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Mini Review

Vitamin B9 and ATP in the control and development of intestinal inflammation

Jun Kunisawa[1,2,3,4)]

[1)]Laboratory of Vaccine Materials, National Institute of Biomedical Innovation, Osaka, Japan

[2)]Division of Mucosal Immunology and International Research and Development Center for Mucosal Vaccines, The Institute of Medical Sciences, The University of Tokyo, Tokyo, Japan

[3)]Graduate School of Pharmaceutical Sciences and Graduate School of Dentistry, Osaka University, Osaka, Japan

[4)]Department of Microbiology and Infectious Diseases, Kobe University Graduate School of Medicine, Kobe, Japan

Intestinal tissues establish homeostatic immune networks to prevent excessive inflammatory responses. The development and regulation of intestinal immune system are mediated at least partly by immunologic crosstalk with gut environmental factors including commensal bacteria and nutrients. Accumulating evidence has demonstrated that the inadequate immune regulation by environmental factors leads to the development of intestinal inflammation. Recent findings have revealed the specific function of vitamins in the development of intestinal inflammation. In addition, nucleotides are currently recognized as a participant in the control of inflammatory responses. In this review, we focus on the immunological functions of vitamin B9 and extracellular adenosine triphosphate (ATP) in the development of intestinal inflammation.

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Correspondence should be addressed to:
Jun Kunisawa, Laboratory of Vaccine Materials, National Institute of Biomedical Innovation, 7-6-8 Saito-asagi, Ibaraki-city, Osaka 567-0085, Japan. Phone: +81-72-641-9871, Fax: +81-72-641-9872, E-mail: kunisawa@nibio.go.jp

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Introduction

Intestinal tissues primary serve as a site to digest and absorb nutrients, but they also simultaneously comprise a unique immune system. The intestinal immune system acts as the first line of defense against pathogens[1)]. Additionally, the intestinal immune system is important to prevent unnecessary inflammatory responses and hypersensitivity to harmless or beneficial materials[2)]. To achieve these opposite but harmonized immunologic functions, the intestinal immune system is composed of various kinds of immunocompetent cells, including the antigen-sampling M cells; antigen-presenting cells (e.g., dendritic cells [DCs] and macrophages); IgA-producing plasma cells (PCs); polarized $CD4^+$ T cells such as regulatory T (Treg), IL-10-

producing Tr1, Th1, Th2, and Th17 cells; mast cells; and innate lymphoid cells[1, 3, 4].

To maintain the immunological homeostasis in the intestine, various regulatory cells are present, including Tr1, Treg, Mreg cells, and regulatory DCs[5]. Among them, Treg cells have a function to suppress other T cells[6]. Also Treg cells are involved in the induction of IgA-producing PCs, which plays a pivotal role in the maintenance of appropriate composition of commensal bacteria[7]. On the other hand, inflammatory cells such as Th1 or Th17 cells, M1 macrophages, neutrophils, and mast cells are involved in the disruption of immunological homeostasis in the intestine and consequent development of intestinal inflammation[8].

Through a physiological function of intestine, they continuously exposed to nutrients, commensal bacteria, and their metabolites. Interaction with these gut environment factors is a critical determinant in the development of intestinal immunity[9, 10]. Recent advances in analytical methods in the identification of commensal bacteria and their metabolites have revealed their pivotal roles in the regulation of host immune responses and the development of immune diseases[11, 12]. In addition to the commensal bacteria, nutritional components and its metabolites clearly are essential and influential exogenous factors for the development, maintenance, and regulation of the intestinal immune system[10, 13]. Indeed, nutrient deficiencies often are coincident with the impaired intestinal immunity[14, 15].

Vitamins are organic compounds that need to be supplied exogenously by the diet or commensal bacteria, because the mammalian species cannot synthesize in sufficient quantities. Vitamins are broadly divided into hydrophilic (e.g., vitamin B family and vitamin C) and hydrophobic (e.g., vitamins A, D, E, and K) one. Both vitamins and their metabolites have diverse functions in many biologic events, including immunologic regulation. Indeed, vitamin deficiency results in high susceptibility to infection and immune diseases[16, 17]. Due to the general biological functions of vitamins, indiscriminate function of vitamins in the control of host immune system was predicted; however, accumulating evidence has revealed specific functions of individual vitamins and their metabolites in immune responses. For example, retinoic acid, a metabolite of vitamin A, has emerged as a critical mediator of intestinal immune responses including the regulation of trafficking of T and B cells toward the intestine by inducing the expression of the gut-homing molecules α4β7 integrin and chemokine receptor CCR9[18, 19], and the differentiation and activation of T cells[20], innate lymphoid cells[21], and mast cells[22]. Another example is vitamin B6, which is required for the metabolic pathway of sphingosine 1-phosphate, a lipid mediator that regulates cell trafficking[23]. Thus, disruption of vitamin B6 function results in aberrant T-cell differentiation and cell trafficking in both systemic and intestinal compartments[24-26].

Nucleotides, especially adenosine 5'-triphosphate (ATP), emerge as key molecules in the regulation of many physiological and pathological processes. Generally, ATP is generated during glycolysis and the tricarboxylic acid cycle in the intracellular compartment and acts as an energy source. However, ATP is occasionally released into the extracellular compartment passively following cellular stress or cell death, inducing the inflammatory responses via signaling through membrane-bound purinergic P2 receptors[27]. P2 receptors can be divided into G-protein-coupled P2Y receptors, and nucleotide-gated ion channel P2X receptors[28]. Different types of P2X and P2Y receptors are expressed on various immune and non-immune cells, leading to the diverse involvement in the several inflammatory responses.

In this review, we focus on vitamin B9 and ATP in the control of immunologic balance between physiologic and pathologic conditions of the intestine.

Homeostatic function of vitamin B9 in the maintenance of immunological homeostasis in the intestine

Vitamin B9 (also known as folate and folic acid) is a water-soluble vitamin abundantly present in the leafy greens such as spinach, broccoli, and parsley (Fig. 1). Like other vitamins, vitamin B9 is not synthesized in mammals, and thus mammalian species must obtain vitamin B9 exogenously. Vitamin B9 is essential for the synthesis, replication, and repair of nucleotides for DNA and RNA and is thus required for cell proliferation and survival[29]. As an immunological function of vitamin B9, it was known that vitamin B9 deficiency reduced the proliferative responses of lymphocytes and natural killer cell activity[30, 31].

It was reported that the vitamin B9 receptor folate receptor 4 (FR4) is specifically expressed on Treg[32], implicating the possible involvement of vitamin B9 in the control of Treg cell function and/or survival. In this issue, we previously demonstrated that vitamin B9 is required for the survival of differentiated Treg cells but not the differentiation of Treg cells from naïve T cells[33]. The impaired survival of Treg cells in the absence of vitamin B9 was associated with the

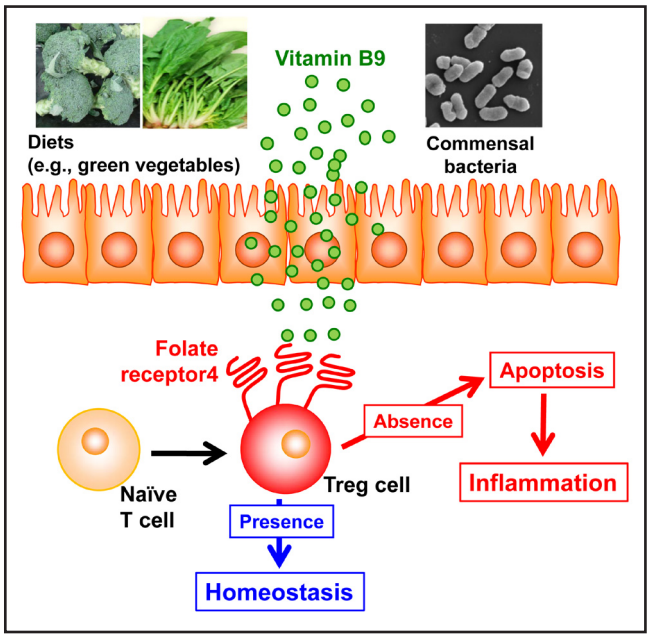

Fig. 1 Vitamin B9-mediated maintenance of regulatory T cells for the immunologic homeostasis in the intestine

When naïve T cells differentiate into regulatory T (Treg) cells, they obtain the expression of folate receptor 4 (FR4). Consistently, they require vitamin B9 for their survival. Vitamin B9 is provided from diets and commensal bacteria. The absence of sufficient amounts of vitamin B9 leads to the induction of apoptosis of Treg cells and consequently development of intestinal inflammation.

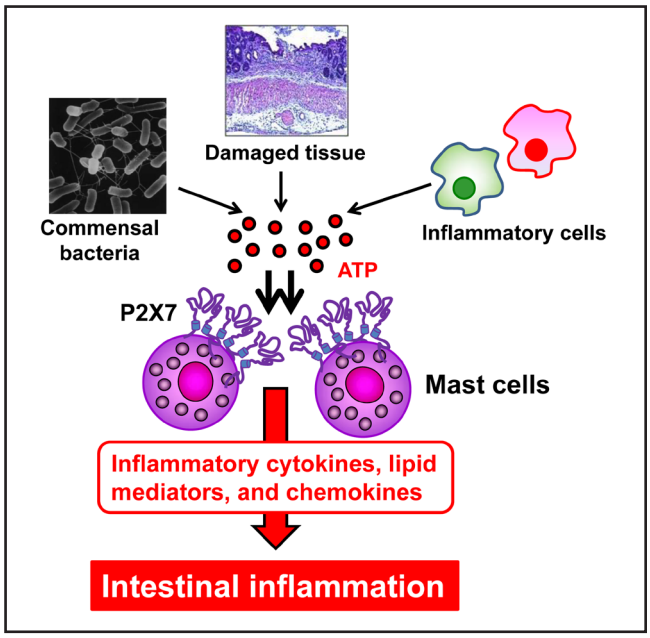

Fig. 2 P2X7^{+} mast cells mediate extracellular ATP-mediated intestinal inflammation

In the inflammatory condition in the intestine, ATP is released into extracellular compartment from damaged tissues, commensal bacteria, and inflammatory cells (e.g., macrophages). The extracellular ATP binds to P2X7 receptors on mast cells and subsequently induces the production of inflammatory chemokines (e.g., CCL2, CCL4, CCL7, CXCL1, and CXCL2), cytokines (IL-1β, IL-6, and TNFα), and mediators (histamines and leukotrienes). These responses exacerbate intestinal inflammation.

decreased expression of Bcl-2, an anti-apoptotic molecule, without affecting inhibitory molecules such as CTLA-4.

Consistent with the requirement of vitamin B9 for the maintenance of Treg cells, *in vivo* deficiency of dietary vitamin B9 resulted in the preferential reduction of Treg cells in the intestine[33, 34]. Due to the regulatory functions of Treg in the maintenance of immunological homeostasis, the reduction of Treg cells in the vitamin B9-deficient condition was accompanied with the high susceptibility to intestinal inflammation (Fig. 1)[34]. These findings suggest that dietary vitamin B9 is a critical factor to keep the appropriate numbers of Treg cells and consequent maintenance of immunological homeostasis in the intestine. It has been proposed that, in addition to diets, commensal bacteria is able to produce vitamin B9 and its production activity was differ among commensal bacteria[35]. Therefore, composition of commensal bacteria is likely to be another determinant of vitamin B9/Treg-mediated maintenance of immunological homeostasis in the gut.

Mast cells mediate the development of intestinal inflammation through the recognition of extracellular ATP by P2X7 receptors

ATP is an energy source in the intracellular compartment, but is occasionally released into the extracellular compartments. Extracellular ATP acts as a danger signal by recruiting and activating immune cells[27, 28]. Major sources of extracellular ATP are damaged tissues, and dead and activated cells including inflammatory cells (Fig. 2). Extracellular ATP is recognized by P2 receptors including several P2X receptors and P2Y receptors[28]. Among them, P2X7 receptors are involved mainly in the induction of inflammatory responses. Indeed, high levels of P2X7 receptor expression was observed in the intestinal mucosa of Crohn's disease patients[36], and treatment with P2X7 inhibitors (e.g., A-740003, Brilliant Blue G, and KN-62) ameliorated experimental colitis[37]. These findings collectively implicate that extracellular ATP-P2X7 pathway is a novel therapeutic target in the treatment of intestinal

inflammation.

Mast cells are generally recognized as pathogenic cells in the development of allergic responses upon the crosslinking among FcεR1, IgE, and relevant allergen; however, several lines of evidence have demonstrated the pathologic function of mast cells in the development of inflammatory responses[38, 39]. To elucidate the unrevealed functions of mast cells, we previously established mast cell-specific antibody libraries and found that one of the monoclonal antibodies (1F11 mAb) was able to inhibit the development of colitis[36]. Proteomics analysis revealed that 1F11 mAb recognized the extracellular domain of P2X7 receptors and thus block the biding of extracellular ATP to P2X7 receptors. Although various immune cells express P2X7 receptors in different tissues[27], P2X7 receptor expression was predominantly observed on mast cells in the colon[36]. Extracellular ATP-mediated mast cell activation through P2X7 receptors leads to the production of inflammatory chemokines (e.g., CCL2, CCL4, CCL7, CXCL1, and CXCL2), cytokines (IL-1β, IL-6, and TNFα), and mediators (histamines and leukotriene) (Fig. 2). Therefore, treatment with 1F11 mAb prevents these pathways and consequently inhibits the development of intestinal inflammation[36]. We recently found that, unlike colonic mast cells, skin mast cells barely expressed P2X7 receptors and skin fibroblasts were involved in the down-regulation of P2X7 receptors on mast cells[22]. Thus, tissue environments determine P2X7 expression on mast cells, which is a critical factor in the development of local inflammation.

Conclusion

Immunological functions of vitamins and nucleotides have been long indicated from both clinical and experimental evidence. Recent achievements allow us to understand molecular and cellular mechanisms underlying their functions in innate and acquired immune responses and its involvement in the development of intestinal inflammation. These findings provide new strategies in the development of pharmacological agents and/or functional foods for the maintenance of a healthy immune condition in the intestine.

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Conflict of interests

No conflicts of interest to be disclosed.

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