博 士(医 学) 佃 馬 正 樹

学 位 論 文 題 名

The mechanisms of plastic conversion of IL-17-producing CD8+T cells into IL-17/IFN-g-double producing-cytotoxic CTL subset and the physiological role in autoimmune diseases

(IL-17産生CD8+T細胞の可塑的変化によるIL-17/IFN-g共陽性CTLの誘導メカニズムと自己免疫疾患における生理的意義の解明)

学位論文内容の要旨

Background and Objectives The immune system is an indispensable mechanism for the host to maintain their homeostasis, which is regulated quantitatively and qualitatively for proper immune responses. T cells are known to proliferate in lymphopenic conditions to maintain the host immune system, which is termed as "homeostatic proliferation (HP)", though disruption of this mechanism may result in unexpected autoimmune diseases by permitting self-reactive T cells to proliferate. The dysregulation of T cell-proliferation induces these cells to differentiate into various effector subsets, according to the cytokine milieu they are exposed. Along with the well-known Type 1/Type 2-immune balance, it is now proposed that Type 17 /Treg balance also plays a pivotal role, and disruption of these sophisticated balances are known to trigger various immune-related disorders. Recently, it has been shown that these effector T cells possess high plasticity to convert into another effector cell fate, though the mechanism of these phenomena has not been elucidated yet. The aim for this research is to reveal (i) the mechanisms of how $CD8^+$ T cells disrupt the quantitative regulation of the T cell pool and vigorously proliferate (termed as "spontaneous proliferation (SP)") to cause autoimmune diseases, (ii) the role of the IL-17-producing $CD8^+$ T (Tc17) cells that is responsible for the inflammation of $CD8^+$ T cell-mediated colitis, and (iii) the plasticity of Tc17 cells to convert into IL-17/IFN-γ-double producing $CD8^+$ T cells that are detected in the local inflammation site.

Methods Naive $CD8^+$ T cells were purified from C57BL/6 mice and transferred into syngeneic $RAG2^{-/-}$ mice. Proliferation of $CD8^+$ T cells was determined by detecting the intensity of CFSE, which were pre-stained before transfer. For analysis of the pathogenesis of colitis, naive $CD8^+$ T cell-transferred $RAG2^{-/-}$ mice were monitored for 6-9 weeks. Lymph nodes and the colon were recovered for analysis of the phenotype of transferred $CD8^+$ T cells. To reveal the mechanisms how IL-17/IFN-γ-double producing $CD8^+$ T cells are induced, naive $CD8^+$ T cells from OVA-Class I peptide-specific TCR transgenic mice were sorted and differentiated into Tc17 cells *in vitro*. These Tc17 cells were further cultured in various cytokines for induction of IL-17/IFN-γ-double producing $CD8^+$ T cells and these cells were used for ^{51}Cr-release assay, adoptive tumor therapy experiments, quantitative PCR, and ChIP assays.

Results Naive $CD8^+$ T cells undergone two types of proliferation, HP, which is induced by self-antigens/MHC complex and IL-7/IL-15, and SP, which precise mechanisms are still remained unknown. SP was induced in IL-7 and/or IL-15-deficient manners, indicating that HP and SP are obviously regulated by different mechanisms. In fact, SP and the subsequent autoimmune colitis were strongly blocked by anti-IL-6 mAb treatment or depletion of the intestinal flora by antibiotics administration. Moreover, these treatments inhibited the induction of Tc17 cells in the mesenteric

lymph nodes. Indeed, this autoimmune colitis was strongly inhibited in IL-17-defecient conditions. Surprisingly, the major IL-17-producing subset was cells that simultaneously produce IFN-γ. To analyze how this IL-17/IFN-γ-double producing $CD8^+$ T cell subset is induced and maintained, OT-1-derived naive $CD8^+$ T cells were differentiated into Tc17 cells, and when exposed to IL-12, these cells acquired IFN-γ-producibility along with strong cytotoxicity, while retaining their Type 17 features. These cells were strongly regulated by the expression of SOCS3, which *Socs3* promoter region was epigenetically modified in a repressive state for modest expression of this molecule, which results in reduced inhibition status of STAT3 activation. This phenomenon consequently induced them to exhibit both Type 17 and Type 1 features.

Discussion Homeostasis of T cells can be defined as mechanisms of restoration of immune balance, and maintenance of immune status after T cell depletion or expansion as a result of immunological responses. Although HP has attracted much attention, the precise mechanisms for SP were unclear. Here, naive $CD8^+$ T cells were transferred into $RAG2^{-/-}$ mice to induce SP, and this SP was strongly regulated by IL-6-signaling and intestinal flora antigens. Moreover, these signals were indispensable for the $CD8^+$ T cell-mediated colitis, and moreover, for the production of IL-17, which accelerate the pathogenesis by inducing the migration of inflammatory cells such as macrophages. Surprisingly, among IL-17-producing $CD8^+$ T cells that are induced in the mesenteric lymph node, the major population was cells that produced both IL-17 and IFN-γ simultaneously, a phenomenon that could not be simply explained by the knowledge up to the present date. IL-17/IFN-γ-double producing $CD8^+$ T cells were strongly induced from Tc17 cells by IL-12 signaling, and this strongly induced IFN-γ-producibility along with strong cytotoxicity. This plasticity of Tc17 cells was regulated by SOCS3, a negative regulator for STAT3 that is a vital molecule for full-differentiation of Type 17 cells. Since SOCS3 is induced by IFN-γ/STAT1 signaling, this molecule is thought to be the key molecule for Type 17/Type 1-immune balance. The promoter region of the *Socs3* gene was in a repressive state in IL-17/IFN-γ-double producing $CD8^+$ T cells for cancellation of STAT3 inhibition, which makes it possible for these cells to exhibit both Type17- and Type 1-immune responses.

Conclusion Dysregulation of T cell proliferation in lymphopenic conditions may induce various immune diseases such as type 1 diabetes, Omenn syndrome, Wiskott-Aldrich syndrome, and SLE. This research shed light on the mechanisms of lymphocyte proliferation to be new therapeutic targets for these disorders, where anti-IL-6R mAb treatment specifically blocked the pathogenic SP, while exhibiting little impact on HP. Furthermore, induction of IL-17/IFN-γ-double producing $CD8^+$ T cells by SP are newly identified effector cells that are induced by dysregulation of SOCS3, the molecule which is the key factor for inhibition of unexpected excess Type 17/Type 1-immune responses. *Socs3* gene in IL-17/IFN-γ-double producing $CD8^+$ T cells are epigenetically modified in a repressive state, though identifying upstream molecules that regulate these epigenetic conditions are issues for the future. In summary, the immune system is strictly regulated quantitatively and qualitatively, and disruption of either or both regulation may cause various immune-related disorders. When considering the immune balance, both aspects should be taken into account, and this may provide us various targets for establishing new therapeutic applications for immune diseases.

学位論文審査の要旨

主　査　教　授　有　川　二　郎
副　査　教　授　西　村　孝　司
副　査　教　授　瀬　谷　　司
副　査　教　授　笠　原　正　典

学位論文題名

The mechanisms of plastic conversion of IL-17-producing CD8+T cells into IL-17/IFN-g-double producing-cytotoxic CTL subset and the physiological role in autoimmune diseases

(IL-17産生CD8+T細胞の可塑的変化によるIL-17/IFN-g共陽性CTLの誘導メカニズムと自己免疫疾患における生理的意義の解明)

免疫系による生体の恒常性の維持には大きく、「質的」制御と「量的」制御があり、この一方、もしくは両方が破綻することで免疫関連疾患が発症すると考えられている。免疫系の中枢を担うT 細胞は、抗原刺激を受ける環境によってさまざまなエフェクター細胞へと分化するが、これらが過剰応答を起こすと自己免疫疾患、アレルギーなどの引き金となり得る。しかし、通常では、分化した種々のエフェクター細胞は、互いが互いを排他的に抑制しあうことで「質的」な制御がなされていることが知られている。一方、生体では、放射線療法などによってT 細胞が激減すると、残ったT 細胞群が元のT 細胞プールのサイズを維持しようと細胞分裂することで「量的」制御がなされていることが知られており、この現象はhomeostatic proliferation (HP)として知られている。HP はnaive T 細胞をRAG$^{-/-}$ マウスやSCID マウスに移入することで誘導できるが、その際、HP よりもはるかに速い速度で分裂を起こす細胞群の存在することが見出され、これはHP に対してspontaneous proliferation (SP) として定義されている。今回の研究により、申請者はCD8$^+$T 細胞が起こすSP がIL-6、さらに腸内細菌叢由来の抗原によって引き起こされることを明らかにした。また、このSP を起こした細胞群がIL-17 を産生するエフェクター細胞へと分化し、大腸において著しい炎症を引き起こすことを明らかにしている。この病態モデルにおいて、本来、排他的な制御によって産生されるべきIL-17 とIFN-γ を同時に産生するIL-17/IFN-γ 共陽性CD8$^+$T 細胞が見出されたことから、申請者はこのユニークな細胞群に注目し、その誘導メカニズムについて検討を行った。申請者はTc17 細胞を *in vitro* にて誘導し、これにIL-12 のシグナルを加えることでIL-17/IFN-γ 共陽性CD8$^+$T 細胞が誘導出来ることを明らかにした。Tc17 細胞は細胞傷害活性を有しないのに対し、Tc17 細胞にIL-12 を加えて誘導した

IL-17/IFN-γ 共陽性 $CD8^+$ T 細胞群は非常に強い細胞傷害活性を獲得していた。この IL-17/IFN-γ 共陽性 $CD8^+$ T 細胞が誘導されるメカニズムとして、本来 IL-12 のシグナルによって誘導されるべき SOCS3 が、IL-17/IFN-γ 共陽性 $CD8^+$ T 細胞においてはその遺伝子プロモーター領域が転写抑制状態にあるために発現が阻害され、STAT3 の抑制を介した IL-17 産生阻害が誘導できないことから Type 1 と Type 17 免疫応答がとも成立していることを明らかにした。

審査会において、副査の瀬谷司教授より、SP を誘導する大腸菌の菌種についての質問と、腸内細菌由来の抗原と大腸炎との関連についての質問を受け、最近の腸内細菌叢について行われている解析の動向を報告するとともに、これまでに知られている腸内細菌と宿主の免疫系との関わりについて述べた。副査の笠原正典教授より、SP と HP との分裂速度についての質問があり、これまでの HP について知られている知見を踏まえ、SP との違いについて述べた。次に主査より、実験に用いた B6 マウスとは異なる系統においても同じような現象がみられるのかどうかについての質問を行い、BALB/c マウスで実験を行っても SP、大腸炎が起きないことから、腸内細菌叢の構成の違いとともに、遺伝的背景もまた炎症性腸疾患の重要な因子である旨を述べた。また、Type 17 免疫応答を誘導することが知られている菌種が日和見感染に関わるものが多い点について質問をし、実際のカンジダの感染による Type 17 免疫応答の誘導メカニズムを例に挙げながらこれまでの報告されている知見を説明した。最後に、指導教員である副査の西村孝司教授より、これらの研究を今後どのように活かしていくのかという質問に対して、今回のマウスモデルで得られた知見について、今後ヒトへの応用を念頭にさらに研究をしていきたい旨を述べた。

この論文は、免疫系を制御するさまざまな恒常性維持機構の破綻がもたらす疾患に対する新たな治療法確立への試みに対して多くの治療ターゲットを供するものであり、今後の研究をもとに臨床的な応用が期待されるものである。

審査員一同は、これらの成果を高く評価し、大学院課程における研鑽や取得単位なども併せ申請者が博士（医学）の学位を取得するのに十分な資格を有するものと判定した。