論 文 の 内 容 の 要 旨

応用生命化学　専　攻
平成 22 年度博士課程 進学
氏　　名　塩河　亜弥
指導教員名　八村　敏志

論文題目　　Studies on immunosuppressive mechanisms in the intestine
（腸管における免疫抑制機構に関する研究）

The intestine is continually exposed to antigens from food proteins and from commensal or pathogenic bacteria. Strict regulation is thus required in the intestine, and oral tolerance represents a unique aspect of the intestinal immune system. Oral tolerance has been classically defined as the specific suppression of cellular or humoral immune responses to an antigen (Ag) by means of prior administration of Ag through the oral route. The response likely evolved as an analog of self tolerance to prevent hypersensitivity reactions to food proteins. Oral tolerance results in antigen-specific T cell deletion, anergy, or induction of regulatory T cells (Tregs). Food hypersensitivity presumably results from either a failure in establishing oral tolerance or a breakdown in existing tolerance. Furthermore, since oral tolerance is a potent way of inducing regulatory cells towards specific Ag, the idea of using the oral route to induce tolerance to Ag involved in autoimmune diseases becomes an important clinical application of the phenomenon.

Chapter 1. Three different CD103$^+$ dendritic cells (DC) subsets in MLN have distinct function in intestinal immune regulation

DC have been revealed as important regulators in oral tolerance induction. It has been proposed that oral tolerance requires mesenteric lymph nodes (MLN), and CD103$^+$ DC and PD-L1$^+$ DC in the MLN are suggested to be critical for the induction of oral tolerance.

However, the relationship of these DC subsets remains unclear. Therefore we aimed to clarify the phenotypes and functions of MLN DC subsets in relation to oral tolerance induction. Flow cytometric analysis demonstrated that $CD103^+$ DC in MLN are divided into distinct three populations by CD11b and PD-L1 expression. $CD103^+CD11b^+PD^-L1^+$ DC and $CD103^+CD11b^-$PD-$L1^+$ DC prominently expressed CCR7, which is the chemokine receptor required to migrate to MLN from the lamina propria. $CD103^+CD11b^+$PD-$L1^+$ DC presented orally administrated Ag to $CD4^+$ T cells and strongly induced T cell proliferation. On the other hand, $CD103^+CD11b^-$PD-$L1^+$ DC prominently expressed retinaldehyde dehydrogenase 2 (Raldh2) compared to other $CD103^+$ DC subsets, and strongly induced Foxp3 expression in $CD4^+$ T cells by producing retinoic acid. $CD103^+CD11b^-$PD-$L1^-$ DC could not present orally administrated Ag, but promptly induced IFN-γ production in $CD4^+$ T cells via IL-12 independent mechanism *in vitro*. These results suggested that the three $CD103^+$ DC subsets have distinctive functions, and may play different roles in inducing oral tolerance.

Chapter 2. IL-10 and IL-27-producing DC capable of enhancing IL-10 production of T cells are induced in oral tolerance [1)]

In addition to MLN, Peyer's patch (PP) is also an important site for establishing oral tolerance. PP DC from tolerized mice induced IL-10 production but not Foxp3 expression in co-cultured T cells. The number of $CD11b^+$DC increased after ingestion of Ag, and $CD11b^+$ DC prominently expressed IL-10 and IL-27 compared with $CD11b^-$ DC. These results suggested that IL-10 and IL-27 producing $CD11b^+$ DC are increased by interaction with antigen specific T cells in PP, and these PP $CD11b^+$ DC act as inducers of IL-10 producing T cells in oral tolerance.

Chapter 3. Th2 suppressive arginase 1 expressing $CD11b^+$ DC are induced in PP after oral Ag administration

Food allergies presumably result from either a failure to establish oral tolerance, or a breakdown in existing tolerance. Allergy results in an excessive Th2-type immune response, characterized by IL-4, IL-13, and IL-5. Therefore, during oral Ag administration, suppression of excessive IL-4 production may be necessary to establish oral tolerance and prevent the onset of food allergy. In addition to the role of PP DC for inducing IL-10 producing T cells, we also found that PP DC from tolerized mice could suppress excessive IL-4 production in T cells. PP DC from tolerized mice prominently expressed arginase 1, and suppressed IL-4 secretion by $CD4^+$ T cells via arginase 1. Arginase 1 expression in PP DC was increased after

oral Ag administration, and the expression was restricted to $CD11b^+$ DC. PP $CD4^+$ T cells prominently expressed IL-4 compared to SPL or MLN $CD4^+$ T cells, and arginase 1 expression in DC was induced by IL-4 *in vitro*. These observations suggested that after oral Ag administration, PP T cells abundantly produce IL-4, and IL-4 induced arginase 1 expression in PP $CD11b^+$ DC. Then PP $CD11b^+$ DC suppress excessive IL-4 production by arginase 1, establishing IL-4-arginase 1 negative feedback loop.

Chapter 4. Th2 suppressive arginase 1 expressing neutrophils are accumulated in PP after oral Ag administration

During examination of $CD11b^+$ DC, we also found that neutrophils were increased in PP after oral Ag administration, and accumulated around T cells in the intrafollicular region (IFR). Numbers of neutrophils in blood was also increased after oral Ag administration, and it was suggested that fibroblastic reticular cells (FRC) in OVA-fed PP could chemoattract neutrophils. These results suggested the possibility of stepwise attraction of neutrophils; Ag-specific $CD4^+$ T cell response promotes certain chemokine secretion from FRC, which promotes neutrophil migration from blood to PP IFR. Similar to $CD11b^+$ DC, accumulated PP neutrophils prominently expressed arginase 1, and suppressed production of IL-4 via arginase 1. Arginase 1 expression in neutrophils was induced by IL-4 *in vitro*. These results suggested that after oral Ag administration, FRC in PP recruited neutrophils, and these recruited neutrophils also were involved in establishing the IL-4-arginase 1 negative feedback loop.

Chapter 5. Stromal cells in gut-associated lymphoid tissue have distinct immunoregulatory function

Non-hematopoietic stromal cells provide structural support to the lymphoid organs. Recent studies have shown that stromal cells also have a crucial role in tolerance induction in the periphery. T cell zone of lymphoid tissue is delineated by FRC and forms a scaffold to provide essential guidance cues to cells of the immune system. It is suggested that stromal cells play important roles in shaping tissue-specific immune responses; however, intestinal tissue-specific phenotypes of stromal cells remain unclear and immunoregulatory function of PP-stromal cells have not been reported. Therefore, the characteristics of stromal cells in MLN and PP, in particular FRC ($gp38^+CD31^-CD45^-$ cells) were examined, focusing on T cell response. For comparison, double-negative cells (DNC; $gp38^-CD31^-CD45^-$ cells) were isolated from mouse MLN and PP. MLN-FRC prominently expressed cyclooxygenase-2

(COX2) compared with PP-FRC or DNC. MLN-FRC strongly suppressed $CD4^+$ T cell proliferation but PP-FRC showed only weak suppression. MLN-FRC suppressed $CD4^+$ T cell proliferation depending partly on COX2 activation. It was reported that cultured lymph node-FRC suppressed T cell proliferation dependent on nitric oxide synthase 2 (NOS2). However, we found that NOS2 expression in freshly isolated MLN- and PP-FRC was extremely lower than that in DNC, and that NOS2 was not essential for suppressive function of T cell response by MLN-FRC. PP-FRC prominently expressed Raldh2 compared with MLN-FRC, and PP-FRC induced Foxp3 expression in $CD4^+$ T cells via producing retinoic acid. These results suggested that phenotypes and functions of FRC are distinct between MLN and PP, and they are involved in intestinal immune response in different manners.

Conclusion

DC have been thought to critical for inducing intestinal tolerance, and this study showed distinct DC subsets have distinct immunoregulatory function in different tissues. In addition to DC, it was shown that neutrophils and stromal cells are also involved in immune regulation. This study suggested that more types of cells than previously assumed communicate with each other, and are involved in intestinal immune tolerance.

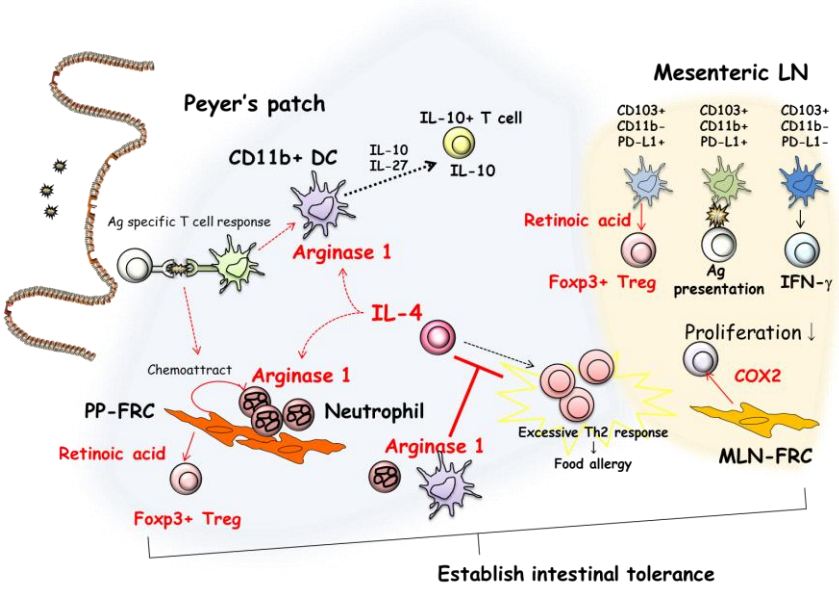

Figure 1. Suggested model of cellular network in intestinal immune regulation

1) Shiokawa A, Tanabe K, Tsuji NM, Sato R, Hachimura S. *Immunol Lett* 2009;125(1):7-14.