ウェブサイト公開用

（様式 10）
（海外特別研究員事業）
令和　３年　１０月　８日

海 外 特 別 研 究 員 最 終 報 告 書

独立行政法人日本学術振興会 理事長　殿

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受 付 番 号　201960201
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海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。
なお、下記及び別紙記載の内容については相違ありません。

記

1. 用務地（派遣先国名）用務地：　メリーランド州ベセスダ　　（国名：　米国　　）

2. 研究課題名（和文）※研究課題名は申請時のものと違わないように記載すること。
新規病原性 T 細胞集団による痒み・くしゃみなどの神経過敏症状の病態形成機構の解析

3. 派遣期間：平成　３１年　　４月　　１日　～　令和　３年　　９月　　３０日（９１４日間）

4. 受入機関名及び部局名
受入機関名：　国立衛生研究所（National Institutes of Health, NIH）
部局名：国立関節炎・骨格筋・皮膚疾患研究所（National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIAMS）

5. 所期の目的の遂行状況及び成果…書式任意　**書式任意（A4 判相当 3 ページ以上、英語で記入も可）**

【記載事項】
- 研究・調査実施状況及びその成果の発表・関係学会への参加状況等
- 新型コロナウイルス感染症の影響にかかる特例措置のうち、国内採用開始・採用期間延長・翌年度渡航のいずれかの適用を受けた場合は、当該措置の適用による影響等

（注）「6. 研究発表」以降については様式 10－別紙 1～4 に記入の上、併せて提出すること。

Overview

This is a newly initiated project based on findings from scRNA-seq in models of type 2 inflammation that began with my arrival last year.　Among the genes that caught our attention was Molecular X an adapter/DNA binding protein whose expression correlated with that of GATA3 (Figure 1).　In muscle, Molecular X is reported to associate with some particular factors and functions to recruit the histone 3 lysine 4 methyltransferase complex including Wdr5.　In addition, Id3 is reported to be a direct target of the Molecular X complex.

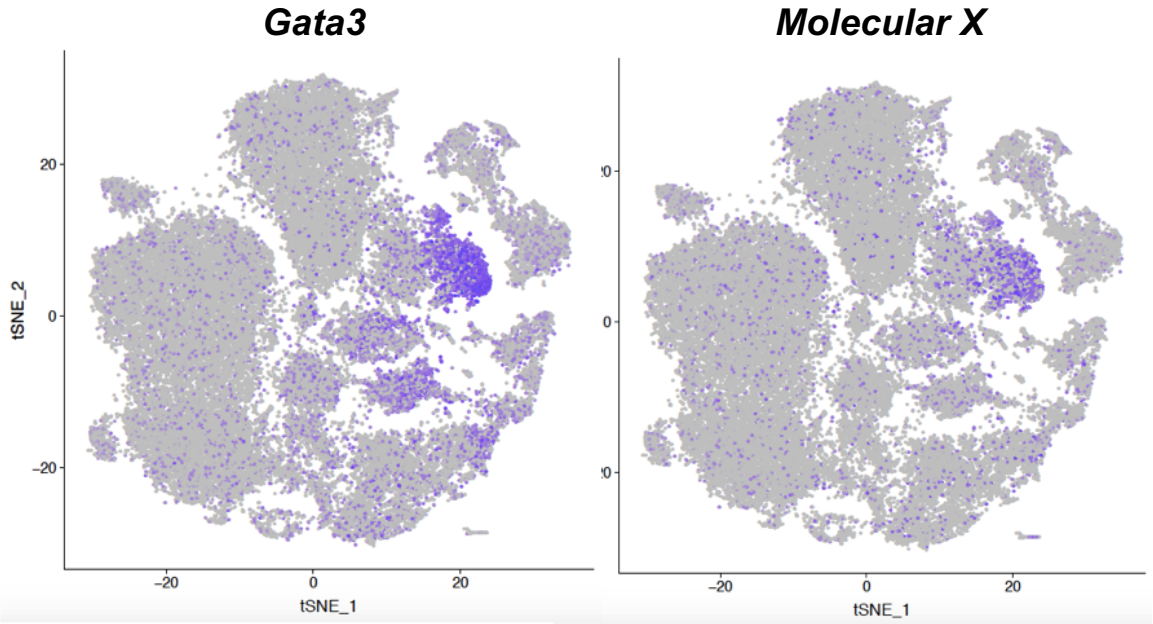
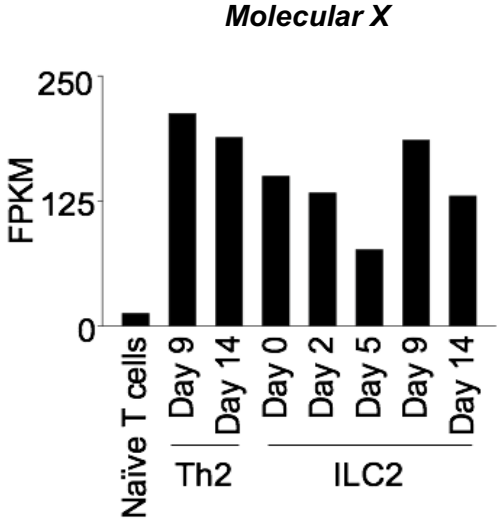


Figure 1. Co-expression of Molecular X and Gata3 in lung lymphocytes

Details

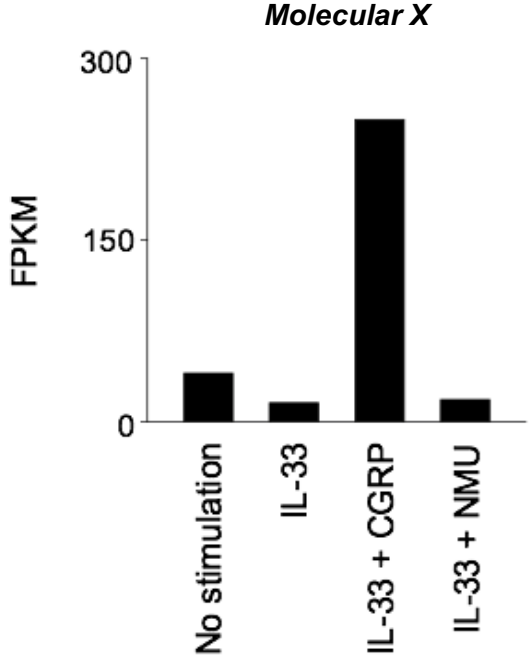
There is a paucity of published information on Molecular X; however, transcriptomic databases indicate expression in bone marrow, high expression of Molecular X in γδ T cells, NK T cells, CD8 T cells and very high expression in small intestine ILC2s. In my experiments, I found that Molecular X is induced relatively late during in vitro Th2 differentiation, but is very abundant (600 RPKM, Figure 2). I also found that Molecular X is expressed in ILC2 cells and upregulated during *N. brasieliensis* infection. Of interest, I found that Molecular X is induced in ILC2 cells in vitro by IL-33 and CGRP (Figure 3). CGRP also promoted the accessibility of the Molecular X locus in ILC2 cells (data not shown). Further attracting my interest locus was the finding that Molecular X is bound Stat6 and dependent upon Stat6 for its expression. In addition, another Molecular Y closely related to Molecular X, which I found is expressed in CCR6+ NKp46- ILC3s and Th17 cells compared to ILC2s.

Figure 2.
Molecular X is induced in lung Th2 cells and ILC2s following *N. brasieliensis* infection



Based on its pattern of expression, its potential direct regulation by Stat6 and induction by neuropeptide CGRP, I concluded that exploration of the function of Molecular X would likely be promising. I therefore plan to determine in detail factors that positively and negatively regulate Molecular X expression, including cytokines that drive Th differentiation. I will further explore the impact of alarmins and neuropeptides on its expression and assess its expression in models of disease. Of interest, will be to assess the effect of Nmu separately and in conjunction with CGRP. The high level of expression of Molecular X in small intestine ILC2s suggest that there may very well be links between Molecular X expression and neural influences. With our collaborators, we will explore induction of Molecular X in models of type 2 inflammation in the skin, lung and gut. Commercial antibodies are available to detect Molecular X by immunofluorescence which will be used to analyze in vivo expression and intracellular localization. I will determine if any of these reagents are suitable for flow cytometry; we generated monoclonal antibodies as the project develops.

Figure 3.
Molecular X is induced in ILC2 by IL-33+CGRP



In my additional experiments, overexpression of Molecular X enhanced IL-4 production and reduced IFN-γ production (data not shown). To verify these preliminary data and to assess the impact of Molecular X, I performed RNA-seq and ATAC-seq about human Th2 cells which were knock-outed by the CRISPR Cas9 systems. In the future, I will work with the analysis about these human T cells. Conversely, I will employ Cas9 transgenic mice to edit Molecular X and Molecular Y in primary lymphocytes with assistance from the Schwartzberg lab. I will assess the impact on in vitro polarization and will study in vivo functions in adoptive transfer experiments. And Molecular X fl/fl mice are obtained from RIKEN and are being crossed with CD4-Cre and Vav1-cre mice initially to begin to define the role of Molecular X in type 2 responses. I hypothesize that Molecular X promotes such responses and that deficiency of Molecular X will limit Th2 differentiation and allergic disease. To study functions of Molecular X in ILCs, we will cross Molecular X fl/fl mice with Il5-Cre and Rora-cre. I hypothesize

that deleting Molecular X in T cells and/or ILC2s will interfere with the ability to clear helminthic infections including *N. brasieliensis* and might limit allergic disease.

Advanced Results

Molecular X is a poorly characterized nuclear factor and germ-line deletion in mice is lethal. We generated conditional deletion (cKO) in mice (CD4-Cre-*Molecular X* fl/fl) to study its function in lymphocytes. *Molecular X* deletion led to T cell maturation defect in thymus and fewer naïve T cells in spleen. Upon OVA asthma challenge, *Molecular X* cKO mice showed impaired T helper 2 (Th2) and enhanced T helper 1 (Th1) responses while comparable numbers of lymphocytes were recovered from wild type and cKO lungs. When differentiated in vitro, *Molecular X* cKO Th2 cells upregulated Th1 genes that were predicted targets of polycomb repressive complex 2 (PRC2). As expression of PRC2 molecules were maintained, Th1 loci specific impairment of PRC2 function was indicated in cKO Th2 cells. High resolution single cell analysis of transcriptomes derived from helminth infected lung identified *Molecular X* as selectively expressed in Gata3+ Th2 subset that represented later stage of Th2 differentiation. Collectively, Molecular X is a late fate-stabilizing factor in Th2 cells for silencing Th1 program.

Conclusion

In this study, roles of Molecular X expressed in T cells were investigated through loss-of-function approach in mice, and various impacts at multiple stages of T cells development were revealed; (1) fetal hematopoiesis (indispensable), (2) adult T cell maturation (~50% reduction), (3) TCR stimulated proliferation (delayed), (4) cell expansion by virus (>70% reduction), (5) cell expansion by repeat OVA Ag sensitization (preserved), (6) Th1 skewed effector response (steady state, LCMV, OVA-asthma), (7) de-repression of Th1 gene expression in Th2, (8) reduced H3K27Me3 marks in Th1 gene loci. Collectively, observed phenotypic alterations in LOF mice and cells can be summed into two principles in biology; (A) proliferation defect, and (B) failure to properly suppress Th1 signature when necessary.

By analyzing WT T cells in vitro and in vivo, it was noted that expression of Molecular X is relatively low across various adult T cell subsets with an exception of Th2 cells. Th2 cells uniquely induce increasingly high levels of Molecular X along their differentiation trajectory. Therefore, it is conceivable that context dependent impact of Molecular X deletion may be partially explained by differential dosage required for signal induce proliferation vs stable Th2 phenotype. In this regard, we could relate our observation to previous reports pointing to differential dosage of TFs as critical determinant of how TFs work, i.e. GATA3, T-bet, RORγt.

Interestingly, the degree of proliferation defect upon activation was similar between Th1 and Th2 cultures, indicating relatively low levels of Molecular X present in naïve cells is essential for initial transition. Nonetheless, activated

Molecular X cKO T cells were eventually able to overcome early delay in cell cycle entry and expand with time. While underlining molecular events leading to cycling T cells in the absence of Molecular X have not been investigated and beyond the scope of this report, it is likely linked to TCR signaling and downstream mTOR activity rather than signals provided by cytokines.

The most unexpected part of our finding relates to Th2 phenotype and selective high expression of Molecular X in Th2. While a hint of Th1 skewing in effector populations was noted in steady state ex vivo cells as well as small fraction of LCMV induced CD44+ T cells, it is most evident in Th2 cells that were generated in vitro or induced in vivo via OVA induced asthma. Aberrantly induced Th1 genes in Molecular X cKO cells is normally repressed by PRC2 via H3K27Me3 marks, but selectively de-repressed in the absence of Molecular X. From the dynamics of Molecular X induction and coregulated genes, Molecular X is predicted to act as a fate stabilizer for Th2 cells along the later part of Th2 differentiation trajectory. Close functional correlation of Molecular X and PRC2 was striking considering that expression of PRC2 complex subunits, specifically EZH2, remained unchanged in the absence of Molecular X. As multiple composition of PRC2 complexes can be formed in a context dependent manner, it is tempting to hypothesize that Th2 cells incorporate Molecular X as a part of PRC2 functional complex to maintain terminally differentiated state. In this regard, physical interaction between Molecular X and EZH2 or SUZ12 was reported using embryonic carcinoma cells, making a possibility of similar PRC2- Molecular X interaction plausible in T cells. Molecular evidence linking Molecular X to PRC2 in Th2 cells as well as mechanism to selectively targets Th1 gene loci would be the focus we envision for our future study.

Impact of COVID-19-related special measures

COVID-19-related special measures extended my dispatch period by six months in addition to the original plan.

Thanks to these measures, the research plan, which had been significantly delayed, was successfully carried out on schedule.