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FIRST PERSON

First person – Bhagawat C. Subramanian

First Person is a series of interviews with the first authors of a selection of papers published in Journal of Cell Science, helping early-career researchers promote themselves alongside their papers. Bhagawat C. Subramanian is the first author on 'The LTB_4–BLT1 axis regulates the polarized trafficking of chemoattractant GPCRs during neutrophil chemotaxis', published in Journal of Cell Science. Bhagawat is a Visiting Postdoctoral Fellow in the lab of Dr Roberto Weigert at the National Cancer Institute, NIH, Bethesda, USA, working on understanding the molecular signaling that drives innate immune cell behavior during inflammation, through the use of subcellular imaging modalities in live animals.

Bhagawat C. Subramanian

How would you explain the main findings of your paper in lay terms?

Neutrophils, an innate immune cell type in our body, act as first responders to inflammation. They do so by sensing and responding to a variety of inflammatory molecules such as parts of bacteria-derived proteins. These protein fragments (peptides) also attract immune cells and bind to a specific class of proteins, the G-protein coupled receptors (GPCRs). Using microscopy, we discovered that in neutrophils, which migrate in a fast amoeboid-like manner, GPCRs bound to peptides that attract immune cells (chemoattractants) get redistributed to the back of stimulated neutrophils. Further, we document that as neutrophils begin to migrate in response to chemoattractant stimulation, the GPCRs move inwards from the retracting cell membrane at the back of the cell. In fact, perturbing back-retraction impacts the extent to which GPCRs are retained on the cell membrane, which in turn affects the ability of neutrophils to persistently migrate in a directed manner. Taking all these results together, our study unravels a fascinating mechanism that drives chemoattractant sensing and the polarized dynamics of GPCRs in the regulation of how neutrophils move in response to chemical signals.

Were there any specific challenges associated with this project? If so, how did you overcome them?

The most challenging aspect of the current study was the use of live-cell imaging to capture early events driving receptor trafficking in human neutrophils, especially under physiological concentrations. We used a combination of peptide conjugation and optimized confocal microscopy settings to capture the earliest events during neutrophil stimulation (from time zero), and receptor dynamics on the plasma membrane during the course of neutrophil polarization and migration. These standardizations led us to further track and analyze vesicles in human neutrophils treated without or with specific small molecule inhibitors of interest.

When doing the research, did you have a particular result or 'eureka' moment that has stuck with you?

The most striking and surprising finding of our study came from our initial live-cell imaging analysis in primary human neutrophils. The first experiment where we captured the binding of the ligand, clustering of the GPCR, re-distribution and the endocytosis of ligand-bound GPCRs from the back of polarized and migrating human neutrophils was the 'eureka' moment that has immensely fascinated and stuck with me.

Why did you choose Journal of Cell Science for your paper?

We wanted our study to reach a broad audience in cell biology, and we also required a quick and balanced assessment of our work. Considering the established standards of Journal of Cell Science, we believed it was the appropriate platform to report our study that addresses a subject involving intersecting fields of research such as innate immune cell behavior, cell migration, receptor trafficking and cell signaling.

Have you had any significant mentors who have helped you beyond supervision in the lab?

Special thanks to Dr Carole Parent for being a fantastic mentor. She gave me immense freedom to try new ideas, and supported each one of them with her own critical input. It was really wonderful of her to continue supporting me and shaping my thought processes despite moving her group to Michigan while I stayed back at NCI. I want to take this opportunity to specially thank Dr Roberto Weigert for guiding me at key moments of my continuing postdoctoral tenure. I want to also highlight the unconditional support and guidance

Bhagawat C. Subramanian's contact details: Laboratory of Cellular and Molecular Biology, National Cancer Institute, NIH, 37 Convent Drive, Bethesda, MD 20892, USA.
E-mail: bhagawatchandrasekar.subramanian@nih.gov

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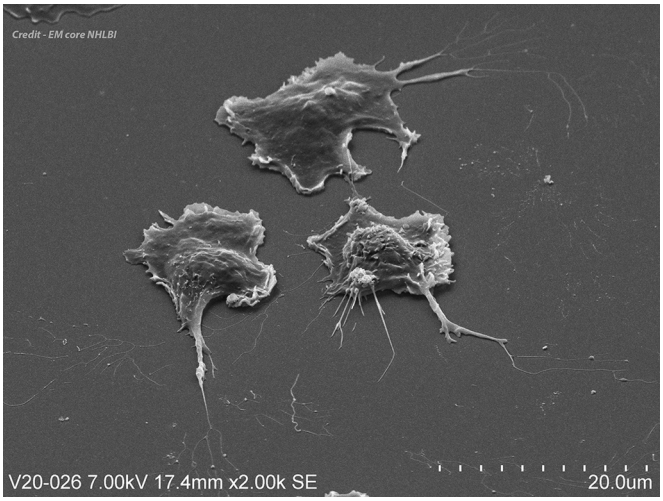

SEM image of primary human neutrophils stimulated with formylated peptide on a fibrinogen-coated culture dish. The image highlights the protrusive fronts (characterized by raft-like projections), the retracting back (characterized with many filopod-like contacts attached to the surface) and the membrane remnants (or tracks) left by the migrating neutrophils (characterized by the migrasome-like structures associated with them).

I receive from Prof. Dipankar Nandi (PhD supervisor at Indian Institute of Science), Prof. D. N. Rao (mentor at Indian Institute of Science) and Prof. Karutha Pandian (Alagappa University), who are always accommodating and have, time and again, supported my scientific and not-so-scientific endeavors.

“[…] it was natural for me to experiment things out rather than to blindly follow textbook instructions.”

What motivated you to pursue a career in science, and what have been the most interesting moments on the path that led you to where you are now?

I am a firm believer in ‘learn as you do’. Therefore, it was natural for me to experiment things out rather than to blindly follow textbook instructions. As a PhD student, I was introduced to the fascinating world of innate immune cell signaling and its impact on our immune responses. The ability to visualize and test immune cell behavior under physiological conditions using microscopy intrigues and captivates my imagination to this day.

What’s next for you?

Currently, I am pursuing my interests in receptor trafficking, signaling and cytoskeleton dynamics in innate immune cells in live animals using intravital microscopy. I intend to eventually establish myself as an independent investigator and further my research on ‘observing’ and ‘testing’ immune cell behavior in its native context and upon injury or inflammation, leading to a better understanding of the impact of such specialized cells on the overall fitness and development of its host.

Tell us something interesting about yourself that wouldn’t be on your CV

I drive, often many hours listening to music, to distinct and less-explored places where nature is at its pristine best. It’s a hobby and a great way for me to unwind, relax and get to know my fellow travelers better. I am also an amateur artist, with a special interest in painting and making caricatures, among other art forms.

Reference

Subramanian, B. C., Moissoglu, K. and Parent, C. A. (2018). The LTB_4–BLT1 axis regulates the polarized trafficking of chemoattractant GPCRs during neutrophil chemotaxis. *J. Cell Sci.* **131**. jcs217422.