研究題目　Thymus medulla development and function during αβT-cell development

研究組織

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【1】研究の概要

［1-1］本研究の目的・概要

The thymus is an essential primary lymphoid organ in the immune system of all vertebrates. It is unique in its ability to support that generation of αβT-cells that play key roles in immune responses to invading pathogens and tumor surveillance. Research in my laboratory has focused on the mechanisms controlling αβT-cell development. In particular, we examine how cortical and medullary thymic epithelial cells (cTEC/mTEC) are able to generate and select self-tolerant T-cells and eliminate autoreactive T-cells that predispose to autoimmunity. The work of my laboratory in this area has been greatly aided by successful collaborations with immunologists at The University of Tokushima. Most recently, by collaborating with Dr Izumi Ohigashi and Professor Yousuke Takahama at The Institute of Genome Research, we co-authored an influential review article in Nature Reviews Immunology on thymic epithelial cell biology.

By continuing to develop our collaborative links with Dr Ohigashi, we are now in the exciting position of being able to exploit our individual and unique skill sets, to explore key unanswered questions in thymus biology. Additionally, these links will allow us to develop new joint research projects, that will train young scientists in Birmingham and Tokushima via reciprocal lab visits, and generate co-authored peer reviewed research articles.

Our overall aims are: (1) To combine fluorescent reporter mouse strains generated in Tokushima (CCL21 Red mice) with those generated in Birmingham (RANK Venus mice) to generate a novel ‘dual reporter’ mouse line, where newly defined thymic epithelial cell subsets can be analysed and isolated from embryonic and adult mice. (2) To train young researchers in cellular (Birmingham) and molecular (Tokushima) immunological techniques, to aid in developing their own research careers and underpin long-term collaborations between next generation scientists in Birmingham and Tokushima.

［1-2］研究の方法・経過

Our main aim is to investigate how cTEC and

mTEC lineages emerge during thymus development, and examine the functional significance of heterogeneity within these lineages in the adult thymus. In the Anderson lab, we recently generated fluorescent reporter mice in which cells expressing RANK, a key regulator of the thymus medulla, are labeled with the green fluorescent protein Venus. The Ohigashi lab has also recently generated a different reporter mouse in which mTEC that express the chemokine CCL21 are carry a red fluorescent label. We crossed these two independently generated mouse strains to generate a new 'dual reporter' mouse line where mTEC heterogeneity can be examined through analysis of red (CCL21) and green (RANK) expression. By sharing our mouse lines, and visiting and collaborating with the Ohigashi lab we did: (1) Examine the emergence of the mTEC lineage during embryogenesis by flow cytometric/confocal analysis of the developing thymus, from embryonic day 12 through to birth. (2) Isolate different subsets of mTEC from dual reporter mice ie. $RANK^{+}CCL21^{+}$: ($green^{+}red^{+}$), $RANK^{+}CCL21^{-}$ ($green^{+}red^{-}$), $RANK^{-}CCL21^{+}$ ($green^{-}red^{+}$), and $RANK^{-}CCL21^{-}$ ($green^{-}red^{-}$) and analyse their gene expression of known regulators of medulla development and mTEC function eg Aire, Relb, LTβR, CD40, Fezf2.

【2】研究成果

[2-1]本共同研究で明らかになった研究成果

Currently, following the importation of CCL21Tomato reporter mice from University of Tokushima into University of Birmingham, we have successfully generated double reporter CCL2Tomato/RANKVenus dual TEC reporter mice. In initial experiments, we have assessed the expression of Venus and Tomato in both thymus sections and by flow cytometry in adult and fetal stages. In early fetal stages (E15-E17) we see two clearly separate populations of mTEC: CCL21Tomato+RANKVenus- and CCL21Tomato-RANKVenus+. In these initial experiments, both populations appear to arise at the same time, suggesting that they may not reflect a precursor-product relationship. Moreover, in the adult, while we see the persistence of these populations, we also see a double positive population of mTEC that is CCL21Tomato+RANKVenus+. Thus, the approach of generating a new TEC reporter mouse line has identified new TEC populations that need to be assessed at both the developmental and functional level.

In addition, by importing the CCL21Tomato mice from Tokushima, we used them to assess the role of CCl21 in formation of the intrathymic dendritic cell (DC) pool. We found that CCL21 is essential to guide the migration of CCR7+ DC into the thymus. This work was recently published in Journal of Immunology as a collaborative publication.

[2-2]本共同研究による波及効果及び今後の発展性

Establishing a novel dual reporter mouse line, in which TEC are defined by the expression of functionally relevant molecules (RANK and CCL21) will provide important insight into the poorly understood field of thymus medulla development and function. Additionally, support obtained from this funding has had a significant impact on the training of young PhD students and postdocs in both Tokushima and Birmingham. By supporting reciprocal visits, scientists have met and discussed future plans, and data generated in this collaboration will be important in applying for future research funding in both the UK and Japan.

【3】主な発表論文等

[3-1]論文発表

1) Takahama Y, Ohigashi I, Baik S, Anderson G Generation of diversity inthymic epithelial cells. Nat. Rev. Immunol. 17:295-305 (2017).

2) Cosway EJ, Ohigashi I, Schauble K , Parnell SM, Jenkinson WE, Luther S, Takahama Y, Anderson G. Formation of the Intrathymic Dendritic Cell Pool Requires CCL21-mediated recruitment of $CCR7^{+}$ progenitors to the thymus. J. Immunol. 201:516-523 (2018).

2) [書籍]

[3-2]学会発表

[3-3]成果資料等

【4】今後の課題等

We now aim to systematically characterize mTEC development in the fetal and adult stages of life in CCL21Tomato/RANKVenus dual reporter mice. We will define the phenotypic properties of Tomato/Venus subpopulations by confocal microscopy and flow cytometry using a panel of markers (EpCAM1, Ly51, UEA1, b5t, MHCII, CD80, CD86). We will also test the developmental potential of individual populations by isolating defined populations on the basis of Tomato/Venus expression, and incorporating them into reaggregate thymus organ cultures, where the developmental potential of purified cells will be revealed.