様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　６ 年　 ９ 月 ２１ 日現在

機関番号：１４５０１
研究種目：若手研究
研究期間：2022～2023
課題番号：２２Ｋ１６３０２

研究課題名（和文）Deciphering regulatory mechanism of trained immunity in aged HSCs and clonal hematopoiesis of indeterminate potential (CHIP)

研究課題名（英文）Deciphering regulatory mechanism of trained immunity in aged HSCs and clonal hematopoiesis of indeterminate potential (CHIP)

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交付決定額（研究期間全体）：（直接経費）　　2,200,000円

研究成果の概要（和文）：本研究課題は、クローン性造血の発症における老化造血幹細胞に対する訓練免疫の役割を明らかにすることを目的に、骨髄を含めた全身でヒト造血の評価が可能なヒト化マウスモデルを作製した。本モデルを用いたがん異種移植モデルにおいて、骨髄から分化した骨髄系細胞の著しい浸潤を認めたことから、担がん下もしくは病原体刺激下でのヒト骨髄系細胞、特にCD34+造血幹細胞に由来するマクロファージの役割の解明を中心に解析を行なった。その結果、訓練免疫で見られるようなヒトマクロファージの代謝の変化を見出したことから、本モデルはヒト造血系における訓練免疫の役割の解明につながることが期待される。

研究成果の学術的意義や社会的意義
ヒト化マウスを用いた本研究により、病原体成分による老化造血幹細胞への刺激がヒト造血幹細胞自身に加えヒトマクロファージの機能にどのように影響を与えるか、訓練免疫の解明につながる成果が得られた。

研究成果の概要（英文）：The original research proposal aimed to investigate the impact of induced trained immunity in aged HSCs and its role in CHIP pathogenesis.
To understand how human HSCs modulates the functions of its progeny populations in immune training in a relevant physiological condition, the generation of a humanized mouse model with robust engraftment of human HSCs that populated bone marrow, blood, and organs like the spleen. Xenograft tumor development in this model confirms CHIP compatibility, revealing significant human myeloid cell infiltration, key in transmitting trained immune responses from HSCs. Therefore, the focus shifted towards elucidating the role of human myeloid cells, particularly macrophages derived from CD34+ HSCs, within this context. Current research aims to induce trained immunity in macrophages via immune modulatory treatments in aged-humanized mice, reflecting human aging. This is expected to provide deeper insights into immune training mechanisms.

研究分野：Immune-hematology

キーワード：Trained immunity　Humanized mouse model　Hematopoietic stem cell　aging　CHIP

１．研究開始当初の背景

Hematopoietic stem cells (HSCs) serve as a continuous source of immune cells throughout life. Initially, the idea of immune memory was primarily associated with the adaptive immune response. However, there has been a recent shift in focus towards recognizing the "memory" capabilities of innate immune cells, such as monocytes, macrophages, dendritic cells, and NK cells. When these cells are stimulated by external or internal factors, they exhibit increased responsiveness to subsequent challenges - a phenomenon referred to as trained immunity. This immune imprinting, which operates independently of lymphocytes, involves epigenetic, metabolic, and transcriptional adaptations in both innate immune cells and their precursor HSCs in the bone marrow. Consequently, upon encountering specific microbial or inflammatory stimuli, these cells and their progeny demonstrate faster and stronger responses to subsequent challenges, leading to broader immune responses, both antigen-specific and non-specific. Moreover, accumulating evidence suggests that the altered transcriptional state in HSCs can be inherited by their progeny, contributing to trained immunity.

Aging is one of the most powerful independent risk factors for the development of various hematological malignancies including clonal hematopoiesis of indeterminate potential (CHIP), and its prevalence unanimously increases with age, with rapid accumulation to >10% of people after age 65. The accumulation of DNA mutations in myeloid related genes within HSCs with aging and the mutant HSC “clone” gradually becomes dominant and overtakes the bone marrow and blood system called clonal hematopoiesis of indeterminate potential (CHIP). Inflammation is another risk potential for CHIP mutation. Dysregulated inflammation confers a selective advantage to the clone and play a key role in perpetuating a cycle of inflammation and clonal expansion acquire aberrant functions of the various gene (TET2, DNMT3A, etc.). Recently, several large genomic studies have identified germ-line mutation in addition to somatic mutation as SNPs (single nucleotide polymorphism) as a key regulator for CHIP progression. Hence, the understanding of trained innate cells mediated immune effects in aging and age-related comorbidities (CHIP) will help us to control, and/or slow-down the aging and CHIP development.

２．研究の目的

The main purpose of this research was to (1) elucidate the molecular mechanism by which HSC conveys immune training to its progeny populations especially in the aging process and CHIP pathogenesis. To unravel the complex links between CHIP,

inflammation, and aging, we need to configure specific candidate molecule(s) to disrupt this vicious cycle. The triad of interconnection between programmed innate immune training, aging, and CHIP mutation will help us to define (2) specific candidate molecule(s) to confer potential interventions.

３．研究の方法

This research project involves the development of a specialized humanized mouse model, where severely immunodeficient mice were engrafted with human hematopoietic stem cells (CD34+ HSCs) derived from human umbilical cord blood. This model aims to closely replicate the human immune system within mice for advanced immunological studies. Human immune cells differentiation and distribution as well tumor microenvironment was analyzed by flow-cytometry.

４．研究成果

(1) Establishment of humanized mouse model:

The immunodeficient mice were successfully engrafted with CD34+ HSCs. This process involves transplanting human stem cells into the mice, allowing the development of a human-like immune system within these animals.

- Human Immune cells differentiation: post-engraftment, the human stem cells differentiated into various immune cells, including both innate (natural killer cells, macrophages, and dendritic cells) and adaptive immune cells (T cells, B-cells).
- Migration and distribution: The human immune cells not only populated the bone marrow but also migrated to peripheral blood and other organs such as the spleen, indicating robust engraftment and functionality.
- Xenograft Tumor Development and CHIP Compatibility:

  Tumor microenvironment analysis:

  The model was used to study the development of xenograft tumors, including both hematological and solid tumors. I observed significant infiltration of human myeloid cells within the tumor microenvironment, suggesting that the humanized model can be used to study cancer-immune system interactions and CHIP (Clonal Hematopoiesis of Indeterminate Potential) compatibility.

(2) Induction of trained immunity:

- Role of Myeloid cells:

  Myeloid cells, derived from the engrafted CD34+ HSCs, were identified as key players in transmitting trained immune responses. Trained immunity refers to the phenomenon where the innate immune system retains a memory of past infections and responds more robustly to subsequent challenges.

- Macrophage focus:

  The research shifted focus to understanding the role of macrophages, a type of myeloid cell, in trained immunity. Macrophages derived from CD34+ HSCs were studied to elucidate their role in immune responses.

- Immune modulatory Treatment:

  Current investigations involve inducing trained immunity in these macrophages using immune modulatory agents such as β-glucan. These treatments are administered to CD34+ HSCs in aged-humanized mice to mimic conditions reflective of human aging. β-glucan is known to enhance the immune response by acting on various immune cells, including macrophages.

This research project establishes a specialized humanized mouse model via CD34+ HSC transplantation, replicating the human immune system for advanced studies. Successful engraftment and differentiation of human immune cells were observed, indicating robust functionality. Analysis of xenograft tumors revealed significant infiltration of human myeloid cells, facilitating the study of cancer-immune interactions and CHIP compatibility. Research now centers on inducing trained immunity in CD34+ HSC-derived macrophages using β-glucan in aged-humanized mice to mimic human aging.

５．主な発表論文等

〔雑誌論文〕　計3件（うち査読付論文　3件／うち国際共著　2件／うちオープンアクセス　3件）

１．著者名 Saito Yasuyuki、Iida-Norita Rie、Afroj Tania、Refaat Alaa、Hazama Daisuke、Komori Satomi、Ohata Shinya、Takai Tomoko、Oduori Okechi S.、Kotani Takenori、Funakoshi Yohei、Koma Yu-Ichiro、Murata Yoji、Yakushijin Kimikazu、Matsuoka Hiroshi、Minami Hironobu、Yokozaki Hiroshi、Manz Markus G.、Matozaki Takashi	４．巻 14
２．論文標題 Preclinical evaluation of the efficacy of an antibody to human SIRPα for cancer immunotherapy in humanized mouse models	５．発行年 2023年
３．雑誌名 Frontiers in Immunology	６．最初と最後の頁 1294814
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fimmu.2023.1294814	査読の有無 有
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１．著者名 Komori Satomi、Saito Yasuyuki、Nishimura Taichi、Respatika Datu、Endoh Hiromi、Yoshida Hiroki、Sugihara Risa、Iida-Norita Rie、Afroj Tania、Takai Tomoko、Oduori Okechi S.、Nitta Eriko、Kotani Takenori、Murata Yoji、Kaneko Yoriaki、Nitta Ryo、Ohnishi Hiroshi、Matozaki Takashi	４．巻 120
２．論文標題 CD47 promotes peripheral T cell survival by preventing dendritic cell-mediated T cell necroptosis	５．発行年 2023年
３．雑誌名 Proceedings of the National Academy of Sciences	６．最初と最後の頁 p.e2304943120
掲載論文のDOI（デジタルオブジェクト識別子） 10.1073/pnas.2304943120	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Mitsuhashi Atsushi, Koyama Kazuya, Ogino Hirokazu, Afroj Tania, Nguyen Na Thi, Yoneda Hiroto, Otsuka Kenji, Sugimoto Masamichi, Kondoh Osamu, Nokihara Hiroshi, Hanibuchi Masaki, Takizawa Hiromitsu, Shinohara Tsutomu, Nishioka Yasuhiko	４．巻 42
２．論文標題 Identification of fibrocyte cluster in tumors reveals the role in antitumor immunity by PD-L1 blockade	５．発行年 2023年
３．雑誌名 Cell Reports	６．最初と最後の頁 112162～112162
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.celrep.2023.112162	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

〔学会発表〕　計3件（うち招待講演　0件／うち国際学会　1件）

1．発表者名 Tania Afroj, Yasuyuki Saito, Satomi Komori, Tomoko takai, Okechi S. Oduori, Takenori Kotani, Yoji Murata, Takashi Matozaki
2．発表標題 Development of a novel cancer immunotherapy model targeting human macrophages
3．学会等名 第21回生体機能研究会
4．発表年 2023年

1．発表者名 Tania Afroj, Yasuyuki Saito, Rie Iida-Norita, Satomi Komori, Takenori Kotani, Yoji Murata, Takashi Matozaki
2．発表標題 Preclinical evaluation of the anti-tumor effect of anti-human SIRPα antibody in a novel humanized mouse model
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1．発表者名 Yasuyuki Saito, Tania Afroj, Rie Iida-Norita, Satomi Komori, Takenori Kotani, Yoji Murata, Markus G Manz, Takashi Matozaki
2．発表標題 Preclinical evaluation of macrophage-mediated anti-tumor effect by using a humanized MITRG mouse model
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〔図書〕　計0件

〔産業財産権〕

〔その他〕

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6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関