論文内容の要旨

論文題目：**Studies on the development and function of dendritic cells**

(樹状細胞の発生、分化機構及び機能の解析)

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Introduction

The immune system protects our life from invasions of various pathogens, e.g. bacteria and virus, and also from various diseases caused by mutations. e.g. tumorigenesis and neurodegeneration. The most basic feature of the immune system is self-tolerance and immune response against non-self antigens. While immune responses are important for protection of our life. excess activation of the immune reactions against self-antigens and pathogens causes various immune diseases such as autoimmunity and allergic reaction. Thus understanding of the regulation of immune system is very important. However, it is a very complex system and many problems still remain to be studied.

Dendritic cells (DCs) are professional antigen presenting cells (APCs) and act as sentinels in peripheral tissues. continuously sampling antigens. and initiate immune responses by presenting antigens to T cells upon encounters with microbial products or tissue damages. In contrast. DCs in the thymus play a major role in central tolerance of T cells. which is achieved by inducing apoptosis of self-reactive T cells. A variety of DC subsets have been described based on their cell surface phenotype and morphology and are widely distributed in various tissues including lymphoid such as the thymus. spleen

and lymph node and non-lymphoid tissues such as the skin, liver and gut. However, the developmental origin and functions of DC subtypes have been a controversial issue. Differences in the cytokine and transcriptional factor requirements suggest different developmental pathways for DCs. In this study, I investigate roles of Oncostatin M (OSM), a member of the IL-6 family cytokines, for the development of DCs in the fetal thymus and for the immune response of peripheral DCs.

Results and discussions

1. Development of dendritic cells in the fetal thymus

1-1. A unique feature of thymic DCs at fetal stage

T cell development occurs most actively in the fetal thymus and thymic DCs are involved in T cell selection. A unique feature of thymic DCs is that they can be derived from intrathymic T/DC precursors and stay in the thymus. However, the exact origin of thymic DCs and factors involved in the DC development still remain unclear. Most DCs are $CD8\alpha^{+}CD11b^{-}$ in the adult thymus, but I found that DCs in the fetal thymus are negative for $CD8\alpha$ and partially positive for CD11b. These $CD8\alpha^{-}CD11b^{+}$ cells express co-stimulatory molecules and MHC class II molecule at high levels and exhibit strong APC activity. Thus, these results suggest that $CD11b^{+}$ DCs are functionally maturated APCs and are involved in T cell selection in the fetal thymus.

1-2. Thymic epithelial cells (TECs) induce the development of fetal-type DCs from thymocytes

In our laboratory, E. Esashi established a fetal thymic epithelial cell line, ORTEC, which depends on OSM for proliferation, and reported that OSM receptor (OSMR)-positive thymic epithelial cells were located at the cortico-medullary junction where T cell selection occurs actively. Thus I examined whether thymic DCs were induced from thymocytes by ORTEC and fount that ORTEC conditioned medium (ORTEC-CM) induced generation of $CD11b^{+}$ DCs from intrathymic T cell progenitors and these DCs exhibited strong APC activity. This indicates that TECs produce a soluble factor that induces thymic DC development.

1-3. Identification of a factor required for DC development and function

To identify a factor produced by ORTEC, I analyzed the expression of cytokines known to stimulate DC development or to maintain survival of thymocytes by using RT-PCR and microarray analysis. Among various cytokines I examined, only IL-18, which

is known to activate the TRAF6 signaling pathway, was clearly expressed in ORTEC and the thymus. I found that the IL-18 receptor subunits were expressed on T cell progenitors and that IL-18 induced the development of $CD11b^+$ DCs from T cell progenitors. Furthermore, I found a significant reduction of $CD11b^+$ DCs in TRAF6-deficient fetal thymus, indicating that TRAF6 is required for the thymic DC development. However, as no such reduction was found in IL-18 receptor-deficient fetal thymus, an additional cytokine that activates TRAF6 is involved in the DC development *in vivo*. I also showed that the IL-18-induced DCs exhibited strong APC activity and preferentially bound $CD4^+CD8^+$T (DPT) cells. Moreover, apoptotic T cells were frequently found in the conjugates formed by these DCs. Taken together, these results strongly suggest that $CD11b^+$ DCs induced by TEC-derived IL-18 are actively involved in T cell selection by inducing apoptosis of DPT cells in the fetal thymus.

2. OSM negatively regulates peripheral immune response via DCs

2-1. OSM-deficient mice exhibit enhanced Th1 response

OSM-deficient mice are born according to the Mendelian rule of inheritance, appear normal at birth and develop normally. Most of immune cells including T cells are developed and distributed normally in OSM-deficient mice. However, the development of thymic $CD4^+$ macrophages, which efficiently phagocytosed apoptotic thymocytes in the thymus, was impaired. In accordance with the reduction of professional scavenger of apoptotic cells in the thymus, apoptotic cells were accumulated in OSM-deficient thymus. It is well known that impaired clearance of apoptotic cell leads autoimmune disease. In fact, OSM-deficient mice exhibited a symptom of autoimmunity by aging. However, it was not clear whether autoimmunity of OSM-deficient mice was caused solely by the impaired clearance of apoptotic thymocytes in the thymus. Therefore I considered another reason for autoimmunity of OSM-deficient mice.

T cells are composed of $CD8^+$ T cells (cytotoxic T cells: Tc) and $CD4^+$ T cells (helper T cells: Th). $CD4^+$ T cells are further divided into Th1 cells and Th2 cells and those subsets regulate distinct types of immune response. Th1 cells, which predominantly secrete IFN-γ, regulate cell-mediated immunity, including the activation of innate immunity. Conversely, Th2 cells, which secrete IL-4, IL-5, IL-6, IL-10 and IL-13, regulate antibody-mediated immunity (humoral immunity). I found that OSM-deficient mice exhibited prolonged IFN-γ production in serum after lipopolysaccaride (LPS)

administration into intraperitoneum (i.p.). On the other hand, IL-10 production was reduced in OSM-deficient mice. These results indicated that Th1 responses were augmented in OSM-deficient mice by microbial infection. I assessed the Th1/Th2 balance of OSM-deficient mice by *in vitro* splenocytes culture and found that OSM-deficient splenocytes produced more IFN-γ and less IL-10 than those of WT. Therefore I concluded that OSM-deficient mice exhibit enhanced Th1 response.

2-2. OSM is a negative regulator of IL-12 production from DCs

Differentiation of Th1 cells requires IL-12 that induces IFN-γ production in T cells. DCs capture foreign antigens and migrate into the secondary lymphoid organs such as the spleen and lymph nodes. DCs then present the antigen by MHC class II to $CD4^+$ T cells and produce IL-12 efficiently for inducing Th1 response. As Th1 response was enhanced in OSM-deficient mice, I assessed whether IL-12 production by LPS stimulation was increased in OSM-deficient mice. As expected, I found that IL-12 production was enhanced in OSM-deficient splenocytes and DCs. In co-culture of $CD4^+$ T cells and DCs, which were isolated from WT or OSM-deficient spleen, in the presence of LPS, OSM-deficient DCs induced production of more IFN-γ and less IL-10 from $CD4^+$ T cells than WT DCs. Activation of DCs was augmented and prolonged in OSM-deficient spleen by LPS injection. Taken together, it is indicated that OSM-deficient DCs are defective in the negative regulation after activation. Using bone marrow-derived DCs (BMDCs), I found that DCs expressed OSM. More interestingly, OSMR expression was dramatically up-regulated in BMDCs and splenic DCs by LPS stimulation. I also found that OSM suppressed IFN-γ production in OSM-deficient splenocytes. It is known that Th1 cells produce OSM as well as IFN-γ. Therefore, it is suggested that OSM negatively regulates Th1 response via directly acting on DCs by both autocrine and paracrine mechanisms.

OSM-deficient mice develop autoimmune diseases. Here, I show that OSM-deficient mice are biased to Th1 response upon microbial infection. In addition, clearance of apoptotic thymocytes is impaired in OSM-deficient thymus. These results suggest that autoimmunity in OSM-deficient mice is induced by alteration in the central (thymus) as well as peripheral (spleen) immune response.