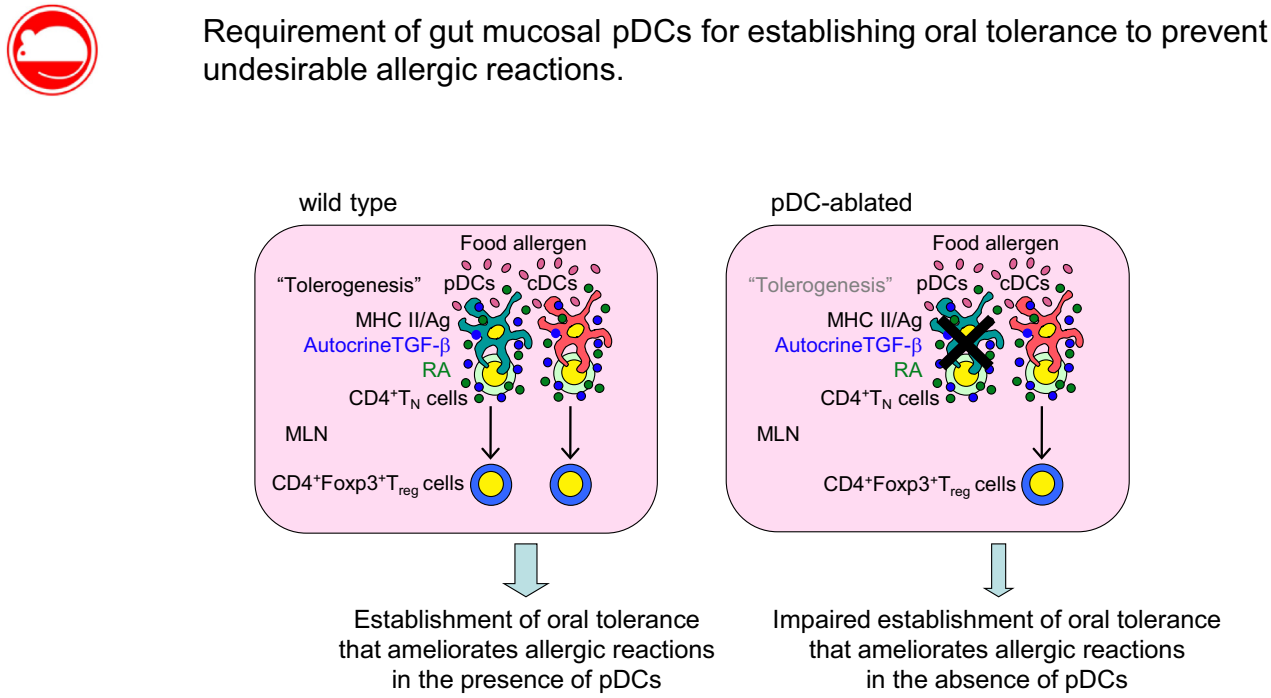
Critical role of plasmacytoid dendritic cells in induction of oral tolerance

Tomofumi Uto, PhD,[a,e] Hideaki Takagi, PhD,[a,e] Tomohiro Fukaya, DVM, PhD,[a,e] Junta Nasu, DDS,[a,b] Takehito Fukui, DDS,[a,b] Noriaki Miyanaga, MD,[a,c] Keiichi Arimura, DDS, PhD,[a,b] Takeshi Nakamura, MD,[a,c] Narantsog Choijookhuu, MD, PhD,[d] Yoshitaka Hishikawa, MD, PhD,[d] and Katsuaki Sato, PhD[a,e] *Miyazaki and Tokyo, Japan*

GRAPHICAL ABSTRACT



Background: Exposure to dietary constituents through the mucosal surface of the gastrointestinal tract generates oral tolerance that prevents deleterious T cell–mediated immunity. Although oral tolerance is an active process that involves emergence of $CD4^+$ forkhead box p3 $(Foxp3)^+$ regulatory T (Treg) cells in gut-associated lymphoid tissues (GALTs) for suppression of effector T (Teff) cells, how antigen-presenting cells initiate this process remains unclear.

Objective: We sought to determine the role of plasmacytoid dendritic cells (pDCs), which are known as unconventional antigen-presenting cells, in establishment of oral tolerance.

Methods: GALT-associated pDCs in wild-type mice were examined for their ability to induce differentiation of $CD4^+$ Teff cells and $CD4^+Foxp3^+$ Treg cells *in vitro*. Wild-type and pDC-ablated mice were fed oral antigen to compare their intestinal generation of $CD4^+Foxp3^+$ Treg cells and induction of oral

From [a]the Division of Immunology, Department of Infectious Diseases; [b]the Department of Oral and Maxillofacial Surgery; [c]the Department of Otolaryngology, Head and Neck Surgery; and [d]the Division of Histochemistry and Cell Biology, Department of Anatomy, Faculty of Medicine, University of Miyazaki; and [e]the Japan Agency for Medical Research and Development (AMED), Tokyo.

Supported by a Grant-in-Aid for Challenging Exploratory Research (to K.S.) and for Young Scientists (B; to T.U. T.F., and H.T.) from the Ministry of Education, Science and Culture of Japan; the Project for Cancer Research and Therapeutic Evolution (P-CREATE) from the Japan Agency for Medical Research and development (AMED; to K.S.); the Mochida Memorial Foundation for Medical and Pharmaceutical Research (to K.S.); the Uehara Memorial Foundation (to K.S. and H.T.); the Takeda Science Foundation (to H.T. and T.F.); the Novartis Foundation (Japan) for the Promotion of Science (to H.T.); the Kato Memorial Bioscience Foundation (to H.T.); the Nagao Memorial Fund (to H.T.); the Daiichi Sankyo Foundation of Life Science (to K.S.); the Naito Foundation (to K.S.); the GSK Japan Research Grant (to T.F. and H.T.); and Bristol-Myers Squibb Foundation Grants (to K.S.).

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication June 12, 2017; revised October 6, 2017; accepted for publication November 8, 2017.

Corresponding author: Katsuaki Sato, PhD, Division of Immunology, Department of Infectious Diseases, Faculty of Medicine, University of Miyazaki, 5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan. E-mail: katsuaki_sato@med.miyazaki-u.ac.jp.

0091-6749/$36.00

https://doi.org/10.1016/j.jaci.2017.11.048

tolerance to protect against Teff cell–mediated allergic inflammation.
Results: GALT-associated pDCs preferentially generate $CD4^+Foxp3^+$ Treg cells rather than $CD4^+$ Teff cells, and such generation requires an autocrine loop of TGF-β for its robust production. A deficiency of pDCs abrogates antigen-specific *de novo* generation of $CD4^+Foxp3^+$ Treg cells occurring in GALT after antigenic feeding. Furthermore, the absence of pDCs impairs development of oral tolerance, which ameliorates the progression of delayed-type hypersensitivity and systemic anaphylaxis, as well as allergic asthma, accompanied by an enhanced antigen-specific $CD4^+$ Teff cell response and antibody production.
Conclusion: pDCs are required for establishing oral tolerance to prevent undesirable allergic responses, and they might serve a key role in maintaining gastrointestinal immune homeostasis. (J Allergy Clin Immunol 2018;■■■:■■■-■■■.)

Key words: *Mucosal immunity, oral tolerance, dendritic cells, regulatory T cells, allergy*

Abbreviations used
ALDH: Aldehyde dehydrogenase
APC: Antigen-presenting cell
BALF: Bronchoalveolar lavage fluid
cDC: Conventional dendritic cells
CFA: Complete Freund adjuvant
DC: Dendritic cell
DT: Diphtheria toxin
DTH: Delayed-type hypersensitivity
EGFP: Enhanced green fluorescent protein
Foxp3: Forkhead box p3
GALT: Gut-associated lymphoid tissues
IDO: Indoleamine 2,3-dioxygenase
iTreg: Inducible regulatory T
LP: Lamina propria
MLN: Mesenteric lymph node
OVA: Ovalbumin
OVAp: $OVA_{323-339}$ peptide
pDC: Plasmacytoid dendritic cell
PGN: Peptidoglycan
RA: Retinoic acid
siLP: Lamina propria of the small intestine
TCR: T-cell receptor
Teff: Effector T
TLR: Toll-like receptor
Treg: Regulatory T

Dendritic cells (DCs) are considered unrivaled essential antigen-presenting cells (APCs) that play critical roles in orchestrating the immune system, linking innate information to tailored adaptive responses.[1-3] DCs serve as sentinels, recognizing the presence of invading pathogens through various pattern recognition receptors to secrete multiple cytokines for induction of inflammatory responses, and they subsequently initiate primary T-cell responses on activation.[1-3] DCs comprise heterogeneous subsets that are functionally classified into classical or conventional dendritic cells (cDCs) and plasmacytoid dendritic cells (pDCs).[1-3] Even at steady state, cDCs have outstanding capacity to prime naive T cells to generate various types of effector T (Teff) cells because of the prominent expression of MHC class II and costimulatory molecules.[4-6] On the other hand, pDCs constitute a distinct group of APCs specialized in endosomal Toll-like receptor (TLR) 7/9–mediated recognition of viral and self nucleic acids and respond with the massive secretion of type I interferon.[7,8] Therefore pDCs have been not only been considered important mediators of antiviral responses[7,8] but also contributors as primary inflammatory cells to the induction of pathogenesis in patients with psoriasis and systemic lupus erythematosus.[9-12]

Conversely, DCs might also be important for maintenance of immune homeostasis by promoting immune tolerance.[13,14] It has been proposed that DCs not only participate in thymic clonal deletion of self-reactive T cells, which is known as central tolerance, but also induce peripheral tolerance mediated through clonal deletion and anergy of antigen-specific T cells and active immune suppression by $CD4^+$ forkhead box p3 $(Foxp3)^+$ regulatory T (Treg) cells, which encompass self-reactive thymus-derived naturally occurring Treg cells and inducible regulatory T (iTreg) cells generated from antigen-specific naive $CD4^+Foxp3^-$ T cells in the periphery.[13,14]

The gastrointestinal immune system is the largest and most complex part of the immune system, in which the gastrointestinal tract is exposed constantly to an enormous variety of foreign materials that might be either harmful or beneficial to the organism.[15,16] Consequently, the gastrointestinal immune system has to balance protective immune responses to potentially pathogenic microorganisms with immunologic nonresponsiveness to commensal flora and dietary constituents to maintain mucosal immune homeostasis in this environment, a phenomenon known as oral tolerance.[15,16] Oral tolerance is a physiologic process that prevents development of local and systemic deleterious T cell–mediated inflammatory responses to commensals and dietary proteins.[15,16] However, disruption of this process is believed to contribute to mucosal inflammation, leading to the occurrence of enteropathy, such as inflammatory bowel diseases and food allergies, as reflected by susceptibility genes and environmental factors.[17-19] Accumulating evidence supports a pivotal role for $CD4^+Foxp3^+$ iTreg cells emerging from gut-associated lymphoid tissue (GALT) in enforcing oral tolerance, which requires antigen presentation by $CD103^+$ cDCs in the lamina propria (LP) of the small and large intestines and migration to the mesenteric lymph nodes (MLNs) after sampling antigen; these are privileged sites in triggering oral tolerance.[20-23] These mucosal $CD103^+$ cDCs imprint characteristic features to favor generation of $CD4^+Foxp3^+$ iTreg cells relative to other cDC subsets in lymphoid and peripheral tissues under homeostatic conditions, including the preferential production of retinoic acid (RA), TGF-β, and indoleamine 2,3-dioxygenase (IDO), as well as prominent expression of the B7 family of costimulatory molecules (B7-H1 and B7-DC).[20-23] On the other hand, pDCs have reportedly conferred peripheral tolerance to certain types of immunopathogenesis through suppression of pathogenic T-cell responses through mechanisms involving expansion of $CD4^+Foxp3^+$ Treg cells,[24,25] whereas the function of pDCs for *de novo* generation of $CD4^+Foxp3^+$ iTreg cells has not been clarified. Furthermore, despite data linking pDCs and $CD4^+Foxp3^+$ Treg cells to oral tolerance,[26,27] the role of these cell types in the development of oral tolerance remains associative. Here we report that gastrointestinal pDC–mediated tolerogenesis through *de*