ウェブサイト公開用

（様式 10）
（海外特別研究員事業）

令和 2 年 2 月 29 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

採用年度 平成２９年度
受付番号 829
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（氏名は必ず自署すること）

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：マックス・プランク免疫生物学エピジェネティック研究所（国名：ドイツ）

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。
代謝スイッチに関連する細胞周期遺伝子に着目したマクロファージの分化機構の解明

3. 派遣期間：平成 ３０ 年 ０２ 月 ２８ 日 ～ 令和 ０２ 年 ０２ 月 ２７ 日

4. 受入機関名及び部局名
マックス・プランク免疫生物学エピジェネティック研究所 Edward Pearce 研究室

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）**
（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）
（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。

< Research Summary >

Immune cells undergo profound metabolic alterations upon activation that are integral to their change for bioenergetic and biosynthetic demands [2]. Enhanced glycolysis together with ancillary pathways supports growth, proliferation, migration and/or secretory state, and therefore it has become evident that metabolic reprogramming of immune cells is pivotal to mount a proper immune response (Fig. 1). Recent findings suggest that intracellular metabolic substrates, checkpoint pathways and post-transcriptional modification determine the differentiation and function of immune cells. Indeed, the finding that effector T cells metabolically reprogram towards glycolysis in order to initiate effector function challenged the conventional knowledge of engaging glycolysis to acquire energy rapidly for proliferation [3]. Furthermore, the molecular mechanisms underlying the switch to glycolysis in inflammatory macrophages are being investigated. Yet, despite advances in the field, there are still many challenges that remain to be addressed. It is not fully understood why activated inflammatory macrophages metabolically reprogram to gain effector function and halt proliferation.

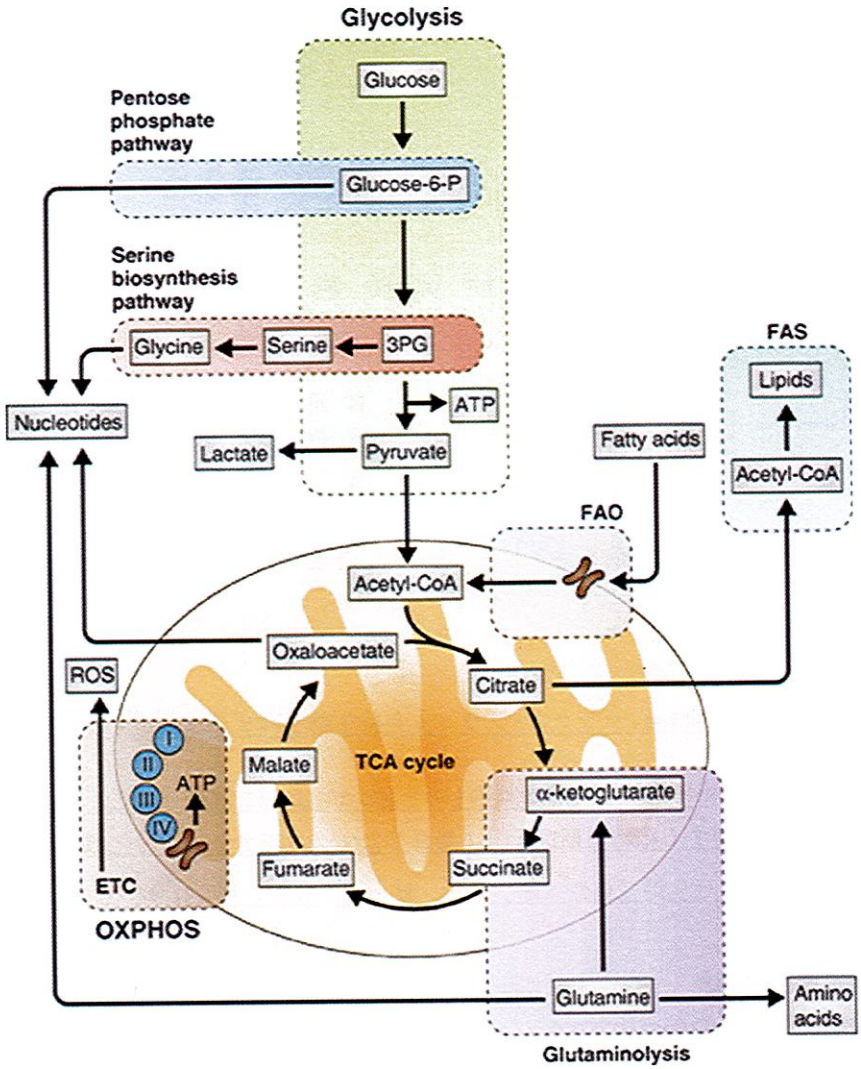

Fig 1: Overview of the different metabolic pathways associated with cellular activation. [1] 3PG, 3-phosphoglyerate; ETC, electron transport chain; ROS, reactive oxygen species; FAO, fatty acid oxidation; FAS, fatty acid synthesis.

The main objective of this project was to elucidate the molecular mechanism of metabolic reprogramming and the link to suppression of cellular proliferation in inflammatory macrophages. Gene expression pattern obtained from RNA sequence analysis of bone marrow derived macrophages, revealed an upregulation of Schlafen (Slfn) gene family upon activation with bacterial-derived pro-inflammatory stimulus, lipopolysaccharide (LPS). Slfn genes have been reported previously to control cell cycle progression in various cell types, suggesting a potential involvement for these genes in the growth regulatory function of LPS stimulated macrophages (Fig. 2). Three major objectives in this research together evaluated the significance of Slfn gene regulation in glycolytic macrophage activation: (i) to determine the key Slfn gene members that regulate proliferation and glycolytic switch, (ii) to identify Slfn gene regulation in type I IFN signaling, and (iii) to define the *in vivo* significance of target Slfn

genes by generating macrophage specific conditional Slfn gene knockout mice.

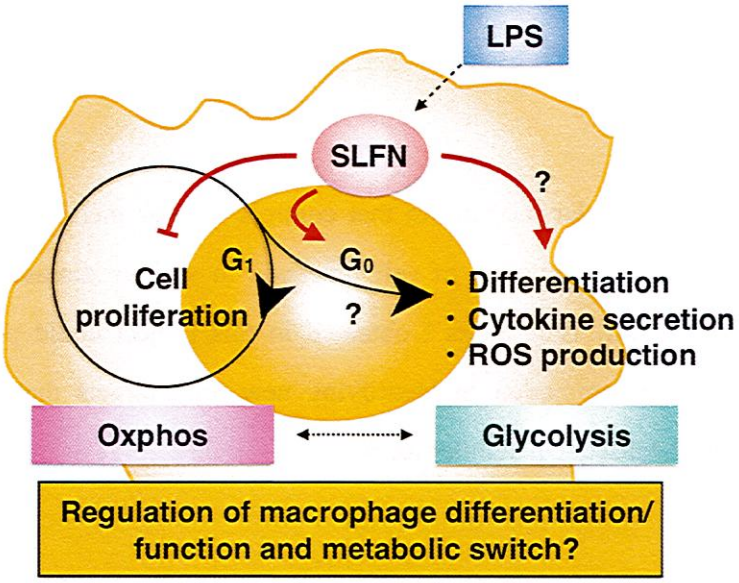

Fig 2: Hypothesis of Slfn gene regulation on cell proliferation and trigger in metabolic switch on LPS-stimulated macrophages. LPS, lipopolysaccharide; SLFN, Schlafen gene; Oxphos, oxidative phosphorylation.

The project first set out to identify critical Slfn gene members that are essential for proliferation and differentiation in LPS-stimulated macrophages. While upregulation of Slfn gene expression was established upon type I IFN-mediated TLR4 stimulation, contrary to previous findings in other cell types, none of the Slfn gene members contributed to cell cycle arrest in inflammatory macrophages. Additionally, the metabolic profile associated with Slfn genes was tested during this initial screening process and found that Slfn3 gene alters the glycolytic profile of LPS-stimulated macrophages. This result suggested that this gene possesses a previously unidentified function in the regulation of activated macrophage metabolism. Knockdown of Slfn3 gene using siRNA resulted in a marked decrease in expression of key glycolysis enzymes and glucose carbon flux through the glycolysis pathway (Fig. 1, green area) into ancillary pathways. Enhanced glucose carbon into the pentose phosphate pathway (Fig. 1, blue area) sustained normal levels of lactate production and those into serine biosynthesis pathway (Fig. 1, red area) supported the production of reducing equivalents to maintain steady redox balance. In addition, glucose carbon into lipids (Fig. 1, aqua area) contributed to the engagement of fatty acid synthesis. Further analysis is currently being performed to gain better insight in the underlying reason and consequence of glucose rerouting into ancillary pathways. Furthermore, the *in vivo* significance of Slfn3 is yet to be evaluated. Currently, conditional myeloid cell-specific Slfn3 knockout mice are being generated

using Crispr-Cas9 technology, which is expected to shorten the time taken to generate a mouse strain, in collaboration with the in-house transgenic mouse core facility. Once the mice are available, these mice will be phenotypically assessed and tested for their pro-inflammatory function and glycolytic switch upon LPS challenge and bacterial infection.

< Outlook >

Understanding the molecular mechanism that controls cellular metabolic states possess great potentials for clinical manipulation of cell functions in various diseases settings caused by immune cell imbalances. The results I obtained in this project could provide a therapeutic promise to chronic inflammation in identifying new drug targets that influence metabolic changes and control consequent cellular function.

< Conference Attendance and Publication >

Findings of this project were presented as a poster entitled "The role of cell cycle-regulating genes in metabolic reprograming in differentially activated macrophages" at the Keystone Symposia on Molecular and Cellular Biology (Myleoid Cells; Breckenridge CO, USA; April 2018). This conference provided me a great networking platform that subsequently led me to invite Dr. Kenneth M. Murphy (Washington University), an eminent immunologist, to Germany for scientific discussion and a seminar at the institute.

Within the duration of this funding period, I contributed to three publications, including a preview in Science and a research article in Cell Reports.

< Reference >

1. Buck MD, O'Sullivan D, Pearce EL (2015) T cell metabolism drives immunity. *J Exp Med* **212**: 1345–1360.

2. Pearce EL, Pearce EJ (2013) Metabolic pathways in immune cell activation and quiescence. *Immunity* **38**: 633–643

3. Chang C-H, Curtis JD, Maggi LB Jr, Faubert B, Villarino AV, O'Sullivan D, Huang SC-C, van der Windt GJW, Blagih J, Qiu J, et al. (2013) Posttranscriptional Control of T Cell Effector Function by Aerobic Glycolysis. *Cell* **153**: 1239–1251.