. Kim HR, Mun Y, Lee KS, Park YJ, Park JS, Park JH, Jeon BN, Kim CH, Jun Y, Hyun YM, et al. T cell microvilli constitute immunological synaptosomes that carry messages to antigen-presenting cells. *Nat Commun* 2018;9:3630-3648.
PUBMED | CROSSREF
21. Yi JC, Samelson LE. Microvilli set the stage for T-cell activation. *Proc Natl Acad Sci U S A* 2016;113:11061-11062.
PUBMED | CROSSREF
22. He HT, Lellouch A, Marguet D. Lipid rafts and the initiation of T cell receptor signaling. *Semin Immunol* 2005;17:23-33.
PUBMED | CROSSREF
23. Harder T. Lipid raft domains and protein networks in T-cell receptor signal transduction. *Curr Opin Immunol* 2004;16:353-359.
PUBMED | CROSSREF
24. Cebecauer M. Role of lipids in morphogenesis of T-cell microvilli. *Front Immunol* 2021;12:613591.
PUBMED | CROSSREF
25. Ikenouchi J, Hirata M, Yonemura S, Umeda M. Sphingomyelin clustering is essential for the formation of microvilli. *J Cell Sci* 2013;126:3585-3592.
PUBMED | CROSSREF
26. Ghosh S, Di Bartolo V, Tubul L, Shimoni E, Kartvelishvily E, Dadosh T, Feigelson SW, Alon R, Alcover A, Haran G. ERM-dependent assembly of T cell receptor signaling and co-stimulatory molecules on microvilli prior to activation. *Cell Reports* 2020;30:3434-3447.e6.
PUBMED | CROSSREF
27. Kim HR, Jun CD. T cell microvilli: sensors or senders? *Front Immunol* 2019;10:1753.
PUBMED | CROSSREF
28. Danielsen EM, Hansen GH. Lipid rafts in epithelial brush borders: atypical membrane microdomains with specialized functions. *Biochim Biophys Acta* 2003;1617:1-9.
PUBMED | CROSSREF
29. Majstoravich S, Zhang J, Nicholson-Dykstra S, Linder S, Friedrich W, Siminovitch KA, Higgs HN. Lymphocyte microvilli are dynamic, actin-dependent structures that do not require Wiskott-Aldrich syndrome protein (WASp) for their morphology. *Blood* 2004;104:1396-1403.
PUBMED | CROSSREF
30. Gupton SL, Gertler FB. Filopodia: the fingers that do the walking. *Sci STKE* 2007;2007:re5.
PUBMED | CROSSREF
31. Yang C, Svitkina T. Filopodia initiation: focus on the Arp2/3 complex and formins. *Cell Adhes Migr* 2011;5:402-408.
PUBMED | CROSSREF

32. Le Clainche C, Carlier MF. Regulation of actin assembly associated with protrusion and adhesion in cell migration. *Physiol Rev* 2008;88:489-513.
PUBMED | CROSSREF

33. Berlin C, Bargatze RF, Campbell JJ, von Andrian UH, Szabo MC, Hasslen SR, Nelson RD, Berg EL, Erlandsen SL, Butcher EC. α4 Integrins mediate lymphocyte attachment and rolling under physiologic flow. *Cell* 1995;80:413-422.
PUBMED | CROSSREF

34. von Andrian UH, Hasslen SR, Nelson RD, Erlandsen SL, Butcher EC. A central role for microvillous receptor presentation in leukocyte adhesion under flow. *Cell* 1995;82:989-999.
PUBMED | CROSSREF

35. Lange K. Fundamental role of microvilli in the main functions of differentiated cells: outline of an universal regulating and signaling system at the cell periphery. *J Cell Physiol* 2011;226:896-927.
PUBMED | CROSSREF

36. Long H, Zhang F, Xu N, Liu G, Diener DR, Rosenbaum JL, Huang K. Comparative analysis of ciliary membranes and ectosomes. *Curr Biol* 2016;26:3327-3335.
PUBMED | CROSSREF

37. Molday RS, Goldberg AF. Peripherin diverts ciliary ectosome release to photoreceptor disc morphogenesis. *J Cell Biol* 2017;216:1227-1229.
PUBMED | CROSSREF

38. Wood CR, Rosenbaum JL. Ciliary ectosomes: transmissions from the cell's antenna. *Trends Cell Biol* 2015;25:276-285.
PUBMED | CROSSREF

39. Veziroglu EM, Mias GI. Characterizing extracellular vesicles and their diverse RNA contents. *Front Genet* 2020;11:700.
PUBMED | CROSSREF

40. Yáñez-Mó M, Siljander PR, Andreu Z, Zavec AB, Borràs FE, Buzas EI, Buzas K, Casal E, Cappello F, Carvalho J, et al. Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles* 2015;4:27066.
PUBMED | CROSSREF

41. Théry C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. *Nat Rev Immunol* 2009;9:581-593.
PUBMED | CROSSREF

42. Osaki M, Okada F. Exosomes and their role in cancer progression. *Yonago Acta Med* 2019;62:182-190.
PUBMED | CROSSREF

43. Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: biogenesis, biologic function and clinical potential. *Cell Biosci* 2019;9:19.
PUBMED | CROSSREF

44. Rudraprasad D, Rawat A, Joseph J. Exosomes, extracellular vesicles and the eye. *Exp Eye Res* 2022;214:108892.
PUBMED | CROSSREF

45. van der Pol E, Böing AN, Harrison P, Sturk A, Nieuwland R. Classification, functions, and clinical relevance of extracellular vesicles. *Pharmacol Rev* 2012;64:676-705.
PUBMED | CROSSREF

46. Gutiérrez-Vázquez C, Villarroya-Beltri C, Mittelbrunn M, Sánchez-Madrid F. Transfer of extracellular vesicles during immune cell-cell interactions. *Immunol Rev* 2013;251:125-142.
PUBMED | CROSSREF

47. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol* 2013;200:373-383.
PUBMED | CROSSREF

48. Corrado C, Raimondo S, Chiesi A, Ciccia F, De Leo G, Alessandro R. Exosomes as intercellular signaling organelles involved in health and disease: basic science and clinical applications. *Int J Mol Sci* 2013;14:5338-5366.
PUBMED | CROSSREF

49. Lee Y, El Andaloussi S, Wood MJ. Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. *Hum Mol Genet* 2012;21:R125-R134.
PUBMED | CROSSREF

50. Meldolesi J. Exosomes and ectosomes in intercellular communication. *Curr Biol* 2018;28:R435-R444.
PUBMED | CROSSREF

51. Wilson HL, Francis SE, Dower SK, Crossman DC. Secretion of intracellular IL-1 receptor antagonist (type 1) is dependent on P2X7 receptor activation. *J Immunol* 2004;173:1202-1208.
PUBMED | CROSSREF

52. Kuo L, Freed EO. ARRDC1 as a mediator of microvesicle budding. *Proc Natl Acad Sci U S A* 2012;109:4025-4026.
PUBMED | CROSSREF

53. Puca L, Chastagner P, Meas-Yedid V, Israël A, Brou C. A-arrestin 1 (ARRDC1) and β-arrestins cooperate to mediate Notch degradation in mammals. *J Cell Sci* 2013;126:4457-4468.
PUBMED | CROSSREF

54. Nabhan JF, Hu R, Oh RS, Cohen SN, Lu Q. Formation and release of arrestin domain-containing protein 1-mediated microvesicles (ARMMs) at plasma membrane by recruitment of TSG101 protein. *Proc Natl Acad Sci U S A* 2012;109:4146-4151.
PUBMED | CROSSREF

55. Saliba DG, Céspedes-Donoso PF, Bálint Š, Compeer EB, Korobchevskaya K, Valvo S, Mayya V, Kvalvaag A, Peng Y, Dong T, et al. Composition and structure of synaptic ectosomes exporting antigen receptor linked to functional CD40 ligand from helper T cells. *eLife* 2019;8:e47528.
PUBMED | CROSSREF

56. Finetti F, Cassioli C, Baldari CT. Transcellular communication at the immunological synapse: a vesicular traffic-mediated mutual exchange. *F1000 Res* 2017;6:1880.
PUBMED | CROSSREF

57. Zakharova L, Svetlova M, Fomina AF. T cell exosomes induce cholesterol accumulation in human monocytes via phosphatidylserine receptor. *J Cell Physiol* 2007;212:174-181.
PUBMED | CROSSREF

58. Cai Z, Yang F, Yu L, Yu Z, Jiang L, Wang Q, Yang Y, Wang L, Cao X, Wang J. Activated T cell exosomes promote tumor invasion via Fas signaling pathway. *J Immunol* 2012;188:5954-5961.
PUBMED | CROSSREF

59. Blanchard N, Lankar D, Faure F, Regnault A, Dumont C, Raposo G, Hivroz C. TCR activation of human T cells induces the production of exosomes bearing the TCR/CD3/zeta complex. *J Immunol* 2002;168:3235-3241.
PUBMED | CROSSREF

60. Dustin ML, Springer TA. T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature* 1989;341:619-624.
PUBMED | CROSSREF

61. Hsu CJ, Hsieh WT, Waldman A, Clarke F, Huseby ES, Burkhardt JK, Baumgart T. Ligand mobility modulates immunological synapse formation and T cell activation. *PLoS One* 2012;7:e32398.
PUBMED | CROSSREF

62. Pelissier Vatter FA, Cioffi M, Hanna SJ, Castarede I, Caielli S, Pascual V, Matei I, Lyden D. Extracellular vesicle- and particle-mediated communication shapes innate and adaptive immune responses. *J Exp Med* 2021;218:e20202579.
PUBMED | CROSSREF

63. Kong H, Kim SB. Exosomal communication between the tumor microenvironment and innate immunity and its therapeutic application. *Immune Netw* 2022;22:e38.
PUBMED | CROSSREF

64. Besnard AG, Togbe D, Guillou N, Erard F, Quesniaux V, Ryffel B. IL-33-activated dendritic cells are critical for allergic airway inflammation. *Eur J Immunol* 2011;41:1675-1686.
PUBMED | CROSSREF

65. Trevejo JM, Marino MW, Philpott N, Josien R, Richards EC, Elkon KB, Falck-Pedersen E. TNF-α-dependent maturation of local dendritic cells is critical for activating the adaptive immune response to virus infection. *Proc Natl Acad Sci U S A* 2001;98:12162-12167.
PUBMED | CROSSREF

66. Schall TJ, Bacon K, Toy KJ, Goeddel DV. Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. *Nature* 1990;347:669-671.
PUBMED | CROSSREF

67. Smith CM, Wilson NS, Waithman J, Villadangos JA, Carbone FR, Heath WR, Belz GT. Cognate $CD4^+$ T cell licensing of dendritic cells in $CD8^+$ T cell immunity. *Nat Immunol* 2004;5:1143-1148.
PUBMED | CROSSREF

68. Zhu DM, Dustin ML, Cairo CW, Thatte HS, Golan DE. Mechanisms of cellular avidity regulation in CD2-CD58-mediated T cell adhesion. *ACS Chem Biol* 2006;1:649-658.
PUBMED | CROSSREF

69. Naseri M, Bozorgmehr M, Zöller M, Ranaei Pirmardan E, Madjd Z. Tumor-derived exosomes: the next generation of promising cell-free vaccines in cancer immunotherapy. *OncoImmunology* 2020;9:1779991.
PUBMED | CROSSREF

70. Sun S, Hao H, Yang G, Zhang Y, Fu Y. Immunotherapy with CAR-modified T cells: toxicities and overcoming strategies. *J Immunol Res* 2018;2018:2386187.
PUBMED | CROSSREF

71. Perica K, Varela JC, Oelke M, Schneck J. Adoptive T cell immunotherapy for cancer. *Rambam Maimonides Med J* 2015;6:e0004.
PUBMED | CROSSREF

72. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K. Immunobiology of dendritic cells. *Annu Rev Immunol* 2000;18:767-811.
PUBMED | CROSSREF

73. Perez CR, De Palma M. Engineering dendritic cell vaccines to improve cancer immunotherapy. *Nat Commun* 2019;10:5408-5417.
PUBMED | CROSSREF

74. Farkona S, Diamandis EP, Blasutig IM. Cancer immunotherapy: the beginning of the end of cancer? *BMC Med* 2016;14:73.
PUBMED | CROSSREF

75. Almåsbak H, Aarvak T, Vemuri MC. CAR T cell therapy: a game changer in cancer treatment. *J Immunol Res* 2016;2016:5474602.
PUBMED | CROSSREF

76. Anurathapan U, Leen AM, Brenner MK, Vera JF. Engineered T cells for cancer treatment. *Cytotherapy* 2014;16:713-733.
PUBMED | CROSSREF

77. Rivière I, Sadelain M. Chimeric antigen receptors: a cell and gene therapy perspective. *Mol Ther* 2017;25:1117-1124.
PUBMED | CROSSREF

78. Ho NI, Huis In 't Veld LG, Raaijmakers TK, Adema GJ. Adjuvants enhancing cross-presentation by dendritic cells: the key to more effective vaccines? *Front Immunol* 2018;9:2874.
PUBMED | CROSSREF

79. McAleer JP, Vella AT. Understanding how lipopolysaccharide impacts CD4 T-cell immunity. *Crit Rev Immunol* 2008;28:281-299.
PUBMED | CROSSREF

80. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998;392:245-252.
PUBMED | CROSSREF

81. Beacock-Sharp H, Donachie AM, Robson NC, Mowat AM. A role for dendritic cells in the priming of antigen-specific $CD4^+$ and $CD8^+$ T lymphocytes by immune-stimulating complexes in vivo. *Int Immunol* 2003;15:711-720.
PUBMED | CROSSREF

82. Gołębiewska JE, Wardowska A, Pietrowska M, Wojakowska A, Dębska-Ślizień A. Small extracellular vesicles in transplant rejection. *Cells* 2021;10:2989.
PUBMED | CROSSREF

83. Dietrich J, Menné C, Lauritsen JP, von Essen M, Rasmussen AB, Ødum N, Geisler C. Ligand-induced TCR down-regulation is not dependent on constitutive TCR cycling. *J Immunol* 2002;168:5434-5440.
PUBMED | CROSSREF

84. Martínez-Martín N, Fernández-Arenas E, Cemerski S, Delgado P, Turner M, Heuser J, Irvine DJ, Huang B, Bustelo XR, Shaw A, et al. T cell receptor internalization from the immunological synapse is mediated by TC21 and RhoG GTPase-dependent phagocytosis. *Immunity* 2011;35:208-222.
PUBMED | CROSSREF

85. von Essen M, Bonefeld CM, Siersma V, Rasmussen AB, Lauritsen JP, Nielsen BL, Geisler C. Constitutive and ligand-induced TCR degradation. *J Immunol* 2004;173:384-393.
PUBMED | CROSSREF

86. Huang JF, Yang Y, Sepulveda H, Shi W, Hwang I, Peterson PA, Jackson MR, Sprent J, Cai Z. TCR-mediated internalization of peptide-MHC complexes acquired by T cells. *Science* 1999;286:952-954.
PUBMED | CROSSREF

87. Park JS, Kim JH, Soh WC, Lee KS, Kim CH, Chung IJ, Lee S, Kim HR, Jun CD. Trogocytic-molting of T-cell microvilli controls T-cell clonal expansion. *bioRxiv*. 2022. doi: 10.1101/2022.05.03.490404.
CROSSREF

88. Lee H, Kim SH, Lee JS, Yang YH, Nam JM, Kim BW, Ko YG. Mitochondrial oxidative phosphorylation complexes exist in the sarcolemma of skeletal muscle. *BMB Rep* 2016;49:116-121.
PUBMED | CROSSREF