and 10 ng ml^{-1} IL-2 (both from R&D Systems) in the presence or absence of 0.1 mM sodium acetate, sodium propionate or sodium butyrate (Sigma-Aldrich) for 1–3 days (Fig. 3). In the experiments described in Fig. 2a, b and Supplementary Fig. 7, T cells (5×10^5 cells ml^{-1}) were cultured for 3 days and then expanded in complete medium supplemented with TGF-β and IL-2 for 2 days followed by analyses. T cells were polarized to T_H1, T_H2 or T_H17 subtypes *in vitro* by the addition of the following: 10 ng ml^{-1} IL-12 (R&D Systems) and 10 μg ml^{-1} anti-IL-4 monoclonal antibody (clone 11B11) for T_H1 cell polarization; 10 ng ml^{-1} IL-4 (R&D Systems) and 10 μg ml^{-1} anti-IL-12 monoclonal antibody (clone: R4-6A2) for T_H2 cell polarization; and 40 ng ml^{-1} IL-6 (R&D Systems) and 0.2 ng ml^{-1} TGF-β1, along with 10 μg ml^{-1} each of anti-IFN-γ and anti-IL-4 monoclonal antibodies for T_H17 cell polarization. IL-2 was added to the medium 48 h after initial stimulation, and T cell were cultured for 2 days followed by analyses.

ChIP. ChIP assays were performed using the MAGnify ChIP system (Invitrogen) according to the manufacturer's protocol with a few modifications. In brief, 500,000 cells were fixed with 1% formaldehyde (room temperature, 10 min) and the reaction was stopped by the addition of glycine to a final concentration of 125 mM. Crude nuclei were isolated in SDS lysis buffer to obtain the chromatin solution. The chromatin solution was sonicated using a focused-ultrasonicator (Covaris) to reduce the chromatin DNA length to approximately 100–300 base pairs. The acoustic parameters were optimized (Duty cycle: 10%, Intensity: 5, Cycle/Burst: 200, 15 cycles) with monitoring the DNA fragment using Agilent 2100 Bioanalyzer. The sheared chromatin was immunoprecipitated (overnight, 4 °C) with magnetic protein A/G beads with immobilized 3 μg of anti-acetyl histone H3 antibody (Millipore) or rabbit IgG on a rotating wheel. After extensive washing, immune complexes were eluted (55 °C, 30 min) and reverse crosslinking was carried out (65°C, 1 h) in the presence of proteinase K. qPCR analysis was performed following magnetic bead-based DNA purification. For ChIP-seq library construction, immunoprecipitated DNA fragments were ligated with adaptors and amplified by PCR. Amplified libraries were subjected to cluster generation and sequencing analysis with the HiSeq 1000 (Illumina). Sequenced reads were mapped to the mouse genome (v. mm9) with BOWTIE. Peaks for each population were called with MACS with *P* value threshold of less than 1×10^{-5}. The numbers of reads in a section were that of total mapped reads and the length of each section. Because butyrate treatment globally enhances histone acetylation, total mapped reads may be increased in butyrate-treated cells compared to control cells. Therefore, the normalized values within each sample were further subjected to cross-sample adjustment on the basis of read counts of the four genomic regions around transcription start sites of the housekeeping gene *Rpl13a*, whose histone acetylation status remained unchanged between butyrate-treated and untreated cells as confirmed by ChIP-qPCR analysis (data not shown). The normalized reads were calculated within fixed base length for upstream and downstream regions, and relative length for genic regions.

Gene expression profiling. Total RNA was extracted by Trizol reagent (Life Technologies) following a standard protocol, and subjected to microarray analysis using the GeneChip Mouse Gene 1.0 ST Array (Affymetrix). Microarray signals were processed using a standard robust multiarray averaging (RMA) algorithm. Observed signals were normalized using quantile normalization method and genes that had no significant signals were ignored to reduce noise. Butyrate-sensitive transcription factors were selected using annotation provided by Gene Ontology, as they have annotation 'nucleus (GO:0005634)' and 'regulation of transcription, DNA-dependent (GO:0006355)'. Their acetylation was represented by the normalized peak height of ChIP-seq reads from 4 kb upstream to 4 kb downstream of the transcription start site. The distribution of log ratio between control and butyrate treatment could be approximated with two Gaussian distributions and fitted curves were determined using expectation–maximization algorithm. The threshold value was $\log_2$ 0.5.

Induction of colitis by adoptive transfer of CD4$^+$ CD45RB^{high} T cells. Colitis was induced in *Rag1*$^{-/-}$ mice by adoptive transfer of CD4$^+$ CD45RB^{high} T cells as described previously[21]. In brief, CD4$^+$ T cells were enriched from splenocytes from C57BL/6 mice by IMag Cell Separation System. Enriched CD4$^+$ T cells were labelled with FITC-conjugated anti-mouse CD3ε (145-2C11), PE-conjugated anti-mouse CD45RB (16A) (all from BD Biosciences), and CD3ε$^+$ CD4$^+$ CD45RB^{high} cells were isolated by cell sorting using FACSAria II flow cytometer (BD Biosciences). The *Rag1*$^{-/-}$ recipients were given 2×10^5 CD4$^+$ CD45RB^{high} T cells via the tail vein, and were euthanized at 6 weeks after transfer. For the T_{reg} cell depletion experiment, CD4$^+$ CD45RB^{high} T cells from *Foxp3*^{hCD2} reporter mice were adoptively transferred into *Rag1*$^{-/-}$ mice. The recipient mice were received intravenous injection of 1 mg anti-human CD2 monoclonal antibody (35.1) at 4 and 5 weeks after the transfer.

Histology. Prefixed colonic tissue sections were deparaffinized, rehydrated and stained with haematoxylin and eosin or with Alcian blue-nuclear fast red. The specimens were examined histologically for scoring the degree of colitis based on the following criteria: inflammatory infiltrates, mucosal hyperplasia and loss of goblet cells.

Immunofluorescent staining. Immunofluorescent staining of cross-sections of colonic tissues was performed as described previously[49].

Immunoblotting. Immunoblotting analysis was performed as described previously[50]. Antibodies against histone H3 (ab1791, Abcam), acetylated histone H3 (06-599, Merck), Stat5 (clone: 3H7, Cell Signaling Technology), Smad3 (clone: C67H9, Cell Signaling Technology), NFAT1 (4389, Cell Signaling Technology), c-Rel (AF2699, R&D systems) and GAPDH (clone: 6C5, MAB374, Merck) were used in this study.

Statistical analyses. Differences between two or more groups were analysed respectively by the Student's *t*-test or ANOVA followed by Tukey's post-hoc test. When variances were not homogeneous, the data were analysed by the non-parametrical Mann–Whitney *U* test or the Kruskal–Wallis test followed by the Scheffé test. The body weight changes of the mice fed with or without HAMSB were analysed using a two-way repeated measures ANOVA followed by Tukey's test. All statements indicating significant differences show at least a 5% level of probability.

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Journal of Gastroenterology and Hepatology

JGHF

doi:10.1111/jgh.12259

NUTRITION AND THE DIGESTIVE SYSTEM

Nutritional components regulate the gut immune system and its association with intestinal immune disease development

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Key words
allergy, intestinal immunity, lipid, nucleotide, vitamin.

Accepted for publication 17 April 2013.

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Abstract

The gut is equipped with a unique immune system for maintaining immunological homeostasis, and its functional immune disruption can result in the development of immune diseases such as food allergy and intestinal inflammation. Accumulating evidence has demonstrated that nutritional components play an important role in the regulation of gut immune responses and also in the development of intestinal immune diseases. In this review, we focus on the immunological functions of lipids, vitamins, and nucleotides in the regulation of the intestinal immune system and as potential targets for the control of intestinal immune diseases.

Introduction

The intestinal mucosa is the largest surface area of the body and is constantly exposed to a vast array of microbes and dietary materials. To withstand this harsh environment, the gastrointestinal tract is equipped with a highly organized mucosal immune system that creates and maintains an immunologically dynamic and harmonized homeostasis between the host and the external environment.[1] The immunological components of the gut not only induce protective immunity against pathogenic microorganisms, but also immunologically ignore beneficial nonself antigens (Ags) such as nutritional materials and commensal bacteria. Thus, gut immune system orchestrates both active and quiescent immune responses and plays a central role in creating and maintaining immunologic homeostasis in the gut. Therefore, normal functioning of the gut immune system and integrity of the epithelial barrier are essential for preventing invasion by pathogenic and commensal microorganisms but at the same time preventing the development of intestinal immune diseases (e.g. inflammatory bowel diseases and food allergies).[1]

Nutritional components derived from the diet or synthesized *de novo* are essential environmental factors for the development, maintenance, and regulation of gut immune responses. Indeed, deficient or inappropriate nutritional intake increases the risk of infectious, allergic, and inflammatory diseases.[2] Accumulating evidence has revealed the immunological functions of nutritional molecules such as vitamins, lipids, and nucleotides. In this review, we first describe the unique features of the gut immune system and then examine its regulation by nutritional molecules and its association with the development of intestinal immune diseases.

Intricate immune system in the gut for maintenance of immunological homeostasis

To protect the vast surfaces of mucosal tissues, higher mammals have evolved a unique mucosal immune system. The surfaces of mucosal tissues are covered by single- or multiple-layered epithelium and are in direct contact with the outer environment.[3] In the gastrointestinal tract, the epithelial layer consists of several subsets of intestinal epithelial cells (ECs) including M cells, goblet cells, Paneth cells, enteroendocrine cells, and columnar ECs. Each of these cells has unique functions, for example, goblet cells secrete mucus, Paneth cells produce anti-microbial peptides such as α-defensins, and other ECs produce β-defensin. All of these components are of central importance in host defense as physical, chemical, and immunological barriers.[4] In addition to ECs, another major resident cell component of the mucosal epithelium is intraepithelial lymphocytes (IELs). IELs consist mainly of various T cell subsets, primarily those expressing the γδ T cell receptor, and they bi-directionally interact with ECs to maintain normal homeostasis.[5]

The gut immune system has many organized lymphoid structures, which can be separated into inductive and effector sites on

the basis of their anatomical and functional properties.[6] In the gastrointestinal tract, the inductive sites are the gut-associated lymphoid tissues (GALT) (e.g. Peyer's patches [PPs], isolated lymphoid follicles, and colonic patches).[7] The GALT contains T cell- and B cell-enriched regions, which harbor large numbers of surface immunoglobulin A $(IgA)^+$ B cells.[7] In the follicle-associated epithelium, the PP M cells take up Ags from the lumen of the intestinal mucosa and transport them to underlying dendritic cells (DCs) on a subepithelial dome region.[7] The DCs carry the Ags into the T cell region and subsequently germinal centers in the GALT or via draining lymphatics into the mesenteric lymph nodes, for the initiation of T and B cell responses.

After emigration from the inductive tissues (e.g. PPs), primed lymphocytes traffic into the lamina propria, where they further differentiate into effector cells such as IgA-producing plasma cells (PCs), regulatory T (Treg) cells, and Th17 cells. The ECs again become central players in subsequent event by transporting polymeric IgA via the polymeric immunoglobulin receptor.[8]

These immunological networks in the gut allow the orchestration of both active and quiescent immune responses for immunosurveillance and immunologic homeostasis in the gut.

Sphingosine 1-phosphate (S1P) regulates cell trafficking in gut immunity and immune diseases

It is generally accepted that nutritional materials are involved in immune regulation. Lipids, after their conversion into lipid mediators, are among the major nutritional components involved in the regulation of intestinal immune responses, and imbalances in lipid mediator signaling pathways contribute to disease induction and resolution phases in inflammation, autoimmunity, allergy, cancer, atherosclerosis, hypertension, metabolic syndrome, and degenerative diseases.[9,10]

Among many lipid mediators, S1P is essential for the trafficking and activation of immunocompetent cells.[11] S1P is a metabolite of sphingomyelin from both the host cell plasma membrane and diet.[12] Sphingomyelin is degraded into ceramide by alkaline sphingomyelinase and subsequently to sphingosine by ceramidase. Sphingosine is then phosphorylated to generate S1P by sphingosine kinases.[11] S1P is formed in most cells, but is simultaneously irreversibly degraded by S1P lyase or dephosphorylated by S1P phosphatases.[11] Therefore, S1P levels are extremely low in most tissues but high in the blood and lymph because of the lack of S1P degrading activity of erythrocytes, platelets, and lymphatic endothelial cells; the difference creates an S1P gradient between these types of tissues.[13,14] Cells expressing S1P receptors sense the S1P gradient and traffic toward high concentrations of S1P.

Among five closely related S1P receptors, the type 1 S1P receptor ($S1P_1$) is preferentially expressed by lymphocytes and thus determines lymphocyte emigration from and retention in the lymphoid tissue.[15] Naïve lymphocytes express high levels of $S1P_1$, and their activation is associated with downregulation of this receptor. However, $S1P_1$ expression recovers in fully differentiated activated lymphocytes. These dramatic changes in $S1P_1$ determine whether the lymphocytes are retained in the lymphoid tissues or emigrate from them into the blood or lymph circulation.

We and others have shown that S1P regulates the innate and acquired phases of gut immune responses and the development of intestinal immune diseases (reviewed in Reference[12]). For instance, S1P regulates the trafficking of B cells in the PPs and subsequent intestinal IgA production.[16] In the PPs, B cells differentiate into IgA^+ plasmablasts. During B cell differentiation in the PPs, the B cells change their expression of $S1P_1$; high expression is noted on immunoglobulin M^+ naïve B cells but is downregulated during class switching to IgA. The low level of $S1P_1$ allows newly formed IgA^+ B cells to be retained in the PPs so that they can differentiate into IgA^+ plasmablasts. The IgA^+ plasmablasts show recovery of $S1P_1$ expression, resulting in their emigration from the PPs.[16] In agreement with this finding, when mice were treated with the immunosuppressant FTY720 to induce downregulation of $S1P_1$ expression,[17] IgA^+ plasmablasts selectively accumulated in the PPs, and their population was decreased in the lamina propria.[16] As a result, FTY720-treated mice showed reduced intestinal IgA responses against orally administered protein Ag.[16] We have also reported that IgA PCs originated from peritoneal cavity, along with unique subsets of IELs require S1P for their trafficking into the intestine.[18–20]

Several lines of evidence have indicated that S1P plays a key role in the development of intestinal immune diseases. In an ovalbumin-induced murine food allergy model,[21] we found that activated T cells migrate into the colon, where they produced large amounts of Th2 cytokines such as interleukin (IL)-4 and IL-5. Our subsequent study has demonstrated that trafficking of pathogenic T cells from the systemic compartments into the colon is mediated by S1P; thus, infiltration of activated T cells into the colon of allergic mice is inhibited by treatment with FTY720 (Fig. 1).[22] In addition, infiltration or proliferation of mast cells, effector cells in the development of food allergy, in the colon is prevented by treatment with FTY720 (Fig. 1).[22] Similar effects of FTY720 on trafficking of pathogenic cells in the development of intestinal inflammation have been reported in some experimental intestinal inflammation models (e.g. IL-10-deficient mice, dextran sulphate sodium treatment, and T cell transfer models).[23–25] Collectively, these findings suggest that in addition to the physiological role of S1P–$S1P_1$ axis in the optimal supplementation of immunocompetent cells to the intestine, it also participates mainly in the development of intestinal immune diseases (e.g. allergy and inflammation) at the stage of pathogenic cell trafficking into the colon, which is a potential target for prevention and treatment of these intestinal immune diseases.

Immunological functions of vitamins in the versatile intestinal immunity

Vitamins are organic compounds that we cannot synthesize in sufficient quantities, and that therefore need to be supplied from the diet or commensal bacteria. Some of these vitamins are water-soluble (e.g. vitamin B family and vitamin C) whereas others are hydrophobic (e.g. vitamins A, D, E, and K). Both hydrophilic and hydrophobic vitamins and their metabolites have diverse functions in many biological events, including immunological regulation (Fig. 2). Indeed, vitamin deficiency results in high susceptibility to infection and immune diseases.[26]

Accumulating evidence has revealed the molecular and cellular mechanisms of vitamins underlying regulation of the immune system. The biggest breakthrough was the discovery of the function of vitamin A in regulating the tissue-tropism of lymphocytes

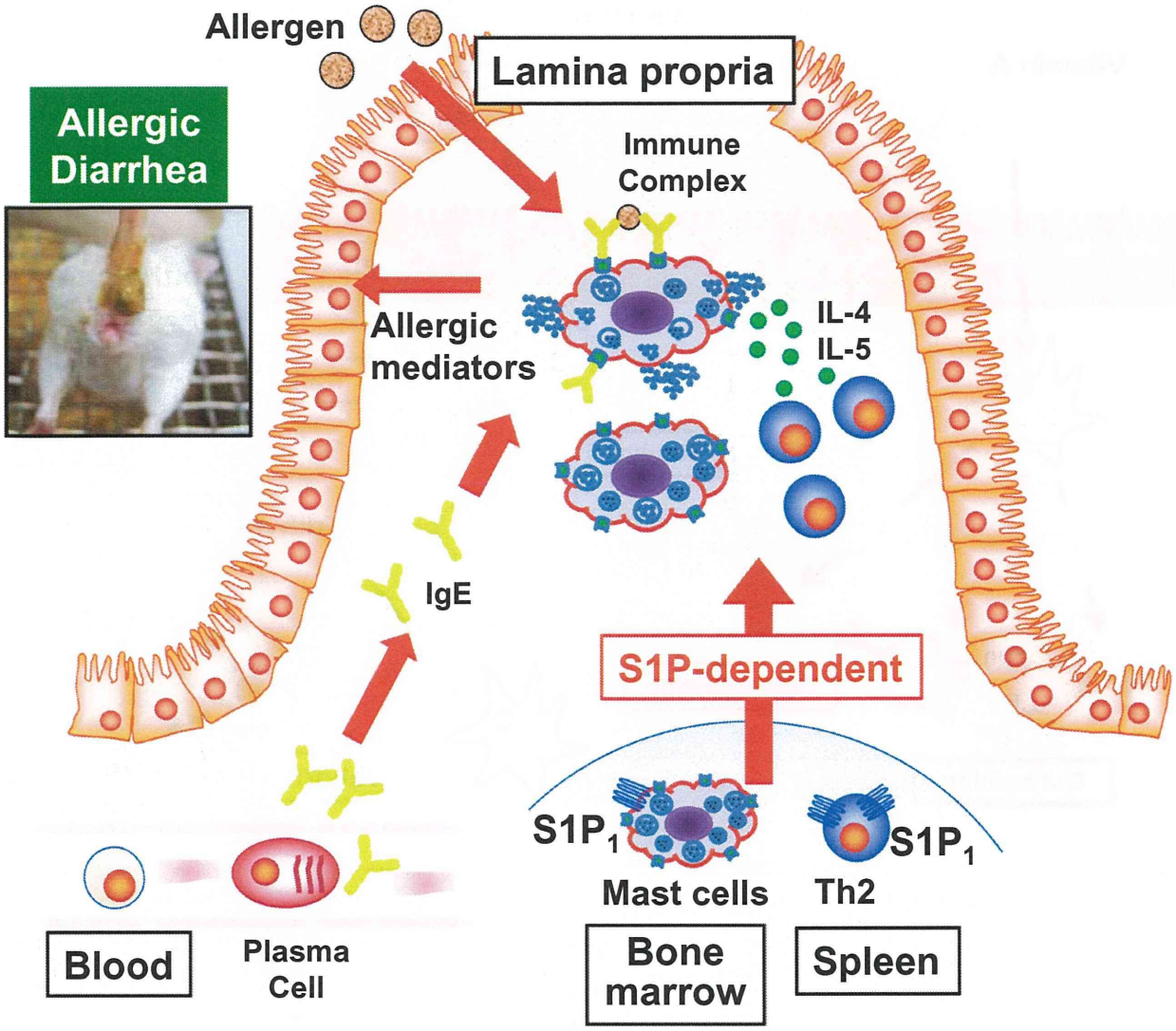

Figure 1 Involvement of sphingosine 1-phosphate in the development of allergic responses. Intestinal allergy is developed by several steps, including the production of allergen-specific immunoglobulin E (IgE) production, Th2 cell infiltration and activation (e.g. interleukin [IL]-4 and IL-5 production), mast cell accumulation and activation via cross-linking among IgE, allergen, and IgE receptor (FcεR1). During these steps, activated T cells and mast cells use sphingosine 1-phosphate (S1P) in their trafficking or growth in the colon.

activated in the gut (reviewed in Reference[27]). Vitamin A is obtained from the diet as all-trans-retinol, retinyl esters, or β-carotene and is metabolized into retinol or retinoic acid (RA) in the tissues.[28] Immunologically, RA induces the expression of $\alpha_4\beta_7$ integrin and the chemokine receptor CCR9 on both T and B cells.[29,30] Because both $\alpha_4\beta_7$ integrin and CCR9 are key molecules in lymphocyte homing into the gut, activated lymphocytes in the presence of RA tend to traffic into the intestinal lamina propria (Fig. 2). In agreement with this, vitamin A-deficient mice show a lack of T cells and IgA PCs in the intestine.[29,30]

RA plays an important role in determining not only the gut-tropism of lymphocytes activated in the intestine but also T cell differentiation. RA potentiates Treg induction with inhibition of Th17 differentiation *ex vivo* (Fig. 2).[31–33] Retinaldehyde dehydrogenase, a key enzyme converting vitamin A into RA, is uniquely expressed on gut-associated DCs, especially $CD103^+$ migratory DCs and ECs.[29,34] Thus, vitamin A metabolism by intestinal DCs and ECs plays a pivotal role in both T cell differentiation and subsequent cell trafficking to maintain the immunological homeostasis in the gut.

Recent studies have revealed the immunological role of vitamin B9 (also known as folate or folic acid) in the maintenance of Treg cells. Vitamin B9 is a water-soluble vitamin derived from both diet and commensal bacteria; the pathways for its *de novo* synthesis are

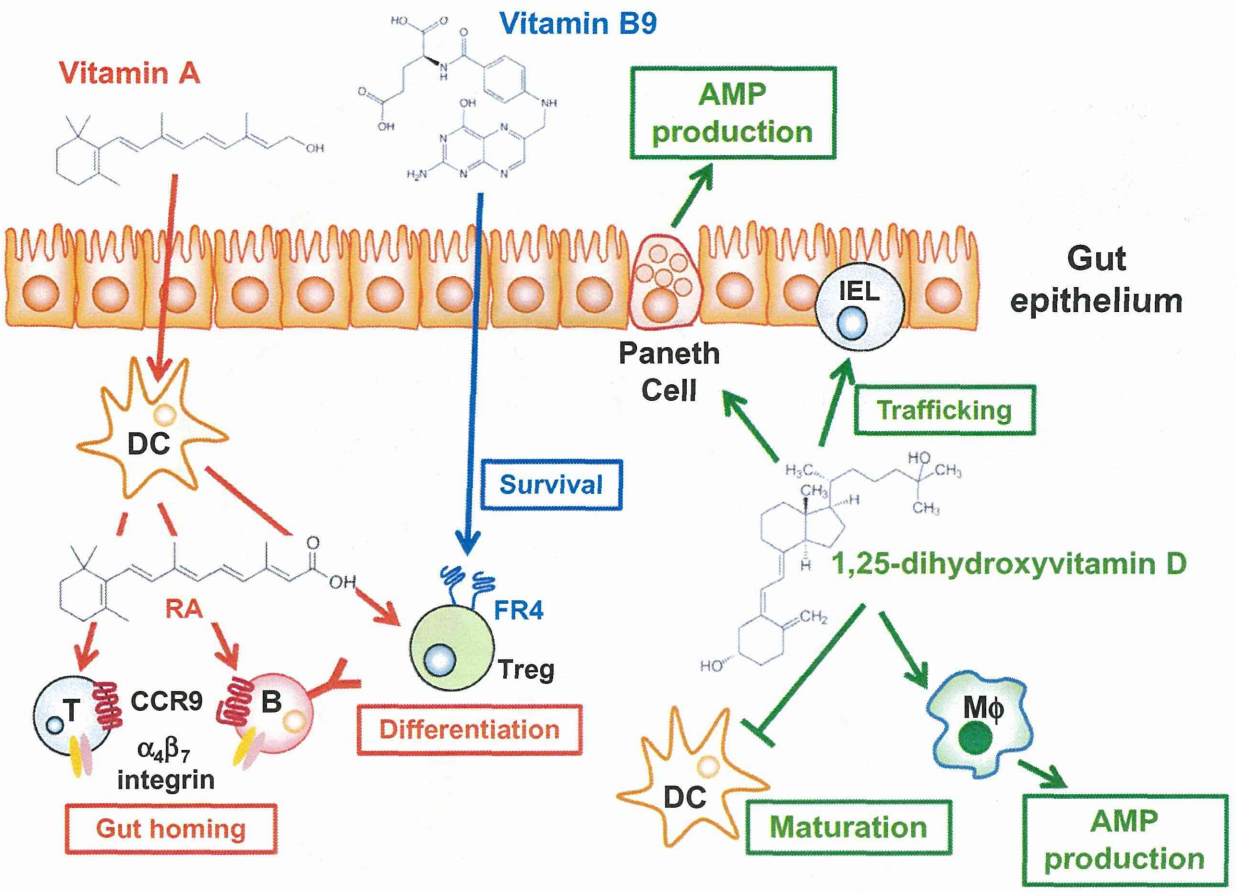

Figure 2 Vitamin-mediated immune regulations in the gut. Vitamin A from diet is converted into retinoic acid (RA) by dendritic cells (DCs). B and T cells primed by DCs in the presence of RA express gut homing molecules ($\alpha_4\beta_7$ integrin and CCR9). RA also promotes the differentiation of naïve T cells into regulatory T (Treg) cells. Upon the differentiation into the Treg cells, Treg cells start to express folate receptor 4 (FR4), a receptor for vitamin B9. The vitamin B9–FR4 axis is required for the survival of Treg cells. Vitamin D acts on Paneth cells on the epithelial layer and macrophages (MΦ) and aids production of antimicrobial peptides (AMPs). It also promotes the trafficking of intraepithelial lymphocyte (IEL) population and inhibits the maturation of DCs.

absent in mammals.[35] The biological functions of vitamin B9 are basically synthesis, replication, and repair of nucleotides for DNA and RNA to maintain cell proliferation and survival.[36] From an immunological perspective, Yamaguchi *et al.*[37] reported that folate receptor 4, one type of vitamin B9 receptor, is highly expressed on the surfaces of Treg cells, implicating the specific function of vitamin B9 on Treg cells. Moreover, we recently reported that Treg cells could differentiate from naïve T cells, but not survive, in the absence of vitamin B9 *in vitro* and *in vivo*, which was associated with the reduced expression of anti-apoptotic molecules (e.g. Bcl-2).[38] Because Treg cells are essential for maintaining immunological quiescence, mice deficient in vitamin B9 have increased susceptibility to intestinal inflammation.[39] These findings collectively suggest that vitamin A is required for the induction of Treg cells and that subsequent maintenance of the differentiated Treg cells is mediated by vitamin B9 (Fig. 2).

In addition to modulating lymphocytes, vitamins regulate innate immunocompetent cells. For example, vitamin D enhances the production of the antimicrobial peptide cathelicidin by intestinal Paneth cells,[40] stabilizes tight-junction structures in ECs,[41] and enhances homing of the IEL population in the gut (Fig. 2).[42] Consistent with these findings, mice lacking vitamin D receptors have increased bacterial loads in the intestine and show intestinal inflammation.[42,43] In addition, vitamin D receptors and CYP27B1, a vitamin D-activating enzyme, are induced in macrophages or DCs upon their activation (Fig. 2). In macrophages, intracrine synthesis of an active form of vitamin D, 1,25-dihydroxyvitamin D, promotes their antibacterial response to infection.[44] Intracrine 1,25-dihydroxyvitamin D in DCs inhibits their maturation, which in turn results in impaired T cell activation.[45] 1,25-dihydroxyvitamin D also acts extrinsically on T cells. 1,25-dihydroxyvitamin D3 inhibits T cell differentiation into interferon-γ-, IL-17-, or IL-21-producing inflammatory T cells but promotes the differentiation of Treg cells.[46] These versatile functions of vitamin D have led to its use in the control of infectious and inflammatory diseases.[47,48]

Nucleotides act as danger signals to induce the inflammatory responses

In addition to vitamins and lipids, our diet also contains sizable amounts of nucleotides. Dietary nucleotides have various effects on the immune responses such as protection from bacterial infections[49] and immune regulations.[50]

With dietary nucleotides, there is an abundance of extracellular nucleotides in the intestinal lumen, mainly in the form of adenosine triphosphate (ATP). Several lines of evidence have demonstrated that extracellular ATP acts as a danger signal to induce inflammatory responses. Therefore, stimulation of macrophages and DCs by ATP induces the production of inflammatory cytokines, which can consequently lead to the development of asthma, contact hypersensitivity, or graft-versus-host disease.[51–53] ATP is also involved in the development of intestinal inflammation through the induction of Th17 cells via intestinal DC activation.[54]

In the intestinal lumen, extracellular ATP is catalyzed by ATP-hydrolyzing enzymes, such as ectonucleoside triphosphate disphosphohydrolases preferentially expressed on intestinal ECs.[55] A recent study demonstrated that mice lacking ecto-nucleoside triphosphate disphosphohydrolases had elevated levels of ATP in the intestinal lumen and consequently high numbers of Th17 cells in the intestinal lamina propria.[56] We recently reported that, in addition to inducing Th17 cells, ATP directly stimulates mast cells in the intestine.[57] Among immunocompetent cells in the intestine (e.g. DC, T and B cells, macrophages, and ECs), mast cells express the highest levels of P2X7 purinoceptor (one type of receptor for extracellular ATP). ATP-mediated stimulation of mast cells results in the production of inflammatory cytokines (e.g. IL-1β and tumor necrosis factor-α), chemokines (e.g. CCL1), and lipid mediators (e.g. leukotriene B4), and thus, inhibition of this pathway by blocking antibody led to the prevention of intestinal inflammation (Fig. 3).[57]

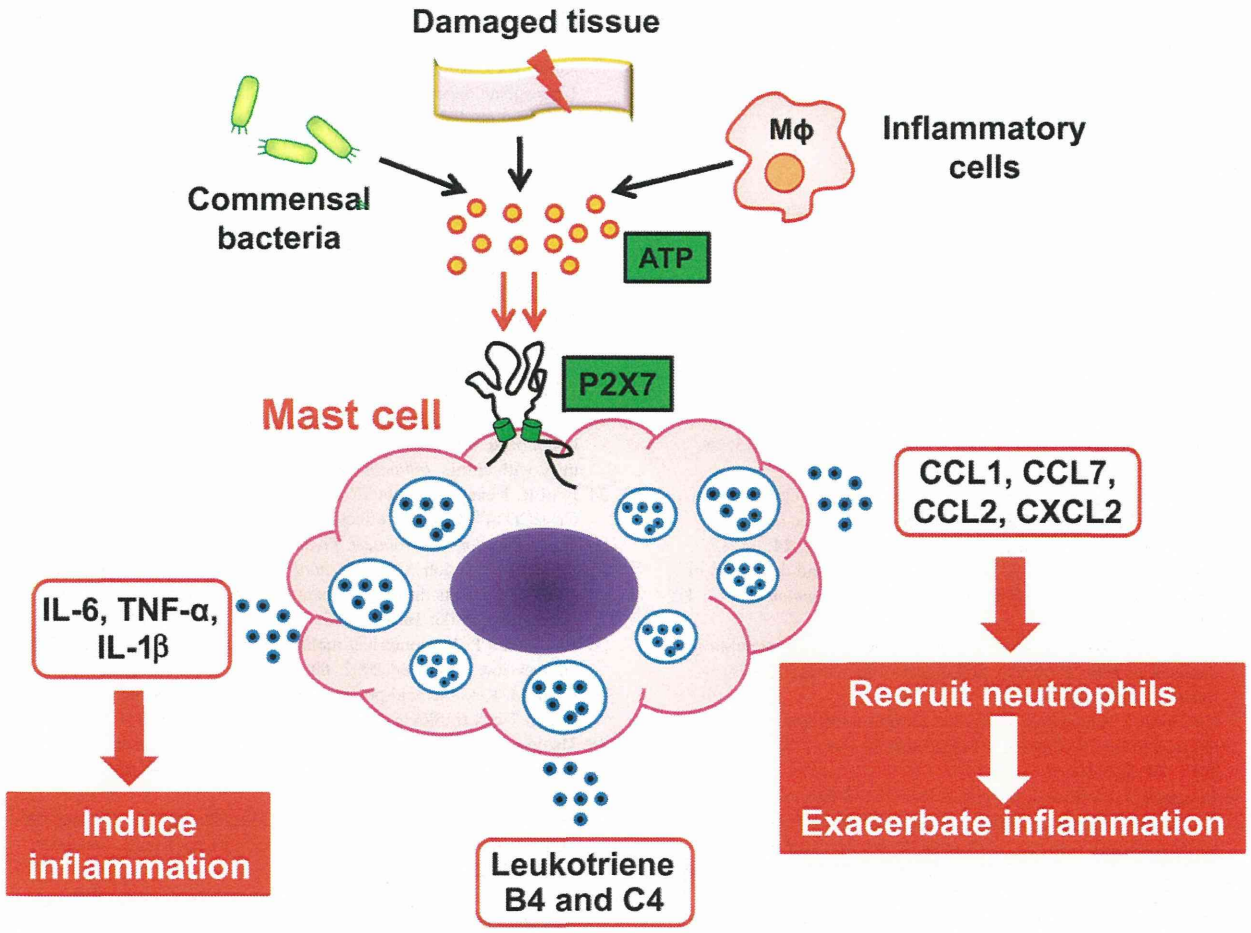

Figure 3 Extracellular adenosine triphosphate (ATP) acts as pro-inflammatory nucleotide through mast cell activation. Extracellular ATP derived from damaged tissues, commensal bacteria, and inflammatory cells can act as danger signals in the intestine. Colonic mast cells express the highest levels of P2X7 purinoceptors, a receptor for extracellular ATP. ATP-mediated activation of mast cells through P2X7 purinoceptors results in the production of inflammatory cytokines (e.g. interleukin [IL]-6, tumor necrosis factor [TNF]-α, IL-1β), chemokines (e.g. CCL1, CCL7, CCL2, CXCL2), and lipid mediators (leukotriene B4 and C4), which in turn leads to the induction and exacerbation of intestinal inflammation.

Conclusion

Immunological homeostasis and immunosurveillance in the gut are achieved by both innate and acquired unique immune systems. Many nutritional components play important roles in the development and smooth functioning of the gut immune system in both the innate and the acquired phase. Further elucidation of the intricate system by which nutrients regulate mucosal immunity by nutrition will allow us to develop functional nutritional materials for controlling the intestinal immune system and thus preventing intestinal immune diseases.

Acknowledgments

The work related to this review was supported by grants from the Program for Promotion of Basic and Applied Research for Innovations in Bio-oriented Industry (to J.K.), the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grants-in-Aid for Scientific Research on Innovative Areas [J.K.], for Scientific Research S [H.K.], Challenging Exploratory Research [J.K.], and for the Leading-edge Research Infrastructure Program [to J.K and H.K.]); and grants from the Ministry of Health and Welfare of Japan (J.K and H.K.); and the Yakult Bio-Science Foundation (to J.K.).

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REVIEW ARTICLE
published: 11 July 2013
doi: 10.3389/fimmu.2013.00189

Vitamin-mediated regulation of intestinal immunity

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The intestine is exposed continuously to complex environments created by numerous injurious and beneficial non-self antigens. The unique mucosal immune system in the intestine maintains the immunologic homeostasis between the host and the external environment. Crosstalk between immunocompetent cells and endogenous (e.g., cytokines and chemokines) as well as exogenous factors (e.g., commensal bacteria and dietary materials) achieves the vast diversity of intestinal immune functions. In addition to their vital roles as nutrients, vitamins now also are known to have immunologically crucial functions, specifically in regulating host immune responses. In this review, we focus on the immunologic functions of vitamins in regulating intestinal immune responses and their roles in moderating the fine balance between physiologic and pathologic conditions of the intestine.

Keywords: intestinal immunity, vitamin, IgA, regulatory T cells, allergy, inflammation

INTRODUCTION

The primary physiologic function of intestine is to serve as the chief site of nutrient absorption into the body. However, intestinal tissues also comprise a unique immune system that can discriminate between pathogens and harmless or beneficial antigens such as commensal microorganisms and dietary constituents (1). To prevent unnecessary inflammatory responses and hypersensitivity to harmless or beneficial materials, the intestinal immune system usually becomes unresponsive to these factors through the induction of oral tolerance (2). At same time, the intestinal immune system acts as the first line of defense against pathogens. For the coordinated operation of this complex network, the intestinal immune system is customized with cooperative immunocompetent cells, including the specialized 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 (T_{reg}), Th1, Th2, and Th17 cells; mast cells; and innate lymphoid cells (1, 3, 4). Accumulating evidence has demonstrated that the disruption of oral tolerance underlies pathogenic conditions such as intestinal inflammation and food allergy (5).

Coordination of the numerous diverse intestinal immunological functions is achieved through the immunological crosstalk among immunocompetent cells via endogenous molecules (e.g., cytokines and chemokines). In addition to these endogenous factors, components of the gut environment, such as commensal bacteria and dietary materials, influence intestinal immunological functions. Recent advances in genetic identification have revealed that commensal bacteria play an important role in the development and maintenance of not only intestinal or mucosal immunity but also the host immune system [reviewed in Ref. (6)]. Although the underlying molecular and cellular mechanisms are not fully understood, nutritional components derived from the diet, either directly absorbed or metabolized or synthesized *de novo* by commensal bacteria, clearly are essential and influential exogenous factors for the development, maintenance, and regulation of the intestinal immune system (7, 8). This idea is underscored by the fact that nutrient deficiencies often are associated with impaired intestinal immunity (9). For instance, a recent study shows that angiotensin I converting enzyme 2 regulates intestinal amino acid metabolism and consequently affects the ecology of commensal bacteria, which leads to the transmittable colitis (10). Another recent study has demonstrated that commensal bacteria from kwashiorkor, a form of acute malnutrition that occurs by inadequate intake of dietary protein, perturb the metabolism of amino acids and carbohydrates (11).

Vitamins are organic compounds that the host organism cannot synthesize in sufficient quantities and that therefore need to

be supplied exogenously by the diet or commensal bacteria. Some vitamins (e.g., vitamin B family and vitamin C) are water-soluble, whereas others (e.g., vitamins A, D, E, and K) are hydrophobic. Both hydrophilic and hydrophobic 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 (12). Previously vitamins were thought to regulate the immune system in an indiscriminant manner, but accumulating evidence has revealed specific functions of individual vitamins and their metabolites in immune responses.

In this review, we discuss recent progress regarding our understanding of the immunologic functions of particular vitamins and their contributions toward maintaining the immunologic balance between physiologic and pathologic conditions of the intestine.

VITAMIN A REGULATES CELL TRAFFICKING AND DIFFERENTIATION IN THE INTESTINE

Vitamin A, especially its metabolite retinoic acid (RA), has emerged as a critical mediator of mucosal immune responses [reviewed in Ref. (13)]. Vitamin A is a fat-soluble essential micronutrient obtained from diets as all-trans-retinol, retinyl esters, or β-carotene and is metabolized into retinol in tissues (14). Retinol then is converted mainly to the all-trans isoform of RA through oxidation by alcohol dehydrogenases (ADH) and retinaldehyde dehydrogenases (RALDH) (**Figure 1**).

The importance of vitamin A in the regulation of intestinal immunity has long been indicated. Indeed, vitamin A deficiency leads to increased susceptibility to various pathogens and vitamin A supplementation reduces the morbidity and mortality due to infectious diseases (e.g., diarrheal infections and measles) (15). During the past few years, our molecular and cellular understanding of the roles of vitamin A in the regulation of intestinal immunity has increased greatly. A key discovery was that RA regulates cell trafficking by inducing the expression of the gut-homing molecules α4β7 integrin and chemokine receptor CCR9 on lymphocytes and thus determining the gut tropism of these cells (16, 17). Epithelial cells and DCs, especially $CD103^+$ DCs, in the intestine uniquely express RALDH and thus are capable of synthesizing RA; therefore the lymphocytes activated by intestinal DCs and epithelial cells express α4β7 integrin and CCR9, which allow them to return to the intestinal compartment (**Figure 1**). In agreement with this understanding, vitamin-A-deficient mice lack T cells and IgA-PCs in the intestine (16, 17). Several lines of evidence have demonstrated that GM-CSF induces the RALDH expression in DCs and RA itself, IL-4, and MyD88-mediated toll-like receptor pathway enhance the induction of RALDH expression (**Figure 1**) (18, 19).

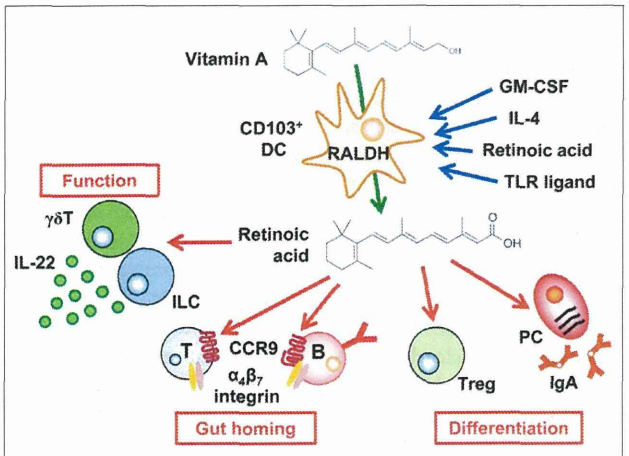

FIGURE 1 | Regulation of cell trafficking, differentiation, and function by the vitamin A metabolite retinoic acid. $CD103^+$ dendritic cells (DCs) express retinaldehyde dehydrogenases (RALDH) by GM-CSF, IL-4, TLR ligand, and retinoic acid (RA), which enable them to convert vitamin A into RA. RA then induces CCR9 and α4β7 integrin in T and B cells, causing them to migrate into the intestine. In addition, retinoic acid affects cell differentiation, such as the preferential differentiation of T cells into regulatory T (T_{reg}) cells and B cells into IgA-producing plasma cells (PCs). RA also enhances IL-22 production from γδT cells and innate lymphoid cells.

Retinoic acid plays an important role in determining not only the gut tropism of lymphocytes activated in the intestine but also cell differentiation. For example, through the cooperative effects of TGF-β, RA promotes class switching of IgM^+ B cells to those expressing IgA (**Figure 1**). Therefore, antagonism of RA results in reduced IgA production (17, 20). Another study demonstrated that Runx proteins mediate effects downstream of RA and TGF-β1 signaling in IgA class switching (21).

In addition to the effects of RA on DCs and B cells, RA affects T cell differentiation. Indeed, preferential differentiation of T cells into T_{reg} cells is mediated by $CD103^+$ DCs that are capable of producing RA and activating latent TGF-β (22–24). Reciprocally, RA failed to enhance differentiation of naïve T cells into Th17 cells in the absence of DCs (25). In this regard, DCs in the intestinal lamina propria of vitamin-A-deficient mice reportedly show impaired production of IL-6, a cytokine that is essential in the differentiation of Th17 cells (26) although there are controversial reports on the production of IL-6 by MLN-DCs from vitamin-A-deficient mice (27). On the other hand, RA–RA receptor α signal in T cells requires T cell effector responses regardless T cell subsets (26), which is in line with a previous report that Th17 cells require a low concentration of RA (20). In agreement with these functions of RA, vitamin-A-deficient mice have decreased numbers of both T_{reg} and Th17 cells in the intestine mainly due to the defect of T cell trafficking into the small intestine (25, 26, 28). In addition, segmented filamentous bacteria, Th17-inducing commensal bacteria, is decreased in vitamin A-deficient condition by high levels of mucin by goblet cells, which also leads to the impaired Th17 cell differentiation (29). Taken together, intrinsic and extrinsic factors for T cell differentiation are affected by the RA.

In addition to conventional αβ T cells, a recent study has demonstrated that RA enhanced IL-22 production by γδT cells and innate lymphoid cells, which are involved in the attenuation of intestinal inflammation (30). RA also affects non-lymphoid cells in the lymph node initiation. Indeed, RA produced by neurons