Response letter to the referees

We would like to thank the referees for their careful assessment of our work. While the four referees had overall positive comments regarding our review, one referee raised concerns, as follows:

"What is missing from this article is a section that would describe the translational implications of these basic immunological mechanisms. The authors should add an additional section to cover how understanding of these pathways may offer therapeutic opportunities and for which conditions. In addition, the effect on these mechanisms by the currently available drugs that target the IL-1-mediated pathways should be covered by the authors."

Our response:

We thank the referee for raising this point and have accordingly revised the conclusion section of our mini-review to expand more on the potential clinical implications of IL-1-driven Th17 and Th9 cell polarization, please see below.

Best regards,

Lionel Apetoh

Concluding remarks

Since the discovery of the lack of IL-1RI on mouse Th1 cells, many studies focused on *in vivo* animal models of Th2 related diseases. Different models of mice either deficient for IL-1α/IL-1β or for their receptor IL-1RI were analyzed to determine the effects IL-1 family members on the Th2 response; and most, but not all, of these studies agree with the idea that both IL-1α and IL-1β promote proliferation and differentiation of Th2 cells *in vitro* and *in vivo*. Surprisingly few studies have investigated the effects of IL-1 on human Th2 cells, and the findings do not enlighten if human Th1 or Th2 cells express a functional IL-1RI and therefore can be modulated by IL-1α or IL-1β cytokines. Furthermore, recent studies

demonstrated that the major IL-1 family cytokines directly acting on Th1 and Th2 cells are IL-18 and IL-33 rather than the classical IL-1α and IL-1β.

In contrast to the discrepant results obtained with Th1 and Th2 cells, animal models and human studies all agree with the concept that IL-1β has a fundamental role in Th17 modulation. The *in vitro* assays clearly demonstrate that IL-1β is able to induce the transcription factors necessary for Th17 development, as soon as its own receptor IL-1RI is upregulated in naïve T cells upon TCR triggering in the presence of γ-chain cytokines. IL-1RI expression is maintained on effector Th17 cells and this signaling is probably responsible for the prolonged survival of these cells during inflammation. This IL-1-driven enhanced Th17 cell activity has *in vivo* relevance. Nakae and colleagues have shown in a mouse model of arthritis that IL-1 drives the secretion of IL-17 from CD4 T cells, resulting in enhanced arthritis development[1]. These findings have possible clinical relevance given the ability of IL-1 neutralization using Anakinra, a recombinant version of IL-1Ra, to alleviate the symptoms of rheumatoid arthritis[2]. However, whether the beneficial effect of IL-1Ra administration in inflammatory diseases results from the inhibition of Th17 responses remains elusive. In a cancer setting, we have shown that the treatment of tumor-bearing mice with the chemotherapeutic agent 5-Fluorouracil leads to the *in vivo* release of IL-1β, the release of IL-17 from CD4 T cells and tumor progression[3]. Importantly, neutralization of IL-1β with IL-1Ra *in vivo* led to synergistic anticancer effects upon combination with 5-FU in mouse models. A clinical trial evaluating the possible benefit of IL-1Ra addition to 5-FU treatment in metastatic cancer patients is ongoing (NCT02090101). Thus, IL-1Ra-driven down-modulation of Th17 cell responses may be clinically relevant for the treatment of cancer and inflammatory diseases.

Finally, we found that IL-1β was able to enhance the differentiation of mouse naive $CD4^+$ T cells into Th9 cells by considerably enhancing expression of IL-9 and IL-21 cytokines and efficiency of antitumor activity in several models of mouse tumors through $CD8^+$ and NK cells. While we found in a mouse model of melanoma that the systemic administration of IL-

1β enhanced the anticancer efficacy of adoptively transferred Th9 cells[4], these findings may be difficult to translate in the clinic because of the high toxicity profile of IL-1 administration in humans. We believe instead that our findings may be relevant in the context of adoptive T cell therapy with Th9 cells treated with IL-1β *in vitro* and given in combination with chemotherapy and/or immunomodulators. Thus, it will be important to determine if comparable IL-1β-modified Th9 cells can be obtained from human T lymphocytes, and if their antitumor activity could be even more increased by association with immune checkpoint inhibitors like PD-1, and whether appearance of autoimmune side effects could be a limitation for their use in cancer therapy.

References

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