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Antigen-specific regulatory T cells are detected in Peyer's patches after the interaction between T cells and dendritic cells loaded with orally administered antigen

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ABSTRACT

Systemic immune tolerance is induced for orally administered antigen, and this phenomenon is called oral tolerance. However, the mechanism of oral tolerance has not been completely elucidated. It has been suggested that antigen presentation and generation of regulatory T cells in Peyer's patches (PPs) are important for induction of oral tolerance. Hence, we orally administered fluorescence-labelled antigen to mice and examined kinetics of the antigen and interaction between antigen-loaded dendritic cells and T cells. It was visualized that dendritic cells in PP rapidly take up antigen. We next transferred antigen-specific naïve T cells from T cell receptor transgenic mice and administered the antigen orally. Antigen-specific T cells accumulated in IFR in PP and DCs that have ingested antigen come in contact with antigen-specific T cells in IFR. The accumulated T cells were then collected and analyzed for the pattern of gene expression by real-time PCR, which revealed a gene expression pattern similar to that of FoxP3-positive regulatory T (T_{reg}) cells. CCR9, an intestinal homing marker, was also strongly expressed. These results suggest that DCs that have captured oral antigens in PPs locally induce antigen-specific naïve T cells to differentiate into T_{reg} cells with the intestinal homing phenotype.

Introduction

Various substances enter the digestive tract which begins at the mouth and ends at the anus, and thus various antigens are found in this area. Of these antigens, those that are pathogenic and cause harm to the organism are prevented from entry or expelled from the body. However, the body does not mount an immune response to substances such as food antigens and indigenous intestinal bacteria that are not only harmless but also beneficial to the organism, rather, immune tolerance is induced. This phenomenon is known as oral tolerance. An understanding of this phenomenon will open the door to treatments for inflammatory bowel disease, said to occur as a result of failure to become tolerant to indigenous intestinal bacteria, as well as for other autoimmune disorders. Moreover, as the capacity to artificially prevent oral tolerance would pave the way to the development of oral vaccines for various infectious diseases, clarification of the mechanisms of this phenomenon are eagerly awaited.

The principal mechanism of oral tolerance is thought to be the formation of antigen-specific $CD25^+CD4^+$ regulatory T cells (T_{reg} cells) in the vital organ known as Peyer's patches (PPs) in gut associated lymphoid tissue (GALT) (Mowat 2003; Strobel and Mowat 2006; Weiner 1997) in the small intestine. However, the details of how these T_{reg} cells are formed in GALT are not yet clear. In addition, the literature contains a report of T_{reg} cell number increasing following oral administration of an antigen (Zhang et al. 2001); however, it is not clear from this report how or where the orally administered antigen is presented to T cells or whether T_{reg} cells are induced. Furthermore, it has been discovered in recent years that dendritic cells (DCs) play an important role in the maintenance of immune tolerance in peripheral T cells (Coombes and Powrie 2008). Three main populations of DCs exist in PP, namely, $CD11b^+$, $CD8\alpha^+$, and $CD11b^-CD8\alpha^-$ (double negative; DN) (Iwasaki and Kelsall 2000, 2001). *In vitro*, $CD11b^+$ DC induces IL-10-producing $CD4^+$T cells, and alongside $CD8\alpha^+$ DCs and DN DCs induces IL-12-producing $CD4^+$T cells, also known as Th1 cells. These DCs are known to be spread unevenly within PP. The fact that the DCs in PP

Abbreviations: PPs, Peyer's patches; GALT, gut associated lymphoid tissue; T_{reg} cell, regulatory T cell; DC, dendritic cell; OVAo, valbumin; SED, subepithelial dome; IFR, interfollicular region.

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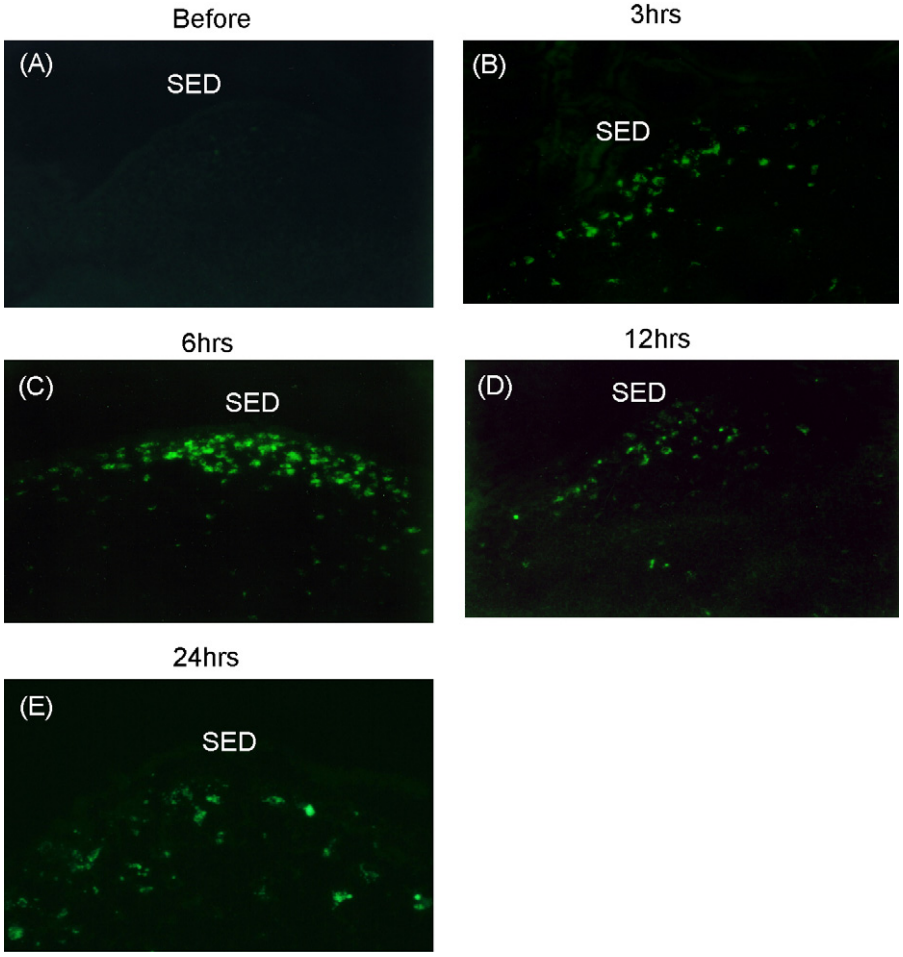

Fig. 1. Kinetics of oral antigen in Peyer's patches after antigen feeding.
We fed 30 mg of FITC-conjugated OVA to Balb/c mice and evaluated the kinetics of FITC-positive cells in Peyer's patches by fluorescence microscopy. (A) Peyer's patches before feeding of FITC-OVA (100×): (B) 3 h after feeding: (C) 6 h after feeding: (D) 12 h after feeding: (E) 24 h after feeding.

and those in the spleen possess different function with respect to differentiation of naïve T cells, plus the fact that oral administration of Flt3 ligand, a growth factor known to specifically simulate and cause propagation of DCs, brings about more efficient induction of oral tolerance, suggests that the DCs present in PP play an important role in inducing oral tolerance (Viney et al. 1998). There are several reports in the literature that examine whether DCs residing in PP and other GALT can induce $CD25^+CD4^+$ T_{reg} cells (Benson et al. 2007; Coombes et al. 2007; Mucida et al. 2007; Sun et al. 2007). However, none contain *in vivo* data regarding the site at which homologous antigen is captured, thereby causing DCs to become regulatory, nor is there data on the site at which the antigen is presented to naïve T cells, thereby causing induction of T_{reg} cells.

Hence, the authors orally administered the antigen fluorescence-labelled ovalbumin (OVA) to Balb/c mice, and examined by fluorescence microscopy the kinetics by which OVA reaches the intestine, and whether OVA is trapped by DCs in PP. Also investigated was the point of contact in PP between T cells and the DCs that ingested OVA. OVA-specific naïve T cells prepared from OVA TCR transgenic mice were transferred to Balb/c mice, then isolated from PP. By doing so, it was possible to investigate, by examining genetic expression measured by real-time PCR, whether the above T cells were being induced to differentiate into T_{reg} cells. Results showed the possibility that DCs induce oral antigen-specific T_{reg} cells in PP *in vivo*, and that these T cells strongly express CCR9, an intestinal homing marker.

Materials and methods

Mice

Female Balb/c mice were purchased from SLC (Shizuoka, Japan) and $OVA_{323–339}$ TCR transgenic mice from Jackson Laboratory (Bar Harbor, USA). All mice were raised in an SPF environment in the animal rooms in the Department of Allergy and Rheumatology of the Faculty of Medicine, the University of Tokyo, and were used in the experiments from eight to ten weeks postpartum. All experiments were conducted with the approval of the Medical Ethics Council of the University of Tokyo.

Fluorescence labelling and oral administration of antigen

Mice were intragastrically administered 30 mg (60 mg/ml, 0.5 ml) of FITC fluorescence-labelled OVA (Grade V, SIGMA, St. Louis, USA) using a stainless steel feeding needle designed for use with animals. Mice used as controls were administered an equivalent dose of water. Fluorescence labelling with FITC was conducted using the FluoroTag™ FITC Conjugation kit (SIGMA) according to the manufacturer's instructions.

Fluorescence staining of PP

PP was isolated from Balb/c mouse small intestine and frozen in O.C.T. compound (Tissue-Tek O.C.T. Compound, Sakura Finetech,