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INTERVIEW

The people behind the papers – Nathalia Azevedo Portilho, Rebecca Scarfò, Andrea Ditadi and Momoko Yoshimoto

Blood cells emerge in several waves through a number of different progenitors during embryonic development. A new paper in Development investigates whether the development of B1 lymphocytes, a type of B cell, is dependent on the differentiation of hematopoietic stem cells. To hear more about the story, we caught up with joint first authors Nathalia Azevedo Portilho and Rebecca Scarfò, and their respective supervisors Momoko Yoshimoto, Associate Professor at the University of Texas Health Science Center in Houston, USA, and Andrea Ditadi, Group Leader at the IRCCS Ospedale San Raffaele in Milan, Italy.

Nathalia Azevedo Portilho (top left), Rebecca Scarfò (top right), Andrea Ditadi (bottom left) and Momoko Yoshimoto (bottom right).

Momoko, can you give us your scientific biography and the questions your lab is trying to answer?

MY: I was originally a Pediatric Hematology-Oncologist in Japan. So, I performed 'real' BM transplantation to leukemic children to cure their diseases. One day, when I attended a small hematology meeting and learned about hematopoietic stem cell (HSC) biology, I became so fascinated by them. I wanted to learn more about HSCs, so I entered Kyoto University Graduate School of Medicine and the first project in my PhD was to produce HSCs from mouse embryonic stem cells (ESCs), which I have not achieved yet! To this aim, I thought that I needed to understand embryonic hematopoiesis because ESC differentiation would recapitulate embryonic development. Since then, I have been working on embryonic hematopoiesis, mouse ESCs and HSC biology. I came to Dr Mervin Yoder's lab as a postdoc and learned a lot from him. I was recruited to the University of Texas Health Science Center at Houston in 2016 and started my lab! So, since 1999, when I entered Graduate School, I have been working on understanding the mechanisms that allow the emergence of the first lymphoid progenitors and HSCs in the mouse embryo. Not all blood cells in an embryo are produced by HSCs. In fact, there are multiple waves of hematopoiesis that emerge in multiple locations and yield progenitors with different properties in order to cope with the increasing needs of the growing embryo. And, strangely enough, HSCs are amongst the last progenitors to be generated. We want to understand what kind of signals and molecular interactions are required for each hematopoietic wave, including the wave that generates HSCs.

And Andrea, what is your scientific background and what does your lab currently work on?

AD: I studied Medical Biotechnology at the University of Padua and then moved to Paris for my PhD where, despite working in a gene therapy lab, I got my feet wet with developmental biology and hematopoiesis. I then joined Gordon Keller's lab in Toronto for my postdoctoral studies, where I started using pluripotent stem cells to model human development. I had seen him talking at a meeting during the first year of my PhD; it was the moment that made me realize that I wanted to become a stem cell scientist. Now, in my lab in Milan, our team wants to understand how the hematopoietic system is established and how blood is generated during mammalian development.

Nathalia, how did you come to work in Momoko's lab and what drives your research today?

NAP: I am fascinated by stem cell and developmental biology. I obtained my MSc and PhD degrees in Cellular and Molecular Biology, and as a graduate student I investigated the hematopoietic potential of mouse placenta. I was interested in continuing to study hematopoiesis when I applied for a postdoctoral position in

NAP & MY: Center for Stem Cell Research and Regenerative Medicine, Institute for Molecular Medicine, McGovern Medical School, The University of Texas Health Science Center at Houston, Houston, TX 77030, USA.
RS & AD: San Raffaele Telethon Institute for Gene Therapy, IRCCS San Raffaele Scientific Institute, Milan 20132, Italy.
Email: ditadi.andrea@hsr.it; momoko.yoshimoto@uth.tmc.edu

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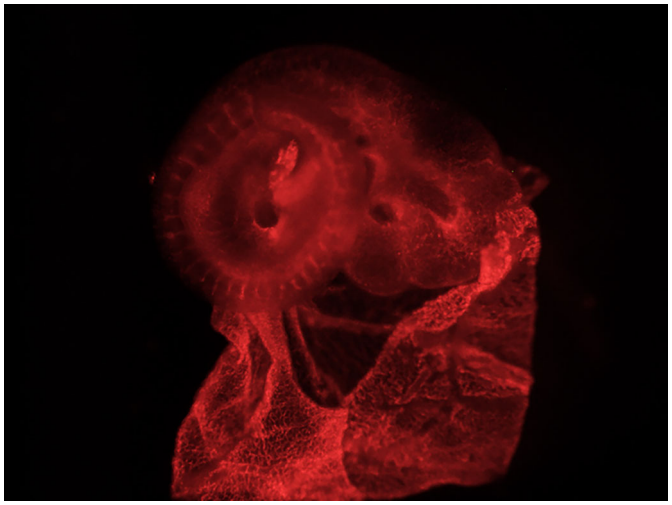
Momoko's lab. I have been following Dr Yoshimoto's research since I was working on my PhD project and her lab at UTHealth seemed the right place for me. My primary project was to investigate the role of Notch signaling in B-cell development. Using mouse ESCs and Cre-lox mouse models, we were able to demonstrate that B1 cells can arise in the absence of Notch signaling, possibly from HSC-independent progenitors. As a postdoc at Momoko's lab, I was also involved in other exciting projects in hematopoietic development. I was able to learn different research techniques and elevate my skill set as a scientist. I continue to work with developmental biology and my research today combines genetic and microengineered biomaterials to study human placenta development and disease. What drives my research is being able to gradually understand the amazing process of development to better understand and improve our ability to treat disease.

And Rebecca, what brought you to work in Andrea's lab and what are you currently researching?

RS: During my Master's studies in Genetics and Molecular Biology, I became fascinated by embryonic development and by one of the most powerful tools to study it: pluripotent stem cells. In fact, I have always found their use in recapitulating embryonic development to understand, in detail, how different organs and tissues are formed and organized captivating. In particular, hematopoeisis captured my attention the most. I found intriguing how the generation of blood cell is tightly spatio-temporally regulated to support the various needs of the embryo during its development. Therefore, I was committed to couple my interest in studying embryonic development, stem cells and hematopoiesis. Andrea's lab was – and is – a perfect fit for my PhD studies. Here, I am modelling hematopoiesis using mouse and human ESCs to dissect and characterize the generation of blood cells and their role in embryonic development.

Before your work, what was known about the developmental origin of B1 lymphocytes?

MY: The study of B1 lymphocyte biology has a long history. B1 lymphocytes were known to be a unique B-lymphoid subset expressing CD5 and CD11b, markers of T and myeloid cells, respectively. They are different from conventional B2 lymphocytes in their B-cell receptor (BCR) repertoire, TdT (terminal deoxynucleotidyl transferase) expression and origins. It was widely known that B1 lymphocytes are derived from fetal liver progenitors, but what precursors provide such B-1 progenitor cells in the fetal liver was unknown.



Blood vessels (red) labeled in an E9.5 Cdh5CreERT2:Td-Tomato mouse embryo injected with tamoxifen at E8.5.

Can you give us the key results of the paper in a paragraph?

AD: Traditionally, all lymphoid cells were thought to be derived from HSCs, but recent studies have indicated the presence of special subsets of lymphocytes, including B1 lymphocytes, that emerge before HSCs can be detected. However, whether they can be produced independently of HSCs was not formally proven. Since HSCs, but not the HSC-independent blood progenitors, are generated in a Notch-dependent manner, we thought we could answer this question by dissecting the Notch dependency of B1 cells. In this report, we show that transplantable innate-like B1 cells do not require Notch signaling to be generated, either *in vitro* or *in vivo*, and can therefore emerge independently of HSCs. Furthermore, we showed that the dose of Notch signaling affects the lineage determination into B1, B2 (conventional adoptive B cells) and T cells, indicating a different Notch signaling requirement for the development of each lymphoid subset.

How do you think Notch signaling regulates the specification of B-1 vs B-2 cells?

MY: It has been controversial how B1 and B2 cells develop from their progenitors, as to whether they have a common differentiation pathway but separate depending on their BCR expression (selection model) or they are different lineages (lineage model). I would like to propose a new model based on our data: at least during embryogenesis, the first B-lymphoid precursors derived from endothelial cells are Notch-signaling independent and biased towards B1 cells. During the maturation of hemogenic endothelial cells, Notch signaling induces B2 and HSC potential in those precursors. Thus, cells having biased B1 cell potential will gain B2 potential in a Notch signaling dose-dependent manner. Once HSCs are established, HSCs are biased to produce B2 cells, rapidly losing B1 cell potential.

What relevance do your data have to HSC transplantation in humans?

AD: Previous studies have shown that B1 cells cannot be reconstituted upon bone marrow transplants. B1 cells are central players in mounting an immune response against microbial infections and in preventing inflammatory diseases, including atherosclerosis. Therefore, it is important that we find strategies to replenish the compartment of B1 cells after HSC transplantation. Human pluripotent stem cells represent a potential source of autologous B1 cells for adoptive transfer protocols in parallel to HSC transplants. However, we need first to understand how to efficiently differentiate pluripotent stem cells into this specific B-cell subset. Our studies refine our understanding of the development of B1 cells and will provide the basis for the design of strategies to differentiate human pluripotent stem cells into functional B1 cells. Based on our results, this will likely involve a stage-specific inhibition of Notch signaling.

When you were carrying out the research, did you have any particular result or 'eureka' moment that has stuck with you?

NAP: It was very satisfying when B-cell progenitors derived from *Rbpj*$^{-/-}$ mouse ESCs engrafted in (sublethally) irradiated newborn mice and differentiated into B1 cell subsets. It was a confirmation that our hypothesis was right and that we were headed in the right direction.

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And what about the flipside: any moments of frustration or despair?

RS: In my experience, ups and downs are frequent in research. In particular, working with stem cells can be extremely demanding due to the delicate nature of the cells. Moreover, recapitulating embryonic development from pluripotent stem cells requires an orchestrated balance of factors, morphogens and environmental cues that can be easily disrupted. Therefore, it has happened (and it will happen again) that, what was scheduled as a 2-week-long experiment, ended up taking 3 months due to trouble with cells, contamination and technical issues. However, the excitement at the end of the experiments – the joy of analyzing the results and speculating on the next steps – unarguably repays the effort.

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What next for you after this paper?

RS: This paper sets an important milestone for my PhD studies that I am planning to conclude in the next months. In the meantime, I am continuing to characterize the ontogeny of different hematopoietic cells, shedding light on the mechanisms discriminating their hematopoietic stem cells (in)dependency.

Where will this story take the Yoshimoto lab?

MY: This story is an important piece of my whole picture. Our lab is currently working on lineage-tracing studies to understand the origin of each blood subset. We will investigate the role of Notch signaling in fetal hematopoiesis and its requirement for each blood subset. We also want to understand the key target of Notch signaling in the specification of B1 and B2 cells.

And Andrea, what is next for the Ditadi lab?

AD: We will focus mostly on human hematopoietic development, always using pluripotent stem cells. We want to understand at a molecular level how much blood cells differ depending on whether they have been generated in the presence versus absence of Notch signaling. In particular, we want to know how an endothelial cell decides to become a blood cell and how Notch signaling affects this transition. All blood cells were endothelial cells once upon a time. I am amazed that, during evolution the production of blood cells, such a key element that allows communication among all the organs (a sort of body 'social network'), has been 'outsourced' to a different lineage. It is yet another story on Notch signaling, since its modulation yields different flavors of this transition.

Finally, let's move outside the lab – what do you like to do in your spare time?

MY: I like reading books, such as mysteries and detective stories, and watching movies, especially Japanese geek movies! Ha-ha-ha. I am very lazy at home. Also, I am a good eater. Unfortunately, the food culture in the USA is very different from the one in Japan. So my husband and I end up cooking various Japanese dishes by ourselves. We enjoy good food at home, not only Japanese food but also Chinese, Indian and Italian food, all of which we make from scratch. Our tiramisu will beat Italian restaurants around Houston!

AD: Spare time? What is spare time? Of course, I am kidding, although when I am not in the lab, I am mostly kidnapped by my two kids, Inés and Gabriel; I love to play with them. If, because of some astral conjunction, there is still some time during the day, I usually invest it in a good book (either fiction to change my ideas or inspirational biographies), a good beer or a glass of good wine and a nice meal (you can always count on me for eating and drinking). It is usually accompanied by some good music. I am also desperately trying to keep waking up in the early hours to go for a run before starting a day, something I started doing only lately during the pandemic. The motivation is not always there, but I force myself into it. It is a good metaphor for life in the lab; it is never easy but with the reward that comes after is really worth it. P.S. Momoko, tiramisu challenge accepted!

Reference

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