厚生労働科学研究費補助金

免疫アレルギー疾患等予防・治療研究事業

食餌性脂質を中心とした生理活性脂質による粘膜免疫制御ならびにアレルギー疾患との関連解明

平成２２年度　総括研究報告書

研究代表者　國澤　純

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I. 総括研究報告書

厚生労働科学研究費補助金（免疫アレルギー疾患等予防・治療研究事業）
総括研究報告書

食餌性脂質を中心とした生理活性脂質による粘膜免疫制御ならびにアレルギー疾患との関連解明

研究代表者： 國澤　純（東京大学医科学研究所　講師）

研究要旨： 本研究においてはこれまでの研究代表者の研究から腸管 IgA の産生や食物アレルギーに関わることを示している脂質メディエーターの一つであるスフィンゴシン１リン酸（S1P）に関する研究を起点に、食餌性脂肪酸を介した腸管免疫制御と食物アレルギーとの関連を検討した。その結果、我々が日常摂取している食用油であってもその脂肪酸組成の違いにより腸管免疫の活性化状態が大きく変化することが示された。特に近年使用量が増えているパーム油は腸管免疫の活性化を引き起こし、腸管 IgA 抗体の産生増強と同時に、食物アレルギーの増悪化を示すことが判明した。さらにパーム油に多く含まれる主要食餌性脂肪酸の一つであるパルミチン酸がその活性化の一端を担っていることを明らかにした。IgA の産生促進に関しては、パルミチン酸は IgA 産生形質細胞に直接作用し IgA 産生を促進すると共に、代謝産物が IgA 産生細胞の遊走を促進することが示された。これらの結果は、食餌性脂肪酸の構成が腸管免疫ネットワークの形成と制御において多彩な経路で関与していることを示す結果であると考えられる。

本研究課題においては分担研究者を配していない。

A. 研究目的

食の欧米化に伴いアレルギー疾患の患者数が増加していることから、食餌性成分を介した免疫制御がアレルギー発症に関与していると考えられている。これまでの国内外のグループによる研究から、アレルギー発症に関わる食餌性成分の一つとして食餌性脂質の関与を示唆する結果が報告されている。その中心概念として、リノール酸を起点とするω6 アラキドン酸系の炎症促進作用とリノレン酸を起点とするω3 系脂質メディエーターによる抗炎症作用のバランスが重要であるとされてきたが、炎症性腸疾患を対象にした大規模な世界的臨床試験の結果から、単にリノール酸とリノレン酸のバランスだけでは説明できない数多くの事例が報告され、現在、第 3 の免疫制御脂質の同定が重要な課題となっている。

申請者はこれまでに脂質メディエーターの一つであるスフィンゴシン１リン酸（S1P）が分泌型 IgA の産生や上皮細胞間 T リンパ球を介した腸管免疫制御を行うのと

同時に、食物アレルギーの発症に関与する活性型 T 細胞やマスト細胞の遊走を制御していることを示してきた。S1P は生体内において細胞膜の主成分であるスフィンゴミエリンやセラミドなどのスフィンゴ脂質を前駆体とし産生されると言われている。しかし食餌性脂質の代謝・吸収部位となっている腸管においては食餌性脂質もその前駆体になり得ると予想されるが、脂質の代謝経路を考えてみると、S1P はこれまで免疫制御に関わっていると提唱されているリノール酸やリノレン酸から産生されるとは考えにくい。すなわち先に記したリノール酸やリノレン酸以外の第３の食餌性脂質の存在が予想されるが、その詳細は不明である。本研究においてはこれまでの研究結果を基盤に、従来提唱されてきたリノール酸やリノレン酸とは異なる食餌性脂肪酸にも腸管免疫制御活性があるという仮説をたて、アレルギー発症との関連も含め実証を行ってきた。過去２年間の研究から、パーム油が腸管免疫を活性化すること、またパーム油に多く含まれるパルミチン酸がその責任脂肪酸であることを見いだした。

3 年計画の 3 年度である本年度は腸管免疫の活性化を示すことを見いだしたパーム油とその責任脂肪酸であるパルミチン酸に焦点を当て、パルミチン酸による腸管免疫制御とアレルギー応答との関連を検討した。

B. 研究方法

1) 食餌性脂質組成の異なる餌でのマウスの飼育

通常のマウス用エサに用いられる大豆油の代わりに、パルミチン酸をパーム油と同量になるように加えた油を重量比で４％含む特殊飼料（パルミチン酸餌）を作製した。これらの餌で 6 週齢の Balb/c マウスを 2 ヶ月間飼育した。

2) 特殊餌で飼育したマウスの免疫学的機能変化と血清中ならびに腸管組織におけるパルミチン酸の定量

免疫学的機能変化について、ELISA やフローサイトメトリー等の免疫学的手法により検討した。また血清中、腸管組織でのパルミチン酸量についてガスクロマトグラフィー等の生化学的手法により測定した。

3) 腸管 IgA 産生形質細胞に対するパルミチン酸の影響

コラゲナーゼ分解法を用い腸管組織より単核球を回収した。FACSAria を用いた cell sorting により IgA 陽性 CD138 陽性形質細胞を単離、精製した。低血清培地に懸濁した細胞を 10 , 100 μM のパルミチン酸で 24 時間培養し、上清中に産生された IgA 量を ELISA 法にて定量した。

4) 食物アレルギーの誘導

特殊餌で飼育したマウスに、フロイント完全アジュバントを用い 1mg の OVA で全身感作を行った。全身感作の一週間後から週 3 回の頻度で 50 mg の OVA を経口投与することでアレルギー性下痢を誘導し、その症状、ならびに免疫学的機能を観察した。

（倫理面への配慮）

動物実験は東京大学医科学研究所のガイド

ラインに則り行った。

C. 研究結果

1) 異なる食餌性脂質を含む餌を摂取した際の腸管免疫応答の変化

コントロールである大豆油の代わりにパルミチン酸をパーム油と同量になるように加えた大豆油を重量比で 4%含む特殊飼料（パルミチン酸餌）を作製し、6 週齢のマウスに 2 ヶ月間与えた。これらのマウスの糞便中 IgA 量を ELISA 法により測定したところ、パルミチン酸餌で飼育した場合は糞便中 IgA の量が約 2 倍に増加した。これらのマウスの腸管から単核球を回収し、フローサイトメトリー法にて解析したところ IgA、CD138 の両陽性の形質細胞の増加が大腸において観察された。

さらにパルミチン酸餌を用いて飼育したマウスの血清、ならびに腸管組織におけるパルミチン酸を定量したところ、血清ではコントロールと比べ変化が見られないものの、腸管組織においては優位にパルミチン酸量が増加していた。

そこでパルミチン酸による抗体産生形質細胞への直接的作用を検討する目的で、腸管から IgA 陽性形質細胞を単離、精製し、in vitro にてパルミチン酸を直接作用させたところ、濃度依存的に IgA 産生の増強が観察された。

2) 異なる食餌性脂質を含む餌を摂取した際の食物アレルギーの発症

パルミチン酸餌で飼育したマウスに食物アレルギーモデルを適応し、その発症状態、ならびに免疫応答を検討した。その結果、IgA の産生増強と同様、パルミチン酸を加えた油を含む飼料で飼育した群で食物アレルギーの増悪化が観察された。これらのマウスにおいては IgE の産生においては大きな変化は観察されないものの、エフェクター細胞であるマスト細胞の増加が認められた。

D. 考察

本研究においては、腸管免疫を活性化する食用油として見いだしたパーム油に多く含まれ、腸管免疫活性化の責任脂肪酸として同定したパルミチン酸に注目した研究を遂行した。その結果、大豆油にパルミチン酸を加えた食用油で作製した餌（パルミチン酸餌）で飼育した場合、生体防御に関わる IgA の産生増強だけではなく、生体にとって有害な免疫応答であるアレルギー反応の増悪化も同時に観察された。これは多くの生理活性脂質が生体防御に働く免疫経路とアレルギーに働く免疫経路の両者に対して促進的に働くという結果により説明される現象であると考えられる。一般食生活において、パーム油は多くの飲食関連業界を中心に使用量が増加している。またパーム油の脂肪酸組成は牛脂に類似している。今回得られた知見は、食の欧米化に伴いパルミチン酸を多く含む食事（パーム油、牛脂）の摂取量が増加したことがアレルギー疾患増加の一因である可能性が考えられる。

パルミチン酸を多く含む餌で飼育したマ

ウスの腸管組織において、組織中の脂質分画に含まれるパルミチン酸が増加していた。この結果は食餌性脂質が生体、特に腸管の脂質構成に直接影響を与えることを示した興味深い結果である。今回の実験において、パルミチン酸による腸管免疫の活性化が観察されるのに２ヶ月を要したが、これは腸管組織の脂肪酸組成が食餌性脂肪酸の違いに伴い変化し、腸管免疫に影響を与えるという過程を経るためだと考えられる。

腸管組織において増加したパルミチン酸が IgA の産生を誘導する機構の一つとして、パルミチン酸が直接 IgA 産生細胞に作用し、IgA 産生を促進していることが示された。またパーム油やパルミチン酸を含む餌で飼育した際に観察されるアレルギー症状の増悪化においては、IgE の産生促進よりもむしろマスト細胞の増加が原因であると考えられた。パルミチン酸はマクロファージ等にも活性化作用があることが知られているので、アレルギー発症におけるマスト細胞等への直接的影響も今後の検討課題である。特に最近、肥満モデルを用いた解析からパルミチン酸が TLR や NLRP3-ASC inflammasome といった自然免疫受容体を介して炎症シグナルを伝えることが報告されていることから、本研究で観察された腸管免疫の活性化におけるこれら自然免疫受容体の関与は今後の重要な検討課題であると考える。またパルミチン酸はそのものが直接作用するだけではなく、代謝されその他の生理活性脂質への変換された後、免疫学的作用を示す経路も考えられることから、今後は代謝経路も踏まえた検討も同じく重要な課題である。

上記の項目に加え、今回見いだしたパルミチン酸による免疫活性化作用とこれまで提唱されている亜麻仁油（or リノレン酸）によるアレルギー抑制のヒエラルキーの解明を行っていくと共に、ヒトにおける影響についても検討することで食餌性脂質を介した腸管免疫制御とアレルギー疾患との関連がより詳細に解明されるものと期待される。

E. 結論

本研究から、日常的に摂取している食用油の一つであるパーム油に多く含まれるパルミチン酸が腸管免疫の活性化を引き起こす責任脂肪酸であることを見いだした。また餌のパルミチン酸含量が腸管組織のパルミチン酸量を決定することも示された。またパルミチン酸による IgA 促進作用のメカニズムの一つとして、腸管組織において増加しているパルミチン酸が IgA 産生形質細胞に直接作用していることが示唆された。

F. 健康危機情報

なし

G. 研究発表

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H. 知的財産権の出願・登録状況 (予定を含む)

1. 特許取得

該当事項なし

2. 実用新案登録

該当事項なし

3. その他

特記事項なし

II. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
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雑誌

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國澤　純	腸管の生体防御や恒常性維持における脂質メディエーター：スフィンゴシン１リン酸の役割	化学と生物	48	827-830	2010

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國澤　純、後藤義幸、 小幡高士、清野　宏	腸内細菌のパイエル板組織内共生	細胞工学	30	409-412	2011

III. 研究成果の刊行物・別冊
（主要なもの）

Curr Allergy Asthma Rep (2010) 10:215–221
DOI 10.1007/s11882-010-0097-z

Aberrant Interaction of the Gut Immune System with Environmental Factors in the Development of Food Allergies

Jun Kunisawa · Hiroshi Kiyono

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Abstract The gastrointestinal immune system is a major component of the mucosal barrier, which maintains an immunologic homeostasis between the host and the harsh environment of the gut. This homeostasis is achieved by immunologic quiescence, and its dysregulation is thought to result from the development of immune diseases such as food allergies. Recent findings have revealed versatile pathways in the development of intestinal allergies to certain food antigens. In this review, we summarize the regulatory and quiescence mechanisms in the gut immune system and describe aberrant interactions between the host immune system and the gut environment in the development of food allergies.

Keywords Food allergy · Mucosal immunology · Vitamin · Commensal bacteria

Introduction

During the past several decades, the number of people suffering from allergic diseases has increased to the point at which it is a major concern worldwide [1]. Food allergy is a serious disease associated with diarrhea; vomiting; drops in body temperature; weight loss; and, occasionally, life-threatening anaphylactic responses. Aberrant responses to dietary materials are due mainly to type I allergic responses, which are mediated by sequential immune disorders (Fig. 1). Initially, allergen-specific IgE production is induced by the T-helper type 2 (Th2) environment along with dysregulation of regulatory immune responses, which promote mast cell infiltration into the intestine. Subsequently, secondary cross-linking by the allergen on mast cells via Fcε receptor results in the production of various allergic mediators by mast cells (e.g., histamine, platelet-activating factor, leukotrienes, and mast cell protease-1). These mediators increase intestinal permeability, exacerbating the allergic symptoms [2].

Although classic immediate food allergies are mediated by mast cells, food allergens lead to the induction of delayed or chronic allergic reactions as well. The mechanisms underlying these delayed reactions are not fully understood but are thought to involve the accumulation of eosinophils in the gut (Fig. 1) [3]. A pathogenic mediator, major basic protein, was detected in the accumulated place of eosinophils in the gut, causing gut tissue damage and associated symptoms, including diarrhea, bloody stools, and blood eosinophilia [3].

In spite of continual ingestion of the same dietary materials, many people show no aberrant reactions to allergens. This unresponsiveness is associated with an immunologic tolerance known as oral tolerance, which involves the specific suppression of cellular and humoral immune responses to ingested antigens [4]. Several lines of evidence indicate that oral tolerance is achieved by a unique gut immune system made up of complex regulatory networks among immunocompetent cells (e.g., dendritic cells [DCs] and T cells) [5]. The establishment of food allergy models using experimental animals allows the investigation of possible pathways involved in the abrogation of the immunologic regulatory network and the consequent development of food allergies [6•]. It also allows the identification of some immunologic characteristics as they appear in human patients, revealing basic

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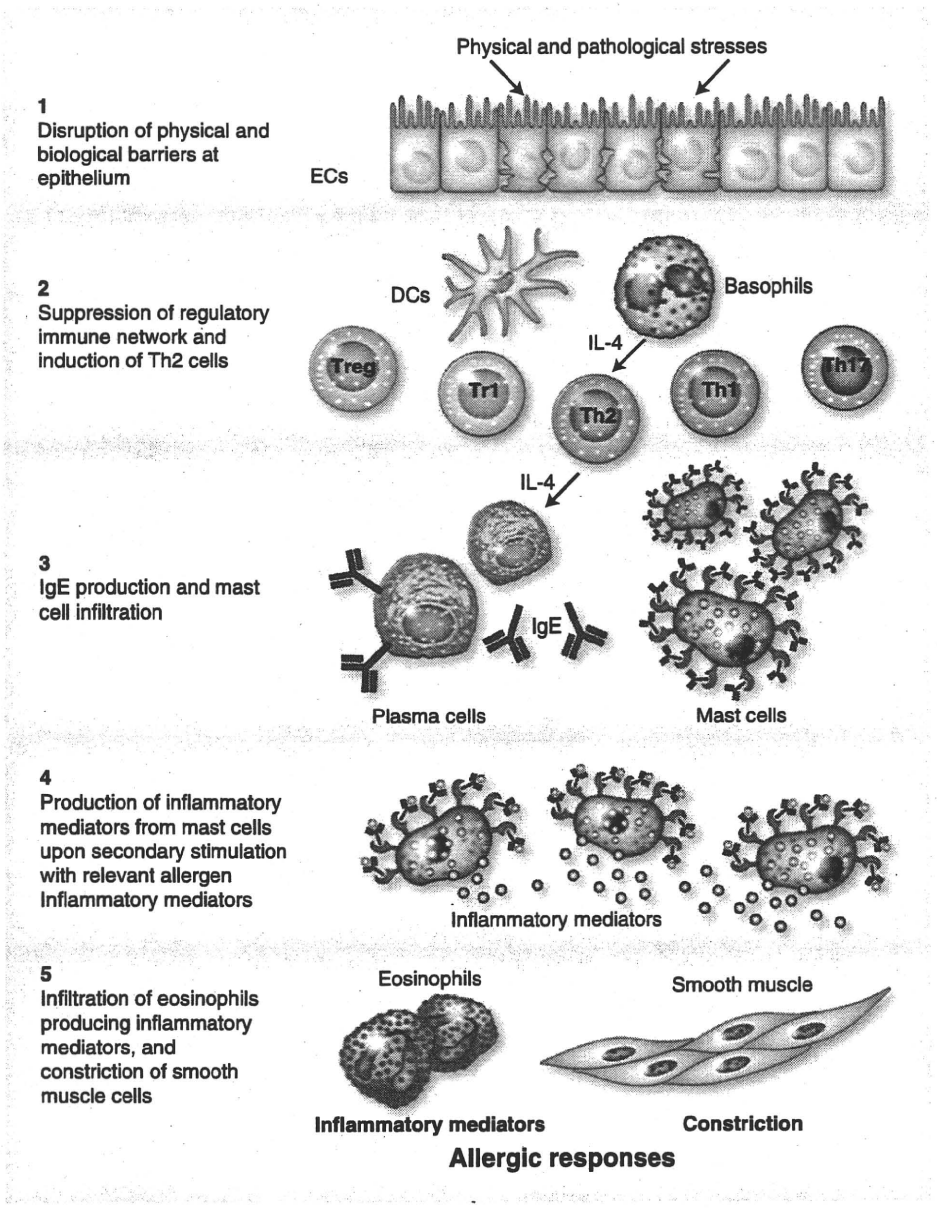

Fig. 1 Multiple steps in the development of allergic responses. **1**, Several stresses, including psychological, bacterial, and cytokine stimulation, disrupt the epithelial barrier, permitting the penetration of allergens. **2**, The immunologic environment mediated by dendritic cells (DCs) and presumably basophils results in the preferential induction of T-helper type 2 (Th2) cells, which leads to **3**, the induction of IgE production and mast cell infiltration. **4**, Mast cells produce inflammatory mediators (e.g., histamine, prostaglandins, and leukotrienes) upon cross-linking of IgE with the allergen, leading to **5**, the constriction of smooth muscle cells and the recruitment of eosinophils. EC—epithelial cell; IL—interleukin; Treg—regulatory T cell

aspects of allergic responses and potential clinical targets against food allergies.

Accumulating evidence indicates that environmental factors in the gut (e.g., commensal bacteria) play an important role in maintenance and disruption of gut immune quiescence [7]. Indeed, previous studies using germ-free mice showed that stimulation by commensal bacteria promotes the development of active and quiescent immune responses [8]. Recent advances in genome-based bacterial analyses have revealed quantitative and qualitative aspects of commensal bacteria, including unculturable bacteria, in the development and dysregulation of the host immune system [9]. Other recent nutritional studies have indicated that diversification in food, particularly Westernized diets, may be associated with the increased number of allergic patients [1].

In this review, we focus on the gut immune system in the development of food allergies from the viewpoint of the quiescent immune system and cross-talk with environmental factors.

Gut Regulatory Immune Networks and Their Disruption in the Development of Food Allergies

The gut immune system is a unique system that can distinguish between harmless and harmful nonself materials [10]. Accumulating evidence shows that various immuno-

competent cells participating in different gut immune responses, including physical, innate, and acquired immunity, use immunologic cross-talk to negatively regulate the immune responses to harmless materials. The tight junction among epithelial cells (ECs) is an example of a physical barrier that prevents the uptake of allergenic materials. Disruption of epithelial barriers promotes the development of food allergies: psychological stress [11], bacterial infection (e.g., by *Candida albicans*) [12], and cytokine stimulation (e.g., by IL-9) [13••] resulted in the increased permeability of epithelial layers, which increased the susceptibility to allergens. Similarly, immature development of the epithelial barrier in infants may explain the prevalence of food allergies in infants younger than 3 years old [1]. Additionally, ECs are not simply a physical barrier; they also influence the biological nature of allergenic macromolecules through the production, formation, and synthesis of secretory IgA and digestive enzymes. Thus, ECs pose physical, physiologic, and immunologic barriers to allergenic materials.

At the T-cell level, the classic paradigm is that Th2 responses favor the development of allergic responses, whereas Th1 responses inhibit them [14]. In this context, our group reported that the homodimeric form of interleukin (IL)-12 p40 (p80) is produced predominantly in the large intestine of allergic mice and plays an important role in the induction of Th2 responses by competing with heterodimeric IL-12 (p40 + p35), an essential cytokine for the induction of Th1 responses (Fig. 2) [15]. Although it is not clear which kinds of cells are responsible for the IL-12 p80 production, it could be worthwhile to examine basophils as immunoregulatory antigen-presenting cells involved in the process of inducing an aberrant Th2-type environment. Recent reports show that basophils express major histocompatibility complex class II and costimulatory molecules (e.g., CD80 and CD86) together with the predominant production of IL-4, initiating Th2 responses (Fig. 2) [16••, 17••, 18••]. Surprisingly, DCs are not required for the induction of Th2 responses; basophils alone are sufficient. Although the role of basophils in the development of food allergies has not yet been tested, this is an important point to be investigated.

The development of allergic responses is not explained simply by the classic Th1/Th2 paradigm. Current attention is focused on the regulatory T-cell (Treg) network. This network, composed of Treg, Tr1, Th3, and CD8$\alpha\alpha$ T cells, plays a key role in the achievement of immunologic quiescence (Fig. 2) [19, 20]. Tregs are abundant in the intestinal compartments for the creation of immunologic quiescent conditions in their harsh environments. As Tregs developing naturally in the thymus, de novo-generated intestinal Tregs express forkhead box P3 (FoxP3), a master transcription factor for the differentiation of Tregs, and have been implicated in the negative regulation of allergic responses [21, 22•]. The de novo differentiation of Tregs from naïve CD4 T cells requires transforming growth factor (TGF)-β, a cytokine that is abundant in the intestine. Importantly, costimulation with IL-6 plus TGF-β leads to the exclusive induction of IL-17–producing T (Th17) cells, which are involved in the induction and inhibition of inflammatory and allergic diseases (Fig. 2) [23–25]. Reciprocally, all-trans retinoic acid (at-RA), a metabolite of vitamin A produced particularly by intestinal $CD103^+$ DCs, prevented the differentiation of Th17 cells but enhanced Treg induction in the intestine (Fig. 2) [26••, 27••, 28••, 29••]. It was reported recently that ECs educate intestinal $CD103^+$ DCs to be tolerogenic through the production of TGF-β and at-RA (Fig. 2) [30•]. Additionally, Tregs reciprocally educate DCs to produce IL-27 for the subsequent induction of Tr1 cells, a distinct Treg population (Fig. 2) [31••]. Like Tregs, Tr1 cells produce IL-10, but unlike Tregs, they do not express FoxP3. These data suggest that the cytokine milieu created by T cells, DCs, ECs, and basophils is critical for the creation and maintenance of immunologic homeostasis in the gut. Further molecular and cellular investigation of this intestinal regulatory system is required for the development of new immunotherapy for food allergies.

Commensal Bacteria in the Regulation of the Gut Immune System

Because the prevalence of food allergies has increased very rapidly in industrialized countries, environmental and host factors are considered to be involved. Among several environmental factors, commensal bacteria are likely to be pivotal in the regulation of the gut immune system because they initiate their intestinal habitation at birth and continuously grow and are required for the maturation of the gut immune system, including the induction of oral tolerance [32]. This idea, known as the *hygiene hypothesis*, suggests that the improvement of hygiene, the development of antibiotics and vaccines, and the intake of almost-sterile food have reduced the gut's exposure to microorganisms and thus have led to the failure of the maturation of the gut immune system [7]. The hygiene hypothesis is supported by several epidemiologic studies, although the issue is still controversial [7]. Supporting the hypothesis, it was reported that mice lacking Toll-like receptor 4 (TLR4), a receptor for lipopolysaccharide, showed high susceptibility to food allergy [33], suggesting that signals dependent on innate immunity influence the allergic responses. Allergic TLR4-deficient mice showed Th2-biased responses in intestinal and systemic (e.g., spleen) compartments. This finding correlated with another finding that a defect in MyD88, an

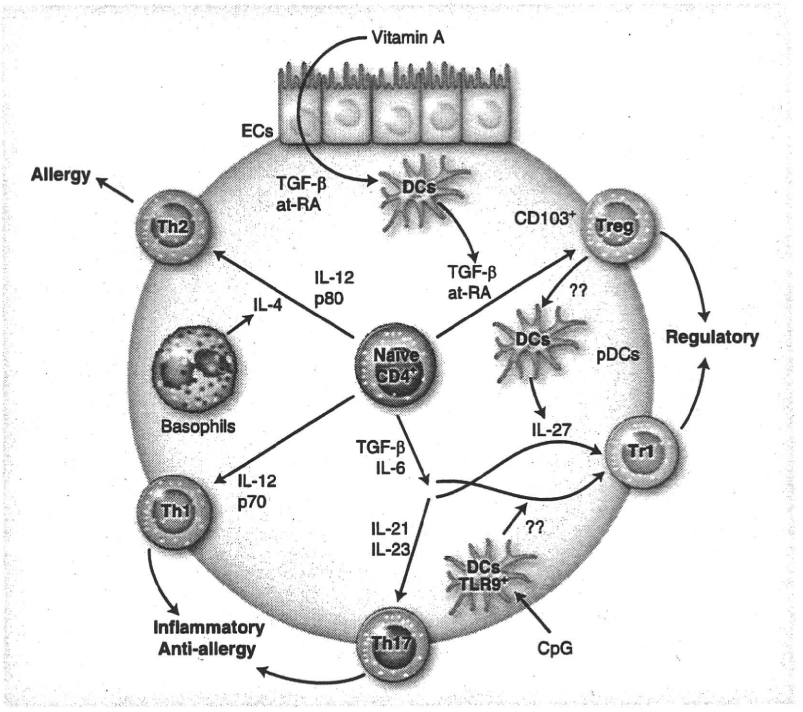

Fig. 2 Versatile pathways for the induction of regulatory and pathological T-cell network. Epithelial cells (ECs) produce transforming growth factor (TGF)-β and all-trans retinoic acid (at-RA), which make $CD103^+$ dendritic cells (DCs) tolerogenic. Naïve $CD4^+$ T cells activated by $CD103^+$ DCs differentiate into regulatory T cells (Tregs) also via at-RA and TGF-β. Tregs subsequently educate plasmacytoid DCs (pDCs) to produce interleukin (IL)-27, which is required for the induction of IL-10–producing Tr1 cells, another type of Treg. Tr1 cells are also induced by CpG-treated DCs. On the other hand, IL-23 and IL-12 p70 are involved in the induction of T-helper type 17 (Th17) and Th1 cells, respectively. Th2 cells, a major T-cell population in the development of allergic responses, require IL-4, which is predictably produced by basophils. TLR—Toll-like receptor

adopter molecule for many TLRs, moved the T-cell responses toward the Th2 type [34]. Reciprocally, stimulation with DNA-containing unmethylated CpG induces Th1-type immune responses via TLR9 [33]. In addition to Th1-type immune responses, a TLR9-mediated signal is a prerequisite for the efficient induction of regulatory-type T cells (e.g., Tregs and Tr1 cells). Indeed, oral administration of a TLR9 agonist inhibited the development of allergic responses to peanuts [33]. In this context, a recent study revealed a reciprocal relationship between retinoic acid and TLR9-mediated signals in the induction of Tregs [35••]. As mentioned previously, costimulation of CD4 T cells with at-RA enhances TGF-β–mediated FoxP3 expression; however, at-RA inhibits IL-10 induction [35••]. On the other hand, stimulation of DCs via TLR9 reduces FoxP3 expression and upregulates IL-10 induction in CD4 T cells (Fig. 2). Although the physiologic roles of the reciprocal regulation systems via at-RA and TLR9 in the development of food allergies are still unclear, these reports reveal a multilayered system involved in the negative regulation of antigen (or allergen)-specific immune responses in the harsh environment of the gastrointestinal tract.

In addition to hematopoietic cells (e.g., T cells and DCs), ECs also express various kinds of TLRs [36]. For instance, the tight junction between ECs is enhanced by a TLR2-mediated signal, indicating that bacterial stimulation is required for the first physical barrier to prevent the penetration of allergens as almost intact protein [37]. In addition to TLR2, TLR9 is a potential innate receptor in the regulation of EC function. TLR9 recognizes unmethylated CpG-containing bacterial DNA and is expressed on the apical and basolateral surfaces of ECs [36]. Intriguingly, TLR9 stimulation at the apical site activates nuclear factor-κB without the production of inflammatory cytokines, whereas basolateral stimulation of TLR9 results in the robust production of inflammatory cytokines [38].

In line with the hygiene hypothesis, probiotic bacteria are used to prevent allergic diseases [39]. Although the precise mechanisms used by probiotics to prevent and treat allergies are not fully understood, several pathways are considered possible mechanisms. In addition to imposing a physical barrier to compete with pathogenic bacteria, probiotics directly stimulate the immune system to establish a regulatory network, particularly in the induction of inhibitory cytokines (e.g., IL-10) [40]. Furthermore, probiotics contribute indirectly to the regulation of the immune system by producing immunomodulatory molecules

through the consumption of foodstuffs. For instance, probiotic bacteria digest exogenous and endogenous materials (e.g., fibers and mucins), and the broken down products affect the host immune system [40]. A recent study reported that short-chain fatty acids produced from fiber by commensal bacteria are required for the normal resolution of inflammatory responses through G-protein–coupled receptor 43 [41•].

Although many bacteria universally produce various TLR ligands (e.g., lipopolysaccharide and CpG-motif DNA) and consume dietary materials, not all bacteria can establish regulatory networks in the gastrointestinal tract. Instead, some commensal bacteria induce inflammatory cells. For instance, recent studies have shown that segmented filamentous bacteria preferentially induce Th17 cells, not Tregs [42, 43]. In line with these findings, it was reported that exogenous adenosine triphosphate derived from commensal bacteria induced Th17 cells [44]. *Lactobacillus* and *Bifidobacterium* are used in the probiotic treatment of allergic diseases on the basis that allergic patients have decreased counts of both [39]. However, among several species of each, only some strains have strong potential as probiotic bacteria. Therefore, the key functions that determine probiotic ability must be determined.

Dietary Materials and Milk in the Development of Food Allergy

The gastrointestinal tissues are vital for the digestion and absorption of nutrients. Because allergic diseases are prevalent in Westernized countries, interactions between dietary factors abundant in Western food and the gut immune system could be involved in the development of food allergies [1]. Among dietary factors, considerable evidence indicates that dietary lipids directly regulate allergic responses, especially omega-3 (e.g., linolenic acid) and omega-6 (e.g., linoleic acid) fatty acid [45]. Mammals must ingest both forms of these essential fatty acids. Some inflammatory lipid mediators (e.g., prostaglandins and leukotrienes) are derived from omega-6 fatty acids, whereas anti-inflammatory mediators (e.g., eicosapentaenoic acid and docosahexaenoic acid) are generated from linolenic acid. Thus, the balance between omega-6 and omega-3 fatty acids in dietary oils seems critical to the development of allergic diseases [45]. In support of this notion, clinical studies have shown that omega-3 dietary supplementation or frequent consumption of fish containing abundant omega-3 fatty acids decreases the risk of allergic diseases [46].

Our group showed an immunologic function of another lipid mediator, sphingosine 1-phosphate (S1P), in the development of food allergy [47]. S1P is generated from sphingomyelin and ceramide and regulates cell trafficking through interactions with its receptors [48]. On the basis of our findings on S1P function in the regulation of the gut immune system [49, 50], we suspect that cell trafficking of pathogenic cells (e.g., activated pathological T and mast cells) is also regulated by S1P. In fact, treatment of an experimental animal model with an S1P inhibitor resulted in the inhibition of allergic diarrhea, which is associated with decreased accumulation of pathogenic T and mast cells in the large intestine, without affecting serum IgE production [47]. Because it is possible that S1P precursors are present in dietary oils, these oils could be additional factors in the determination of allergic diseases.

Milk is the major dietary material for neonates. Previously, breast milk was thought to be responsible for the allergic responses in neonates as a source of allergens; however, several studies demonstrated that removing allergens from the diet during pregnancy and lactation did not prevent allergies [51]. On the other hand, recent evidence has revealed that breast milk contains molecules that induce tolerance, including IL-10, TGF-β, and immunoglobulins [51]. In agreement with this idea, mouse pups suckled by allergen-exposed mothers showed tolerance to those allergens [52••, 53]. A recent study showed that feeding of breast milk induced tolerance that was dependent on TGF-β but was not dependent on the transfer of immunoglobulins or IL-10 [52••]. The nucleus and biological nature of dietary materials, including lipids and milk, may provide us with new candidate regulatory molecule(s) that can mimic the mucosal Treg cell network system.

Conclusions

Progress in our understanding of immunologic tolerance and its abolition in the development of food allergies suggests several strategies against food allergies [54]. One is the re-education of the disordered gut immune system to induce oral tolerance. Although the prevention of food allergies still requires the prolonged elimination of the allergenic diet, several studies have already achieved immune therapy to prevent food allergy. Immunologic homeostasis between the host immune system and the gut environment is maintained by complex pathways. In particular, interactions among host immunocompetent cells (e.g., T cells, DCs, ECs, and basophils) and immunologic modification via dietary materials (e.g., vitamin A and short-chain fatty acids) and bacterial products (e.g., CpG and adenosine triphosphate) are critical events for the formation and maintenance of immunologic quiescence, and their dysregulation leads to the development of food allergies. Further studies of immunologic cross-talk with gut environments are needed to develop novel strategies for the prevention and treatment of food allergies.

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