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

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研究種目：若手研究(B)
研究期間：2017～2019
課題番号：１７Ｋ１５３８８

研究課題名（和文）自然リンパ球を産生する新たな免疫器官－縦隔内脂肪関連リンパ組織の機能とその異常－

研究課題名（英文）Novel immune organs producing innate lymphoid cells - function and abnormality of mediastinal fat-associated lymphoid clusters -

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：報告者らは、MRL/MpJ-fas lprマウスにおける自己免疫性肺病変の性差を明らかにし、その縦隔関連リンパ組織（MFALCs）との関連性を示し、科学論文として公表した（Elewa et al. 2017, Autoimmunity)。加えて、ブレオマイシン投与マウスの肺病変の病理発生を明らかにし、それらはMFALCsの形態変化と関連することを明らかにし、科学論文として公表した（Elewa et al. 2018, Front. Immunol.）。一方、国内外の研究者と共同研究を進め、筆頭著者として共著論文を公表し(Elewa et al. 2019)、また30篇以上の共著論文を公表した。

研究成果の学術的意義や社会的意義
We clarified the contribution of MFALCs in the pathogenesis of lung injury in several mice models. Therefore, this study could open the bright future of human respiratory diseases by introducing innovative therapeutic strategy for respiratory diseases via interfering with some pathways via MFALCs

研究成果の概要（英文）：I revealed the sex-related differences in autoimmune-induced lung lesions in MRL/MpJ-fas lpr mice and its relationship with the development of a novel mediastinal fat-associated lymphoid clusters (MFALCs). I could publish these data in one high impact (Elewa et al. 2017, Autoimmunity). Additionally, I revealed the pathogenesis of lung lesion progression, and their correlation with MFALCs morphologies in bleomycin-induced pneumonitis mice. From the data analysis of this experiment, I could publish in one high impact Journal (Elewa et al. 2018, Front. Immunol.). Also, I collaborated with doing research with other researchers within my lab and other institutions inside Japan and outside Japan and from this collaboration, I could publish the data in high impacted Journal (Elewa et al. 2019, ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY) as first author as well as in more than 30 Journals as co-author .

研究分野：immunity and respiratory diseases

キーワード：autoimmune disease　Lung fibrosis　pneumonities　natural helper cells　innate immunity

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Recently, the association between adipose tissue and immunity has been established and fat-associated lymphoid clusters (FALCs) are considered as a source of several immune cells. Interestingly, I discovered novel FALCs associating with the mediastinal fat tissue of healthy mouse and termed it as "mediastinal fat-associated lymphoid clusters (MFALCs)" (**Fig. 1, Elewa et al, Cell Tissue Res 2014**). Such MFALCs consists mainly of macrophages, B-cells, and T-cells, Moreover, the size of the MFALCs differed among healthy mouse strains, indicating that genetic factors affect the development of this novel immunological unit in mice. Additionally, we revealed the presence of a new innate lymphoid cells (ILCs) named as natural helper cells in such clusters. However, the major role of such cells in the disease progressions remains unclarified. My data suggests a potentially important role for MFALCs in intrapleural environment, but their functional details remain unclear. Interestingly, we also revealed that the MFALCs were significantly larger in autoimmune disease mouse models (e.g. MRL-*lpr*) than in their corresponding control strains, and the pathological correlation was observed between the size of MFALCs and cell infiltrations such as macrophages, B-cells or T-cells, and granulocytes in lung (**Fig. 2, Elewa et al, Immunology 2016**). Therefore, I suggested that such clusters could play an important role in the progression respiratory diseases organs via production of pathogenic cytokines and immune cells including ILCs. However, their role in the progression of other respiratory diseases such as septic and aseptic induced- pneumonitis, asthma, lung cancer required further investigations.

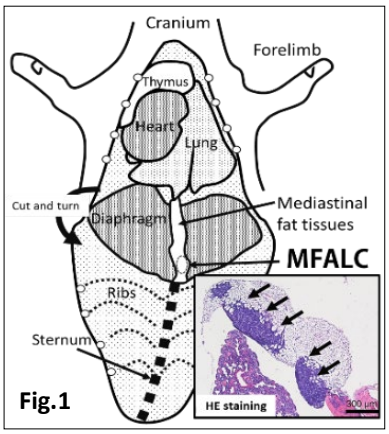

Fig.1

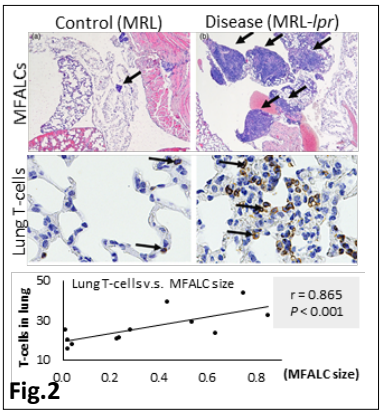

Fig.2

２．研究の目的

The purpose of our investigation is to elucidate the functional attributes of MFALCs, and their pathological contribution in several diseases by using mice model. Additionally, our aim is to clarify if the genetic variations in MFALCs size could contribute to variation in the susceptibility/ resistance for the development of respiratory diseases. Moreover, our study aimed to examine the sexual dimorphism of MFALCs development and their role in the variations of severity of lung injury among both sexes in autoimmune disease models. Therefore, our study could present cutting-edge research in innate immunity that could help to update FALCs immunity and clarify the pathogenesis of several respiratory diseases.

３．研究の方法

Various mouse models for respiratory diseases were prepared and used in this study to clarify what kind of diseases could alter MFALCs. Such mouse models include septic/ chemical induced pneumonitis (e.g. bleomycin intranasal administration), aseptic induction (e.g. *Mycoplasma pulmonis* infection) in both resistant (DBA) and susceptible (B6) mice. Additionally, lung cancer mice model was induced and analyzed with targeting for the endothelium using nanoparticles as an innovating therapeutic strategy for the lung cancer. Also, we examined the sex difference in the development of MFALCs and lung injury in autoimmune disease models. Histopathological examination including immunohistochemical analysis and gene expression of MFALCs and their adipose tissues, and cell populations and immune responses of MFALCs are compared between healthy and diseased groups. Further, histopathology of lungs and MFALCs are examined in all groups. Furthermore, pathological correlations between lung pathological scores (e.g. inflammation, alveolar structures) and MFALC structural changes (e.g. size, cell population) are clarified.

４．研究成果

Through our study we could address the following points:

- Sex related difference in the development of MFALCs and lung injury in autoimmune disease mice model (MRL-*lpr*), where the female revealed more significant lung injury and MFALCs size than that of male. Furthermore, we clarified significant positive correlations between the parameters of lung injury and that of MFALCS **(Elewa et al, Autoimmunity 2017).**
- We Analyzed the role of MFALCS in the inflammatory lung diseases by septic induction such as bleomycin induced-pneumonitis mouse model. Interestingly, we clarified more developed MFALCs in bleomycin group than the control group at both early stage (7d, inflammatory phase) and late phase (21d, fibrotic stage). Furthermore, we clarified the role of high endothelial venules in the development of both lung injury and MFALCs size in the bleomycin group **(Elewa et al., Front. Immunol. 2018).**
- Additionally, we revealed the morphofunctional analysis of antigen uptake mechanisms following sublingual immunotherapy with beads in mice **(Elewa et al. 2018, PLOS ONE).**
- Through my collaborative research, we clarified the effect of genetic variations in MFALCs size (mice strain differences) on the susceptibility to *Mycoplasma pulmonis* infection in DBA mice (that has less developed MFALCs) and B6 mice (that has well developed MFALCs) with the former susceptible and the later resistant strain. Suggesting that such MFALCs could provide a protective role against respiratory tract infection **(Boonyarattanasoonthorn et al., Infection, Genetics and Evolution 2019).**

- Also, through a collaborative research on lung cancer, we could provide an innovative therapeutic approach for lung cancer through targeting the lung endothelium with nanoparticles and the data of such research were published in one of the 10% top Journal **(Abd Elwakil et al., ADVANCED FUNCTIONAL MATERIALS 2019).**

５．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　4件／うち国際共著　4件／うちオープンアクセス　3件）

１．著者名 Elewa Yaser、Mizoguchi Tatsuya、Ichii Osamu、Nakamura Teppei、Kon Yasuhiro	４．巻 13
２．論文標題 Morphofunctional analysis of antigen uptake mechanisms following sublingual immunotherapy with beads in mice	５．発行年 2018年
３．雑誌名 PLOS ONE	６．最初と最後の頁 1-15
掲載論文のDOI（デジタルオブジェクト識別子） 10.1101/370163	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 Elewa, Y. H. A., Ichii, O., Takada, K., Nakamura, T., Masum, Md.A., Kon, Y.	４．巻 9 (271)
２．論文標題 Histopathological correlations between mediastinal fat-associated lymphoid clusters and development of lung inflammation and fibrosis following bleomycin administration in mice.	５．発行年 2018年
３．雑誌名 Frontier Immunology	６．最初と最後の頁 1-10
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fimmu.2018.00271	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 Yaser Hosny Ali Elewa, Osamu Ichii & Yasuhiro Kon	４．巻 50
２．論文標題 Sex-related differences in autoimmune-induced lung lesions in MRL/MpJ-faslpr mice are mediated by the development of mediastinal fat-associated lymphoid clusters.	５．発行年 2017年
３．雑誌名 Autoimmunity	６．最初と最後の頁 306-316
掲載論文のDOI（デジタルオブジェクト識別子） 10.1080/08916934.2017.1344973	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Mahmoud M. Abd Elwakil, Ikramy A. Khalil, Yaser H. A. Elewa, Kenji Kusumoto, Yusuke Sato, Nour Shobaki, Yasuhiro Kon, Hideyoshi Harashima	４．巻 18
２．論文標題 Lung-Endothelium-Targeted Nanoparticles Based on a pH-Sensitive Lipid and the GALA Peptide Enable Robust Gene Silencing and the Regression of Metastatic Lung Cancer	５．発行年 2019年
３．雑誌名 ADVANCED FUNCTIONAL MATERIALS	６．最初と最後の頁 1807677
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fimmu.2018.00271	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

〔学会発表〕　計5件（うち招待講演　0件／うち国際学会　4件）

1．発表者名
Y. H. A. ELEWA, O. Ichii, Y. Kon.

2．発表標題
Innate immunity through mediastinal fat-associated lymphoid clusters in papain induced lung asthma mouse model.

3．学会等名
The 42nd Annual Conference of the Egyptian Society of Histology and Cytology. “NANOPARTICLES BENEFITS AND HAZARDS”. (国際学会)

4．発表年
2018年

1．発表者名
Y. H. A. Elewa, T. Nakamura, O. Ichii, Y. Kon.

2．発表標題
Mediastinal fat-associated lymphoid cluster and lung asthma development in papain induced lung asthma mice model.

3．学会等名
The 64th annual meeting of the Japanese the Anatomical Society（国際学会）

4．発表年
2018年

1．発表者名
Yaser Hosny Ali Elewa, Osamu Ichii & Yasuhiro Kon

2．発表標題
Dual effects of bleomycin on the intra-thoracic immune hemostasis and lung injury in autoimmune disease model mice.

3．学会等名
The 41st Annual International Conference of Medical Histology & Cell Biology, Regenerative Medicine & Cell Transplantation. December 28th（国際学会）

4．発表年
2017年

1．発表者名
Yaser Hosny Ali Elewa, Osamu Ichii & Yasuhiro Kon

2．発表標題
Dual effects of bleomycin on the intra-thoracic immune hemostasis and lung injury in autoimmune disease model mice.

3．学会等名
The 6th Congress of Asian Association of Veterinary Anatomists (Asian AVA). Kuching, Sarawak, Malaysia.October 15th- 16th, 2017（国際学会）

4．発表年
2017年

１．発表者名
Yaser Hosny Ali Elewa, T. Nakamura, Osamu Ichii & Yasuhiro Kon

２．発表標題
Dual effects of bleomycin on the intra-thoracic immune hemostasis and lung injury in autoimmune disease model mice.

３．学会等名
The 63th annual meeting of the Japanese the Anatomical Society. Hirosaki, Aomori, Japan. September 9th-10th, 2017

４．発表年
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〔図書〕　計0件

〔産業財産権〕

〔その他〕
Laboratory of Anatomy
https://www.vetmed.hokudai.ac.jp/en/research/detail/
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https://www.scopus.com/authid/detail.uri?authorId=35763517200
Research Gate
https://www.researchgate.net/profile/Yaser_Elewa3
Orcid
https://orcid.org/my-orcid
Google Schoolar
https://scholar.google.com/citations?user=rXDl_3cAAAAJ&hl=en

６．研究組織

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