学位論文内容の要旨

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学 位 論 文 題 名

The mechanisms of plastic conversion of IL-17-producing CD8$^+$ T cells into IL-17/IFN-γ-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-γ 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）

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 l 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.