JCB Journal of Cell Biology

SPOTLIGHT

Tubulin with a twist: Acetylated highways at the immune synapse

Srishti Mandal[1] and Sudha Kumari[1]

In this issue, (Aceitón et al. https://doi.org/10.1083/jcb.202407181) uncover a pathway that ties together rigidity sensing at the B cell immunological synapse to molecular shuttling of ATAT1, leading to microtubule acetylation and lysosome repositioning, ultimately tuning the efficiency of antigen uptake and presentation by B cells.

In physiology, lymphocytes scan through diverse tissues, presenting biophysical and biochemical contexts in search of antigens. Once a cognate antigen is detected, they form a highly specialized cell–cell contact interface, termed the immunological synapse, that allows exchange of not only biochemical information but also biophysical information. It is known that for an optimal response, lymphocytes must sense, process, and transduce the biochemical antigen information in the context of biophysical cues. While the mechanosensory processes at the plasma membrane have been a focus of extensive investigation in the recent decade (1), the intracellular mechanotransduction pathways that integrate mechanosensing to diverse signaling cascades are still poorly understood (2, *Preprint*). Improved understanding of lymphocyte cell-intrinsic molecular mechanisms relaying mechanical information is extremely important, not only to gain insights into their unique and specialized cell biology, but also to augment rational design principles for improved therapeutic outcomes, where the lymphocytes must function in diverse mechanical microenvironments.

The immunological synapse is a highly dynamic interface, and proper sub-synaptic positioning of the cytoskeleton in space and time is crucial for lymphocyte activation and the subsequent immune response. During the entire synaptic phase, there is a constant remodeling and repositioning of cytoskeletal machinery as the cell responds and adapts to the extracellular biomechanical and biochemical stimuli, as well as changes in intracellular signaling programs. The role of the actin cytoskeleton is very well established in this regard, where an increase in mechanical stiffness of the substrate enhances synaptic contact area, recruitment of signaling molecules, and overall cellular activation by increasing actin polymerization and remodeling (3, 4, 5). However, insights into the role of other mechanoadaptive cytoskeletal elements such as microtubules are scant, although microtubules contribute significantly to sustained antigen receptor signaling and lymphocyte activation (6) and directed vesicular trafficking (7) through reorientation and translocation of the microtubule-organizing center at T cell synapses. In the case of B cells, perturbation of microtubules leads to a loss of stiffness-induced signaling benefits (4), warranting a deeper investigation into the underlying mechanotransduction pathways coupling antigen receptor mechanosensing to optimal B cell activation.

In their study, Aceitón et al. (8) addressed the cytoskeleton–mechanotransduction mechanistic gap by characterizing the changes in the microtubule network as the B cells experienced antigen-presenting substrates of varying rigidities. They found that B cells spread more, showed higher localization of the mechanical effector YAP in the nucleus, and generated a higher number of actin structures termed actin foci (9, 10) as the substrate stiffness increased. While this observation indicated that B cells tuned their cytoskeleton in response to changes in substrate stiffness, it also opened a larger question of how the cell communicated mechanical information of substrate rigidity from its cortex to deep within the cytoplasm and ultimately to the nucleus, and what could be the cytoplasmic ramifications of it? The authors found that stiffer gels augmented the nucleus-to-cytoplasm translocation of the α-tubulin acetyltransferase ATAT1. Previous work has shown that microtubules acetylated via ATAT1 aid mechanosensing at focal adhesions in rat astrocytes during migration (11); however, this pathway had not been explored in the context of immune cells. The authors found that microtubule acetylation by ATAT1 scaled with stiffness, eventually affecting the positioning, activity, and dynamics of lysosomes at the B cell immune synapse, where centrally placed lysosomes with high proteolytic activity are typically attributed with better capability of antigen processing. Indeed, B cells seeded on stiffer gels displayed higher immobilized antigen extraction and presentation. This is particularly interesting when put in the context of previous works that highlighted the importance of a microtubule acetylation-deacetylation balance, where targeted inhibition of the microtubule-deacetylating activity of HDAC6 led to disorganized T cell immune synapses (12) and impaired cytotoxic functions due to defects in lytic granule transport and delivery (13). Considering

[1]Department of Microbiology and Cell Biology, Indian Institute of Science, Bengaluru, India.

Correspondence to Sudha Kumari: sudhakm@iisc.ac.in.

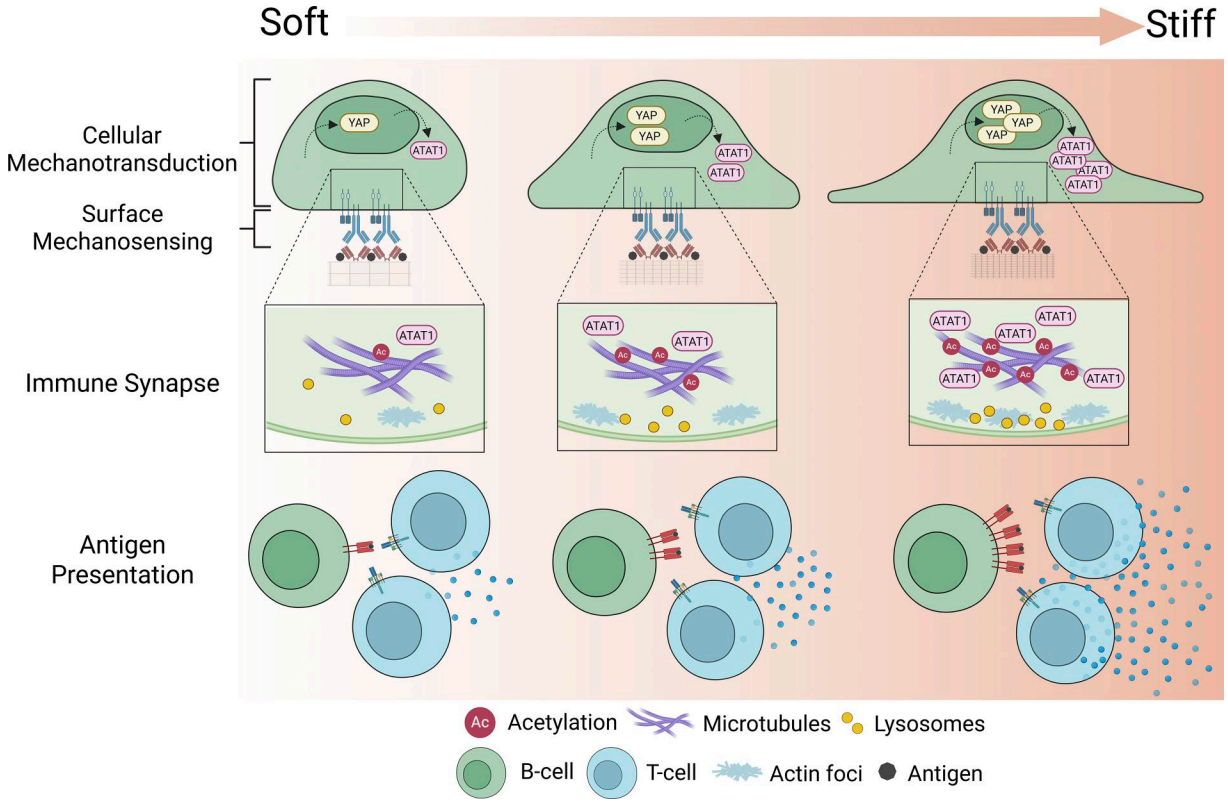

Figure 1. **The B cell mechanoresponse includes mechanosensing via the antigen receptor and adhesion molecules at the cell surface and mechanotransduction via the intracellular signaling pathways that convey mechanical information to the nucleus and cytoskeleton.** This response manifests visibly on stiffer stimulatory substrates, with higher cell spreading observed during immunological synapse formation. Stiffer substrates, upon antigen receptor cross-linking, elicit higher cytoplasmic translocation of ATAT1, leading to an increase in acetylated microtubules. These modified microtubules dictate lysosomal and actin foci positioning at the center of the synapse, which further augments antigen uptake, processing, and presentation to T cells.

that the acetylation of microtubules renders them more resistant to depolymerization, what organizational function do the acetylated, and therefore more persistent, microtubules play at the dynamic interface of an immune synapse? The authors observed that higher microtubule acetylation was accompanied by slower lysosomal dynamics, implying that microtubule acetylation constrains lysosomal movement to the central zone of synapses. Interestingly, heightened acetylation of microtubules on stiffer substrates was also correlated with more centrally localized actin foci at the immune synapse, raising the possibility that a cross talk of microtubules and filamentous actin may exist for an optimal mechanoresponse. Such cross talk has been observed in the Rho–ROCK signaling pathway, where release of GEF-H1 upon microtubule acetylation triggers a cascade modulating actomyosin contractility (14). Indeed, the authors found a reduced association of GEF-H1 with filamentous actin when endogenous ATAT1 was silenced. Finally, B cells extracting antigens from stiffer substrates were able to trigger an enhanced antigen-specific T cell cytokine response than those on softer substrates. This result reinforced the key message that an effective B cell mechanoresponse is required for the proper initiation of adaptive immune response.

Overall, the study by Aceitón et al. (8) advances the idea that lymphocyte activation is tuned by diverse mechanical contexts not just via a collaboration of the actin cytoskeleton and mechanosensitive immunoreceptors on the cell surface, but also by nuclear communication of mechanical information via cytosolic molecular pathways assisted by microtubular networks (Fig. 1). The study also opens doors to numerous mechanistic questions. For instance, how does communication between proximal BCR signaling and the nucleus proceed to facilitate nuclear export of ATAT1? Do other post-translational modifications of microtubules than acetylation impact lysosomal dynamics and vesicle sorting for the uptake and presentation of antigens? Lastly, while the *ex vivo*–reconstituted experimental systems that the authors have used to interrogate specific mechanical cues are indispensable, the mechanical landscape within tissues and lymphoid organs is far more complex. How do the lymphocytes integrate diverse mechanical stimuli to modify and spatiotemporally coordinate cytoskeletal components for an optimal immune response? Perhaps *ex vivo* experiments using multiplexed mechanical cues in the future will shed light on these complex and fascinating cytoskeletal mechanisms of mechanotransduction in lymphocytes.

Acknowledgments

Author contributions: S. Mandal: Conceptualization, Investigation, Visualization, Writing - original draft, Writing - review & editing, S. Kumari: Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Visualization, Writing - review & editing.

Disclosures: The authors declare no competing interests exist.

References

1. Rogers, J., et al. 2024. *Biophys. J.* https://doi.org/10.1016/j.bpj.2024.05.020
2. Mandal, S., et al. 2023. *Elife*. https://elifesciences.org/reviewed-preprints/91854
3. Judokusumo, E., et al. 2012. *Biophys. J.* https://doi.org/10.1016/j.bpj.2011.12.011
4. Wan, Z., et al. 2013. *J. Immunol.* https://doi.org/10.4049/jimmunol.1202976
5. Blumenthal, D., and J.K. Burkhardt. 2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.201911058
6. Martín-Cófreces, N.B., et al. 2008. *J. Cell Biol.* https://doi.org/10.1083/jcb.200801014
7. Stinchcombe, J.C., et al. 2006. *Nature*. https://doi.org/10.1038/nature05071
8. Aceitón, P., et al. 2025. *J Cell Biol*. https://doi.org/10.1083/jcb.202407181
9. Roper, S.I., et al. 2019. *Elife*. https://doi.org/10.7554/eLife.48093
10. Kumari, A., et al. 2019. *Nat. Commun.* https://doi.org/10.1038/s41467-019-10751-7
11. Seetharaman, S., et al. 2022. *Nat. Mater.* https://doi.org/10.1038/s41563-021-01108-x
12. Serrador, J.M., et al. 2004. *Immunity*. https://doi.org/10.1016/s1074-7613(04)00078-0
13. Núñez-Andrade, N., et al. 2016. *J. Cell Sci.* https://doi.org/10.1242/jcs.180885
14. Pineau, J., et al. 2022. *Elife*. https://doi.org/10.7554/eLife.78330