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Central trained immunity is modulated by stimuli mimicking various type of infection

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Hematopoietic stem cells (HSCs) are rare and quiescent cells able to give rise to the entire blood system. During emergency hematopoiesis, HSCs mediate the regeneration of the hematopoietic system through direct sensing of infection 1. We and others have recently shown that stimuli such as LPS, BCG and beta-glucan confer an epigenetic memory in HSCs referred to as « central trained immunity » 1-3. In case of second stimulation, these trained HSCs have better proliferation and differentiation capacity biased toward the myeloid lineage. Chronic or repeated stimulations are known to impair HSCs functions but little is known about how successive combinations of different types of stimuli affect HSC memory and function 4,5.

To answer this question, we have first tested whether HSCs are activated and trained by stimuli mimicking different type of micro-organism infection. We tested whether a single stimulation with zymosan, Lyovec Poly(I:C) (Lyo-PI:C) mimicking respectively fungi and dsRNA viruses can activate HSCs using flow cytometry. We observed that 1 day after the injection of zymosan and Lyo-PI:C, HSCs as well as MPP3, MPP2 (MultiPotent Progenitors) and GMP (Granulo-mono progenitors) increase in number and frequencies showing an emergency myelopoiesis. HSCs show also increase of Sca1 and CD41 expression and a better proliferation capacity as shown by increase of EDU incorporation. Interestingly, 1 week after the simulation, changes observed in HSCs are back to steady state. These results suggest that zymosan and Lyo-PI:C activate transiently HSCs that respond with an inflammatory response and a myeloid bias differentiation.

We then tested whether these stimuli can train HSCs by changing their response to LPS. Mice were pre-stimulated with Zymosan, Lyo-PI:C and were re-stimulated with LPS 4 weeks later. Our preliminary results show an increase of CD41 and a decrease of proliferation in the HSC of pre-stimulated mice compared to naïve mice. This indicate that both stimuli enhanced the myeloid differentiation of HSCs with a moderate proliferation during LPS exposure and suggest that HSCs can be trained by these 2 stimuli. Epigenetic analyses are ongoing to further confirm that this is related to an epigenetic memory.

Finally, we used our new selected stimuli to induce what we call "memory combination" by adding one LPS injection one week before the stimulation with zymosan, Lyo-PI:C or LPS. 4 weeks later, we tested the effect of these different "memory combination" by analyzing the response of HSC to a last LPS injection. Our preliminary results showed that HSCs pre-stimulated twice (LPS+LPS / LPS+Zymosan / LPS+Lyo-PI:C) showed increased frequency compare to those stimulated only once. In addition, this increase is even more important for the "memory combination" LPS+Zymosan. These results suggest that adding a second stimulation to the training modifies the memory acquire in response to the first LPS stimulation and that it is dependent of the nature of the stimulus.

In conclusion we found that new stimuli mimicking fungi and dsRNA virus, able to activate HSCs directly. This will modify their response to subsequent LPS stimulation indicating a trained memory. We also found that stimuli combination seems to modify HSC training and further experiments are needed to described how this is affecting HSC memory and function.

1 Chavakis, T., *et al*. Hematopoietic progenitor cells as integrative hubs for adaptation to and fine-tuning of inflammation. *Nat Immunol* (2019). 2 de Laval, B. *et al.* C/EBPβ-Dependent Epigenetic Memory Induces Trained Immunity in Hematopoietic Stem Cells. *Cell Stem Cell et al.* (2020). 3 Divangahi, M. *et al.* Trained immunity, tolerance, priming and differentiation: distinct immunological processes. *Nat Immunol* (2021). 4 Esplin, B. L. *et al.* Chronic exposure to a TLR ligand injures hematopoietic stem cells. *J Immunol* **186**, 5367-5375, doi:jimmunol.1003438 (2011). 5 Bogeska, R. *et al.* Inflammatory exposure drives long-lived impairment of hematopoietic stem cell self-renewal activity and accelerated aging. *Cell Stem Cell* (2022)