Cytokine-mediated regulation of immunity during persistent viral infection

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Chapter 5

Summary and general discussion

Persistent viral infection remains an unsolved public health problem that results in devastating morbidity and mortality worldwide(1). T cell exhaustion is established as a consequence of chronic immune activation and high antigen load during persistent viral infection and cancer (2). Given that T cells play critical roles in promoting antiviral immunity(2-4), developing efficient immunotherapeutic strategies that can reverse T cell exhaustion and accelerate clearance of viral infection remains a major subject of intense research. Advances in the field of immunology led to discoveries of both immunosuppressive and immunostimulatory cytokines that influence functions of antigen-specific T cells(5, 6). Targeting these cytokines have long been a strategy for developing new therapeutic agents for humans and mice. Given that cytokine signaling networks are complex, more scientific effort is needed to better understand their production, mechanism of action as well as their interactions with other cells or cytokines. In this thesis, we described our studies aimed at investigating the roles of IFN-I, IL-27 and Jak inhibitor ruxolitinib on regulating T cell immunity during persistent LCMV infection and cancer.

The opposing roles of IFN-I and IL-27 on T cell expansion

Although IFN-I is known for their positive antiviral functions during acute viral infection, studies showed that IFN-I possess potential deleterious effects during persistent viral infections(7, 8). Blockade of IFN-I has been shown to increase the expansion of the recently identified subset of stem-like $TCF1^+$ CD8 T cells with strong proliferative potential that drives effector functions after anti-PD-1 treatments(9-11). Corroborating these results, we experimentally demonstrated in **chapter 2** that IFN-I signaling through STAT2 restrains the expansion of stem-like $TCF1^+$ CD8 T cells while STAT1 is required for the expansion of these cells. Moreover, we identified IL-27 as a positive regulator that drives STAT1-dependent $TCF1^+$ CD8 T cell expansion following IFNAR blockade through maintaining cell cycle and protecting them from apoptosis. This finding is relatively surprising for us since the role of IL-27 in promoting control of persistent LCMV infection has been associated with accumulation of virus-specific CD4 T cells and antibody class switch (12). On the contrary, the role of IL-27 on regulating CD8 T cells is complex; IL-27 promotes expression on inhibitory receptors such as PD-1 and Tim-3 on CD8 T cells(13, 14) during cancer and LCMV Cl-13 infection. In addition, IL-27 suppresses virus-specific CD8 T cell functions during acute influenza and Corona viral infections(15, 16). Together, this makes IL-27-mediated induction of negative immune regulatory molecules and preservation of a stem-like CD8 T cell state appears quite contradictory at first glance. However, others have demonstrated that genetic deletion of PD-1 from antigen specific T cells results in more severe exhaustion which is coupled to decreased cell survival and reduced long-term maintenance of the exhausted T cell pool(17). One intriguing hypothesis is that IL-27 promotes T cell stemness in part by promoting expression of negative immune regulatory molecules which restrain terminal T cell differentiation. Multiple studies have demonstrated that IL-27 promotes the generation of memory CD8 T cells during vaccination (18) and CD8 T cell reconstitution following antibody-mediated lympho-ablation, indicating that IL-27 may be necessary for sustained survival and proliferation of CD8 T cells(19). Taken together, our findings suggest that the role of IL-27 on augmenting anti-PD-1 immunotherapy in both persistent infection and cancers should be further assessed. In addition, given that chronic viral

infection lasts several weeks, it would be quite interesting to determine the role of IL-27 in promoting expansion of stem-like TCF1^{+} CD8 T cells during the persistent phase of infection. This can possibly be assessed by using IL-27 blocking antibody *in vivo* or crossing IL-27ra$^{flox/flox}$ mice with CD4CreERT2 mutant mice to generate tamoxifen-induced, Cre-mediated IL-27ra deletion(19, 20).

The newly identified cellular source of IL-27

Although IL-27 has been shown to be essential for promoting functions and accumulations of both CD4 and CD8 T cells during persistent LCMV infection, the cellular source of IL-27 required for long term control of viral persistence remains unknown. Previous study showed that the cellular sources of IL-27 required for early containment of persistent LCMV infection are DCs and myeloid cells(21). Using IL-27p28-eGFP reporter mice, we discovered that B cells produce IL-27 during persistent LCMV infection (**chapter 3**). This finding is consistent with previous findings which showed that B cells can express IL-27p28 upon *in vitro* activation(22, 23). Since multiple studies showed that B cell derived cytokines play essential roles during bacterial infection as well as autoimmune disease(22, 24-26), we crossed IL-27p28$^{flox/flox}$ mice to MB1 cre mice to specifically delete IL-27p28 in B cells and assessed the importance of B-cell-derived IL-27 during persistent LCMV infection. Mice lacking B-cell-derived IL-27 failed to control the infection and displayed reduced virus-specific IgG2a/2c antibody. This finding is quite surprising as B cells are not major producers of IL-27 at any time during infection, suggesting that the location where the cytokine is produced is crucial to promote antiviral immunity. Moreover, B cell-derived IL-27 induces viral control by promoting accumulations of virus-specific T cells and production of IL-21 and IFN-γ by Tfh cells. However, B-cell-intrinsic IL-27 signaling is not required for control of persistent LCMV infection while T-cell-intrinsic IL-27 signaling is indispensable. Our finding supports a model where B cells produce IL-27 during T-B cognate interaction to maintain functions of Tfh cells meanwhile cognate Tfh cells simultaneously promote GCB differentiation and antibody class switch. Since recent findings have reported that B cells in human tumors' tertiary lymphoid structures (TLS) are associated with better response to immunotherapy and increased survival (27-29), the role of B-cell-derived IL-27 should be examined in tumors. Although our study has revealed a central role of B cells in producing IL-27 that induces T-cell-mediated antiviral immunity during persistent viral infection, we are still unable to conclude whether B cells are the only source of IL-27 required for control of persistent LCMV infection. To determine the role of other cellular sources of IL-27 during LCMV Cl-13 infection, IL-27p28$^{flox/flox}$ mice should be crossed with XCR1 cre mice to specifically delete IL-27p28 in DCs, or Lyz2 cre mice to specifically delete IL-27p28 in or myeloid cells. Finally, the roles of B-cell-derived IL-27 on the expansion of stem-like TCF1^{+} CD8 T cells following IFNAR blockade should also be assessed.

Pharmacological manipulation of CD8 T cells during infection and cancer

Despite the success of anti-PD-1 immunotherapy in reversing T cell exhaustion and treating cancer, a large subset of patients fails to respond to this treatment(30). Hence, there is a need to develop novel therapeutic approaches to improve T cell functions and increase the number of patients responding to anti-PD-1 immunotherapy. In **chapter 4,** we employed previously

described *in vitro* chemical screen(31) to identify small molecules that can regulate CD8 T cell exhaustion. Specifically, our findings uncovered that inhibition of Jak signaling with ruxolitinib re-shapes the distribution of exhausted CD8 T cells subsets, favoring expansion of the stem-like $TCF1^+$ CD8 T cells capable of responding to anti-PD-1 immunotherapy. A combination of ruxolitinib with PD-L1 inhibition reduces viral load during chronic viral infection while a combination of ruxolitinib with PD-1 and CTLA-4 inhibition reduces tumor burden in MC38 model. However, unlike anti-IFNAR, which enables massive proliferation of the $TCF1^+$ P14 cells early on during LCMV infection in an IRF1-STAT1 dependent manner, ruxolitinib slows down proliferation and enhances the $TCF1^+$ CD8 subset through STAT3 and Myc inhibition. Given the importance of STAT3 and Myc pathways in promoting accumulation of $TCF1^+$ CD8 T cells, STAT3 and Myc knockout P14 cells should be further examined to settle their CD8 intrinsic roles in differentiation with and without ruxolitinib. Moreover, previous study showed that chronic IFN signaling promotes acquired resistance to both ICB and a combination of radiation therapy with ICB by inducing increased expression of T cell inhibitory receptor ligands on cancer cells. The authors further demonstrated that ruxolitinib was able to reverse this effect and improve tumor rejection exclusively in checkpoint-resistant melanoma and breast tumors(32). Given that we observed synergy between ruxolitinib and ICB in our MC38 tumor model, further study of Jak knockout MC38 cells and their effect on ruxolitinib would be useful in determining whether the effect is direct on immune cells. Finally, in collaboration with the university of Minnesota, peripheral blood mononuclear cells (PBMCs) from patients in a phase 1/2 trial of non-classical Hodgkin lymphoma (cHL), who previously failed to response to chemotherapy as well as immunotherapy, were examined before and 1 week after ruxolitinib treatment. Our data showed that ruxolitinib enhanced absolute lymphocyte counts in these patients. However, no significant difference in stem-like $TCF1^+$ CD8 T cells and cytokine-producing T cells was observed. Although this study likely has clinical implications for humans, with limited access to patient samples, findings that we obtained in mice are not fully aligned with patient data. In addition, it is possible that not only T cells are affected by the combination of ruxolitinib and immune checkpoint blockade. A recent finding revealed that tumor microenvironment (TME) in cHL inhibits NK cell proliferation and functions(33), suggesting NK cells could be another cell type affected by the combination therapy. It would be interesting to assess functions of NK cells in PBMCs from these patients. Taken together, our study revealed the central role of Jak signaling on rescuing CD8 T cell exhaustion and potentially promoting responses to immune checkpoint blockade.

Concluding remarks

T cell exhaustion remains a major challenge that needs to be tackled in both chronic viral infections and cancers. Recent advances in the field of immunology have improved our understanding of how cytokines influence different aspects of T cell responses. Cytokines including IL-2, IL-6, IL-7, IL-10, IL-21, IL-27, and IFN-I have previously been shown to play essential roles in regulating T cell functions(7, 8, 12, 34-39). Although immune checkpoint inhibitors have been successfully used to overcome T cell exhaustion and treat certain types of cancers, a large group of patients fail to respond to ICB and a subset of patients develop resistance to the treatment. Combination therapies are therefore essential to improve the efficacy of ICB and overcome the resistance. Recently, a study using LCMV showed that IL-2 therapy synergizes with anti-PD-L1 therapy in reversing T cell exhaustion and promoting faster viral clearance(40). Another study showed that a combination of PD-1 blockade and IL-10

neutralization in a mouse model of ovarian cancer improves T and B cell responses, reduces tumor size and prolongs survival(41). This thesis further extended this understanding by initially exploring mechanisms of action and signaling network of IL-27 and IFN-I in regulating CD8 T cells. Crucially, we showed in chapter 2 that IL-27 is required for the expansion of the $TCF1^+$ CD8 T cells upon IFNAR blockade. Through activation of STAT1 and IRF1, IL-27 promotes accumulation and survival of stem-like $TCF1^+$ CD8 T cells. Although the role of IL-27 in promoting T cell functions and clearance of LCMV infection has been established, cellular source of this cytokine required for the control of the virus remained unknown. Using mouse models with cell-specific deletion of IL-27p28, B cells were identified as an essential cellular source of IL-27 required for the control of persistent LCMV infection. Our findings in chapter 3 demonstrated that B-cell-derived IL-27 supports T cell functions through inducing IL-21 and IFN-γ productions. These findings clearly demonstrated the complexity of cytokine signaling in regulating T cells immunity. Given that B cells produces IL-27, which subsequently induces IL-21 and IFN-γ productions, it would be very interesting to study the role of B cells during immunotherapy in both persistent infection and cancers. Following our findings in chapter 2, we were encouraged to further search for therapeutic targets that could potentially rescue T cell exhaustion. Using chemical *in vitro* screen in chapter 4, we identified ruxolitinib as a small molecule capable of reversing T cell exhaustion by improving cytokine-producing functions of exhausted CD8 T cell in addition to promoting accumulation and survival of the stem-like $TCF1^+$ CD8 T cells. With our collaborator at the university of Minnesota, we finally reported that ruxolitinib treatment leads to increased lymphocyte counts in patients with cHL. Taken together, our findings demonstrated an effective application of fundamental discoveries in clinical setting. Overall, the studies presented in this thesis have broadened our understanding of how IL-27, IFN-γ, and IFN-I signal and interact to regulate T cell functions and overcome exhaustion. We hope that our findings will provide fertile ground for future designs of effective immunotherapies for human disease.

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