Cytokine-mediated regulation of immunity during persistent viral infection

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Appendices

English summary

Nederlandse samenvatting

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Acknowledgement

Summary in English

Viruses that cause chronic viral infections such as HIV, HBV, HCV and EBV have evolved complicated mechanisms that help them avoid host immune surveillance. As host immune response fails to contain the infection, T cells face sustained high levels of antigen. Similarly, intratumoral T cell population is chronically stimulated by high level of antigens in the tumor microenvironment (TME). Consequently, T cells enter the state of exhaustion where they lose their effector functions and start to express inhibitory receptors. To cope with T cell exhaustion, various strategies targeting inhibitory receptors to reverse T cell exhaustion have been employed. Immune checkpoint blockades (ICB) that inhibits PD-1 and CTLA-4 have shown success in reinvigorating antigen-specific CD8 T cell functions and promoting clinical improvements. Despite their clinical benefits that revolutionized the field of oncology, only a small subset of patients responds to these treatments. Hence, better understanding of the mechanisms that regulate T cell exhaustion is required for the development of new strategies that reverse T cell exhaustion and improve the efficacy of immunotherapy. Recently, large number of cytokines that play pivotal roles in T cell regulations were identified. The aim of this thesis is to obtain better understanding of the roles of different cytokines on regulating antiviral and antitumoral T cell immunity. The ultimate goal of this study is to be able to apply new knowledge gained from this project to develope effective cytokine-directed immunotherapies targeting both persistent viral infections and cancer.

Chapter 1 provides general introduction for the thesis. It covers topics spanning chronic viral infection, T cell exhaustion, LCMV as a model to study chronic viral infection, and the impacts of cytokines on regulating T cells. **Chapter 2** describes the opposing roles of IFN-I and IL-27 in promoting expansion of stem-like $TCF1^+$ CD8 T cells that give rise to proliferative burst upon anti-PD-1 treatment. STAT1 is essential for the expansion of this CD8 subset while STAT2 retrains it. Cell-intrinsic IL-27 signaling is required to positively regulates STAT1 to promote the expansion of these cells via maintaining their differentiation and survival. We found that IRF1 is also a transcription factor required for the expansion of the $TCF1^+$ CD8 subset. Moreover, we showed that IL-27 is required for IRF1 expression. Overall, our findings suggest the potential use of IL-27 in augmenting anti-PD-1 immunotherapy during viral infections and cancer. Given the importance of IL-27 in regulating T cells during persistent LCMV infection, in **chapter 3** we discussed the cellular source of IL-27 required to promote control of persistent LCMV infection. B cells were identified as essential source of IL-27 that drives T and B cell immunity to promote control of persistent viral infection. We demonstrated that T cell-intrinsic IL-27 signaling is required to promote control of viral persistence. Finally, IFN-I blockade was able to increase total number of Tfh cells but failed to improve Tfh functions and promote clearance in IL-27 deficient mice. **In chapter 4** we tested whether we can pharmacologically reverse T cell exhaustion. Using a chemical screen for small molecules that affect T cell exhaustion we identified Jak inhibitors as a class of compound that rescues T cell functions. We further confirmed by testing Jak inhibitor ruxolitinib *in vivo* to show that this compound enhances the stem-like TCF1+ CD8 subset and synergizes with checkpoint blockade therapy during persistent viral infection and cancer. Further, we described that ruxolitinib exert its T cell enhancing

function through suppression of Myc and STAT3. Finally, we examined patient PBMCs from a phase ½ of classical non-Hodgkin Lymphoma trial that received ruxolitinib in combination with checkpoint blockade therapy. Intriguingly, patients displayed increased in total lymphocyte counts after receiving ruxolitinib. Taken together, this study showed that inhibition of Jak using small molecules re-shapes T cell functions during chronic viral infection and cancer.
Chapter 5 provides summary and general discussion for the thesis. Although all three studies presented in this thesis have their own discussion section, here in chapter 5 we further discussed strengths, weaknesses, and better way to improve our study or interpret the data.

Conclusion:

In summary, the three studies described in this thesis demonstrate the roles of cytokines in regulating T cell exhaustion. We have shown that IFN-I and IL-27 affects stem-like CD8 T cell differently. Then, we identified B cells as essential producers of IL-27. Finally, we showed that using small molecule jak inhibitor we can rescue T cell exhaustion and promote antiviral and antitumoral immunity. Overall, the fundamental observations made during these studies provide insights of how immunotherapy can potentially be improved.