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研究課題名（和文）Role of NR4A2 in pathogenic Th cells in autoimmune diseases

研究課題名（英文）Role of NR4A2 in pathogenic Th cells in autoimmune diseases

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研究成果の概要（和文）：自己免疫疾患である多発性硬化症(MS)は、免疫細胞が中枢神経系内で活性化し、神経系を含む自己組織を傷害することで発症する。私たちは以前に、MS患者末梢血中の免疫細胞でNR4A2の発現が増えることを見出した。本研究では、MSの組織傷害に密接に関わり、その性質が多くの興味を集めているヘルパーT(Th)細胞の活性化におけるNR4A2の役割を調べた。
私たちの解析から、NR4A2がMS初期の中枢神経系でのTh細胞活性化を制御することが分かった。とくにNR4A2は、MSを引き起こす病原性Th細胞の機能決定因子であり、この病原性Th細胞が持つ複数の特徴が、新しい治療標的となりうることが明らかとなった。

研究成果の学術的意義や社会的意義
This study provides new information about features of authentic damage-associated T cells in autoimmune disease. This will aid future study of the process these cells are involved in and increase understanding of cellular immunology in diseased tissues as well as providing new targets for treatment.

研究成果の概要（英文）：The autoimmune disease multiple sclerosis (MS) occurs when immune cells enter the brain and spine, become activated, and cause damage to self-tissues, leading to peripheral and neurological disabilities. Previously, we found that a particular gene, called NR4A2, was increased in immune cells in the blood of MS patients. In this study, we investigated how this NR4A2 gene was involved in the activation of T helper cells, a type of immune cells strongly associated with damage in MS disease, although currently the nature of these T cells that cause disease is hotly debated.
Our results showed that NR4A2 controls the type of response that T helper cells make during the initiation of autoimmune disease whey they become activated in brain/spine tissues. In particular, we discovered that NR4A2 determines if T helper cells become a pathogenic cells type, which cause damage in MS-like diseases and that these damage-associated T cells expressed novel features the allow targeting for treatment.

研究分野：Autoimmunity

キーワード：Immunology　Autoimmune diseases　T cells　Th17

１．研究開始当初の背景

Autoimmune diseases are caused by the inappropriate activation of an individual's immune responses against their own tissues, leading to inflammatory damage and loss of function. The incidence of such diseases has been rapidly increasing in Japan and other developed countries over recent decades and lack of autoimmune disease treatment has become a growing medical problem. In many cases these inflammatory immune responses are initiated by T cells responding to self-antigens expressed in particular tissues. Thus understanding how self-reactive T cells become activated is key to developing new treatments to combat autoimmunity. Multiple sclerosis (MS), the human organ-specific autoimmune disease of the central nervous system (CNS), has long been known to associate with self-reactive T helper (Th) cells. Recent findings using genome wide-association studies (GWAS) have demonstrated that the vast majority of MS susceptibility genes are associated with cellular immune responses and Th cell function and new immune specific treatments are effective in controlling some forms of MS (Ransohoff *et al.*, ***Nat Rev Neurol***, 2015). Therefore, research towards clarifying the development, differentiation, and functions of Th cells is highly relevant in identifying new therapeutic targets for MS. We previously reported that amongst circulating peripheral blood T cells from MS patients there was a significant increase in the expression of NR4A2, an orphan nuclear receptor with previously unknown function in T cell activation and highlighted a critical role for NR4A2 in T cell-mediated autoimmunity in a mouse model of MS (Experimental autoimmune encephalomyelitis; EAE). To investigate the function of NR4A2 in (EAE) and Th17 development, the principal investigator developed conditional knockout mice on a B6 background, in which NR4A2 was deleted under the control of Cre-CD4. These NR4A2 cKO mice lacked NR4A2 in peripheral Th cells. When NR4A2 cKO mice were immunized with $MOG_{35-55}$ to induce EAE, although large numbers of Th cells infiltrated into CNS tissue, NR4A2 cKO failed to develop acute/peak EAE (Raveney *et al.*, ***Nat Commun***, 2015). Without NR4A2, a much stronger immunization is required to induce disease, suggesting an important role for NR4A2 signalling in determining if self-reactive Th cells form pathogenic responses following physiological stimulation. A number of pathways to pathogenic Th17 cells have been proposed, our preliminary work indicates that the T cell receptor (TcR) stimulation level is regulated by different factors and is sensitive to NR4A2 signalling, which may explain how the exquisite control of pathogencity by NR4A2 is achieved by modulating particular types of Th17 cell activation under relevant conditions.

２．研究の目的

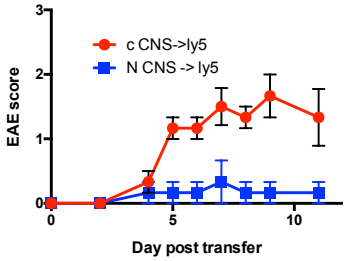
To identify the pathogenic Th cells that generate autoimmune diseases and how this process is controlled by the nuclear receptor NR4A2. In particular, investigate the role of NR4A2 in mediating T cell activation signals against self-tissues under physiological conditions and elucidate the mechanisms by which such T cells develop self-reactive pathogenic responses. To discover how this pathway can be manipulated to alter outcomes and provide novel targets for therapeutic intervention for the treatment of autoimmune diseases in man.

３．研究の方法

EAE was induced in mice on a B6 background by immunization with MOG-peptide in CFA. We generated mice lacking NR4A2 in T cells (NR4A2 cKO) as previously reported (Raveney *et al.*, ***Nat Commun***, 2015). CNS T cells were isolated by mechanism disruptiuon and enzymatic digestion of brain and spine tissues. Cell were sorted for ongoing expression analysis or analyzed for protein expression by flow cytometry. For, in vitro differentiated T cells, naïve T cells were cultured for 96 hours with polyclonal stimulation in the presence of recombinant cytokines. To identify pathogenic signatures of autoreactive Th cells, we used single cell gene expression analysis of CNS-infiltrating cells in EAE, comparing mice lacking NR4A2 with control mice. To understand the development of such pathogenic cells, we utilized *in vitro* Th cell differentiation under physiological stimulation conditions where NR4A2 singalling tunes outcomes.

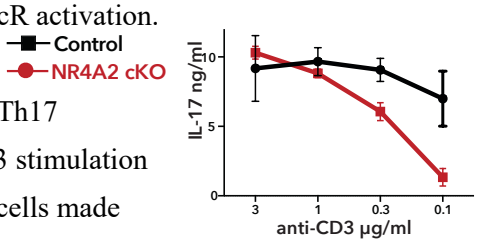
４．研究成果

We have previously shown that NR4A2 is upregulated by CD4+ T cells in relapsind/remitting MS (RR-MS) and in EAE. Further, knocking out NR4A2 in T cells, NR4A2 cKO, abrogates active disease following EAE induction. Although the kinetics of T cells activation and entry to the CNS tissues are similar in the presence or absence of NR4A2, in NR4A2 cKO mice, CNS-infiltrating T cells fail to rapidly expand as observed in diseased mice. However, NR4A2 cKO Th cells are able to proliferate normally in culture. Thus, firstly we wished to test if a lack of NR4A2 in itself was required for disease induction and so NR4A2 cKO Th cells are unable to mount pathogenic responses. Therefore, we transferred CNS-infiltrating Th cells from EAE mice at peak disease time, from both control and NR4A2 cKO mice. Cells were activated polyclonally in vitro and equal numbers were injected i.v. in congenic Ly5.2 recipients, to allow donor cells to be distinguished at a later time. Mice also received PTX at the time of transfer.

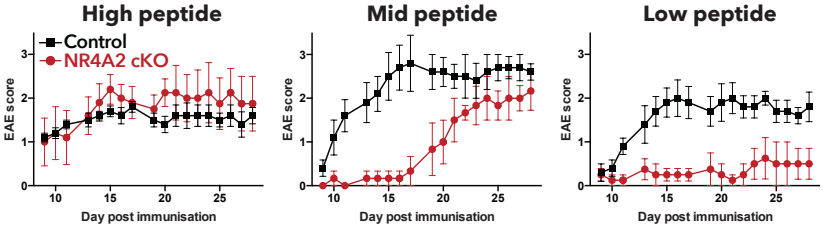



Whilst control CNS Th cells were able to drive passive transfer EAE, NR4A2 cKO Th cells failed to induce any disease symptoms in most mice. These data indicate that NR4A2 controls pathogenicity of CNS-infiltrating Th cells.

Some reports in the literature have associated NR4A family members with TcR signal transduction and we also observe a rapid upregulation of NR4A2 upon TcR ligation. We therefore tested the effect of the absence of NR4A2 on TcR activation. Under low and intermediated levels of TcR activation, Th cells lacking NR4A2 had reduced disease-associated Th17 responses. However, higher doses of polyclonal anti-CD3 stimulation were able to rescue TcR activation and NR4A2 cKO Th cells made similar responses to control cells. However, these levels are likely non-physiologic. Therefore, it is conceivable that NR4A2 does modulate TcR stimulation in vitro.



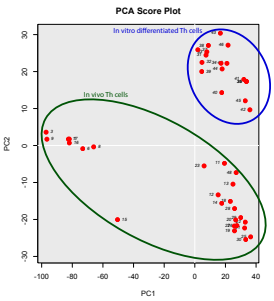
To further test this in vivo, we immunized groups of mice with increasing levels of MOG peptide.

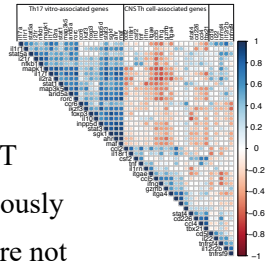



Similar to the in vitro results, the lack of disease in the absence of NR4A2 could be overcome by increasing the level of activation. In conclusion, this key role for NR4A2 in controlling the nature of the Th response, pathogenic or maintaining tolerance, could be critical for determining if autoimmunity results from an inflammatory insult. Under the steady state, low levels of stimulus, combined with a repertoire of lower affinity TcRs for self-antigen resulting from negative selection means that potentially autoreactive Th cells do not become inappropriately activated. However, NR4A2 mobilization can allow autoimmune pathogenic responses under these conditions. Therefore, NR4A2, as a master switch for self-reactive cells, is an excellent target for therapies to modulate Th cells responses, without having to consider the gamut of potential autoantigens

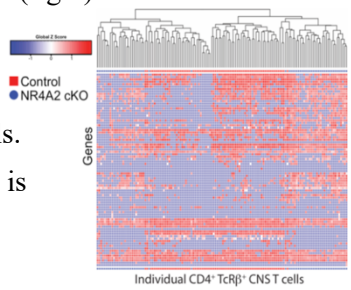
NR4A2-mediated control of autoreactive responses can also be used as a tool to answer important questions about the biology of pathogenic autoimmune responses. For many years, the phenotype and functional responses of autoreactive Th cells generating autoimmunity in MS has been researched and refined. Previously, Th cells infiltrating into CNS tissue and initiating damage have been defined as the Th1 type; then later, Th17 cells were instead implicated; subsequently exTh17 or Th1-like were considered as the most important, whilst at the same time Th17 earlier classed as pathogenic became termed as non-pathogenic Th17 cells; most recently GM-CSF-producing Th cells have been strongly associated with CNS autoimmunity. However, most of these studies rely on somewhat artificial models of disease. In particular, Th cells bearing a single T cell receptor with relatively low affinity against a single CNS antigen given at non-physiologic levels (2D2 CD4+ T cells, responding to a MOG 35-55 epitope) differentiated in vitro using recombinant cytokines to skew development of responses. Depending on the exact conditions used, many publications report particular T cell subsets as pathogenic or not, and indeed some cell types have been published which are not found in active models of disease or human MS itself. For the treatment of human disease, it is critical that research is focused on authentic pathogenic cells, to understand the processes involved in their development and activity, thus to allow monitoring of these harmful responses and target them with new drugs. Animal models and in vitro experiments can play an important role in this work, but only if realistic responses are studied. To this end, we carried out targeted expression analysis of transcriptomes from CNS-infiltrating Th cells during active disease. Our expression targets were chosen from analysis of big data from published reports and our own previously obtained from transcriptome studies.

Using our NR4A2 cKO mice, which after EAE induction undergo T cell activation and expansion then infiltration into the CNS tissue, but do not cause disease, we were able to identify key molecules associated with active disease. Further, comparing these to Th cells developed in vitro under differentiation conditions previously associated with EAE, we were able to identify core components of pathogenicity, versus in vitro only molecules.




With the gene set studied, different groups of in vitro differentiated Th cells that are pathogenic on transfer cluster together, but away from genuine CNS-infiltrating T cells (left). Indeed, a considerable number of genes, previously thought to be pathogenic Th cells correlate together and are not associated with authentic pathogenic cells in the CNS (right).




Finally, these data when extrapolated to CNS systems using NR4A2 cKO mice immunized for EAE, we were able to confirm previously unappreciated genes that were found only in pathogenic Th cells. Early data have indicated that blocking these genes decrease disease and it is hoped that oncoming studies will confirm these data and generate exciting publications shortly.

５．主な発表論文等

[学会発表]（計10件）

1. Max Planck Institute of Psychiatry, Munich, Germany - **Raveney, B.J.E.,** Workshop on Biomarkers in Neuropsychaiatric Disorders. Title: Eomes-positive cytotoxic T helper cells: a new pathogenic player in secondary progressive multiple sclerosis? (INVITED, 18th October, 2017)
2. University of Bristol, Bristol, UK – **Raveney, B.J.E.,** Invited seminar: Joint meeting Division of Immunology and Department of Ophthalmology. Title: NR4A2 and Eomes: novel pathogenic factors in acute and chronic autoimmune neuroinflammation (INVITED, 1st December 2017)
3. University of Nottingham, Nottingham, UK – **Raveney, B.J.E.,** Invited seminar: Department of Clinical Neurology Title: Identification of novel pathogenic factors in acute and chronic autoimmune disease (INVITED, 4th December, 2017)
4. British Society for Immunology Annual Congress, Brighton, UK **Raveney, B.J.E.,** Lin, Y., Sato, W., Oki, S., Yamamura, T. Characterization of novel Eomes-positive cytotoxic T helper cells in chronic autoimmune CNS inflammation (Selected for Oral presentation, December, 2017)
5. Japanese Society for Immunology Annual Meeting, Sendai, Japan **Raveney, B.J.E.,** Sato, W., Oki, S., Yamamura, T. Eomes-positive cytotoxic T helper cells: a new pathogenic immune cell subset in secondary progressive multiple sclerosis? (Selected for Oral presentation; Winner of presentation award, December, 2017)
6. FOCIS, San Francisco, USA **Raveney, B.J.E.**, Sato, W., Takewaki, D., Lin, Y., Okomoto, T., Araki, M., Oki, S., and Yamamura, T. Title: Increases in Eomes-expressing Th cells in secondary progressive multiple sclerosis reveal patients at risk of increased disability Selected for Oral presentation, 23rd June 2018)
7. International Society of Neuroimmunology, Brisbane, Australia **Raveney, B.J.E.**, Sato, W., Takewaki, D., Lin, Y., Okomoto, T., Araki, M., Oki, S., and Yamamura, T. Title: Increases in Eomes-expressing Th cells in secondary progressive multiple sclerosis reveal patients at risk of increased disability (Selected for Oral presentation, 29th August 2018)
8. ECTRIMS, Berlin , Germany **Raveney, B.J.E.**, Sato, W., Takewaki, D., Lin, Y., Okomoto, T., Araki, M., Oki, S., and Yamamura, T. Title: Worsening symptoms in secondary progressive multiple sclerosis are linked to increased eomes Th cells (October, 2018)
9. Japanese Society of Neuroimmunology, Fukushima, Japan **Raveney, B.J.E.**, Sato, W., Takewaki, D., Lin, Y., Okomoto, T., Araki, M., Oki, S., and Yamamura, T. Title: Level of Eomes+ Th cells predicts worsening disease in secondary progressive multiple sclerosis (Selected for Oral presentation, September 2018)
10. Japanese Society of Immunology, Fukuoka, Japan **Raveney, B.J.E.**, Sato, W., Takewaki, D., Lin, Y., Okomoto, T., Araki, M., Oki, S., and Yamamura, T. Title: Eomes+ Th cells in patients with secondary progressive multiple sclerosis are associated with actively progressing disease (Selected for Oral presentation, December 12, 2018)