厚生労働科学研究費補助金

食品の安全確保推進研究事業

肥満・脂質代謝を標的にした機能性健康食品の免疫学的機能・安全性評価

平成２２年度　総括研究報告書

研究代表者　國澤　純

平成２３（２０１１）年５月

目　次

I.　統括研究報告

肥満・脂質代謝を標的にした機能性健康食品の免疫学的機能・安全性評価

國澤　純 ----------1

II.　研究成果に関する一覧表 ----------7

III.　研究成果の刊行物・別刷（主要なもの） ----------9

I. 総括研究報告書

厚生労働科学研究費補助金（食品の安全確保推進研究事業）

総括研究報告書

肥満・脂質代謝を標的にした機能性健康食品の免疫学的機能・安全性評価

研究代表者： 國澤　純（東京大学医科学研究所　講師）

研究要旨： 現在、脂質代謝を標的にし、“やせる”“太らない”を謳った多くの機能性健康食品が開発されている。脂質は免疫制御において重要な役割を担っており、かつその異常が各種免疫疾患の発症ともつながっていることを考慮すると、脂質代謝を標的とした機能性健康食品が特に油の吸収部位である腸管免疫に影響を与え、その結果、各種免疫疾患につながる危険性が考えられる。本事業においては脂質代謝を標的とした機能性健康食品、特に機能性食用油の免疫学的機能・安全性を評価することを目的とする。初年度である 22 年度は、機能性食用油のうちヤシ科食用油とジアシルグリセロールに焦点を当て、これらの食用油を含む餌でマウスを飼育した際の腸管組織における基礎免疫機能について解析した。ヤシ科の食用油のうちパーム油に腸管免疫の活性化を示すこと、その責任脂肪酸がパルミチン酸であることを見いだした。またジアシルグリセロールはトリアシルグリセロールに比べ腸管免疫が活性化している傾向が認められた。

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

現在、“やせる”“太らない”を謳った機能性健康食品が開発されているが、その多くは脂質代謝を標的にしたものである。一方、脂質の代謝や吸収を司る腸管には多くの免疫担当細胞が存在し、生体の免疫学的防御と恒常性維持において重要な役割を担っている。申請者の研究を含む最近の研究結果から、食餌性脂質の量と質が腸管免疫の制御に深く関わっていること、ならびに腸管免疫を介した恒常性維持機構の破綻が各種炎症・アレルギー性疾患などの免疫疾患の発症につながることが示されている。これらを考え合わせると脂質代謝を標的とした機能性健康食品が腸管免疫に影響を与え、その結果、各種免疫疾患につながる危険性が考えられる。しかしながら、食の安全性が危惧されている現在においても、脂質代謝を標的とした機能性健康食品と各種免疫疾患との関連に関する実験的検証はほとんど行われていないのが現状である。本研究においては、研究代表者がこれまで行ってきた脂質を介した腸管免疫の制御と各種免疫疾患との関連に関する研究から得られた知的・技術基盤を発展させ、免疫学的手法やイメージングシステムを含む組織学

的解析、脂質組成解析などの生化学的手法を駆使することで、脂質代謝を標的にした機能性健康食品、特に機能性食用油の免疫学的機能・安全性を評価することを目的とする。

本事業の初年度である22年度は、機能性食用油のうちヤシ科食用油とジアシルグリセロールに焦点を当て、これらの食用油を含む餌でマウスを飼育した際の腸管組織における基礎免疫機能について解析した。

B. 研究方法

各種機能性食用油を重量比で4%含む特殊餌を作製し、その餌で2ヶ月間飼育したマウスにおける定常状態での腸管免疫応答を解析した。以下に本実験で用いた食用油を示す。

1. ヤシ科食用油（ヤシ油、パーム油）、コントロールとして大豆油を用いる

2. ジアシルグリセロールを90%、トリアシルグリセロールを10%含む油をジアシルグリセロールとして用いた。コントロールとしてトリアシルグリセロールで98%構成されている油を用いた。どちらの油もパルミチン酸（3-5%）、ステアリン酸（1-2%）、オレイン酸（35-37%）、リノール酸（47-49%）、リノレン酸（8-9%）から構成される。

これらのマウスにおいて、以下の項目を解析した。

1. 腸管IgAの産生を糞便中のIgA量をELISA法にて測定することで評価した。

2. IgA産生形質細胞をIgAとCD138を指標にしたフローサイトメトリーにて検出した。

3. T細胞からのサイトカイン産生を測定するためにin vitroで抗CD3抗体を用い刺激した際に培養上清中に産生されるサイトカインをCBA kit (BD Bioscience)により測定した。

（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. ヤシ科食用油

ヤシ科の食用油の免疫学的作用を明らかにする目的で、通常の大豆油の代わりにヤシ油、もしくはパーム油を4%含む餌を作製し、その餌で2ヶ月間飼育したマウスの糞便中IgA産生量を測定した。その結果、燃焼しやすいために体に脂肪がつきにくいとされている短鎖脂肪酸を特異的に多く含むヤシ油から構成される特殊餌で飼育したマウスにおいては、2ヶ月間飼育後もIgAの産生量に変化が認められなかった。一方で同じヤシ科であるパーム油においては腸管免疫の活性化が観察され、IgAの産生が約2倍に増加していた。

パーム油の特徴としてパルミチン酸が多く含まれるという性質に着目し、大豆油にパルミチン酸をパーム油に同程度となるよう加え、その油で作製した餌で飼育したマウスにおいて、パーム油と同様のIgAの産生増加が認められた。これらの結果から、パーム油が有するIgA産生の増強を担う責任脂肪酸の一つががパルミチン酸であるこ

とが示唆された。

パルミチン酸による IgA 産生増強機序を明らかにする目的で IgA 産生形質細胞をフローサイトメトリーにより検出したところ、小腸では大きな変化が認められなかったが、大腸において IgA 産生細胞数の増加が認められた。一方、大腸組織から回収した T 細胞のサイトカイン産生については、測定した IFN-g, IL-4, 5, 6, 10, 17 のいずれにおいても大きな変化が認められなかった。

2. ジアシルグリセロール

腸管内においてグリセロールに代謝されることで吸収されにくいとされるジアシルグリセロールを用いて飼育したマウスにおいては、コントロールであるトリアシルグリセロールに比べ体重の増加が 1 割程度抑えられていた。これらのマウスの IgA 産生を測定したところ、有意な差ではないものの IgA 産生の増強が認められた。また IgA 産生形質細胞をフローサイトメトリーにより検出したところ、パルミチン酸を加えた餌の場合と同様、小腸では大きな変化が認められなかったが、大腸において IgA 産生細胞数の増加が認められた。一方、大腸組織から回収した T 細胞のサイトカイン産生については、測定した IFN-γ, IL-4, 5, 6, 17 では大きな変化が認められなかったが、抑制型サイトカインとして知られている IL-10 の産生抑制が認められた。

D. 考察

初年度である 22 年度においては、ヤシ科食用油とジアシルグリセロールに注目した研究を中心に行った。ヤシ科食用油は近年東南アジアを中心に輸入が増加している油であり、日本国民においても使用量が増えていると考えられる。今回の検討から腸管免疫の活性化を示すことが判明したパーム油は菜種油、大豆油に次いで日本国内で使用されている油であり、近年増加している免疫疾患との関連も含め検討が必要であると考える。また今回の検討から腸管免疫の活性化を担う責任脂肪酸として同定したパルミチン酸は Toll 様受容体 4 などの自然免疫受容体のリガンドとなることで炎症を誘導することが最近報告されている。これらを考えると食餌性パルミチン酸の免疫学的作用と炎症性疾患の関連は重要な検討課題だと思われる。

一方、パーム油と同じヤシ科に属するヤシ油はパーム油とは異なり、カプリル酸、カプリン酸、ラウリン酸、ミリスチン酸などの中鎖脂肪酸を多く含む。これは日本国内において頻用されている菜種油や大豆油には全く含まれない脂肪酸であるため、その免疫学的作用が注目されたが、少なくとも 2 ヶ月間の投与では免疫学的機能に違いは認められなかった。今後はヤシ油を長期間摂取した場合の影響や免疫疾患モデルに適用した際の影響が重要な検討課題だと思われる。

またもう一つの機能性食用油であるジアシルグリセロールもパーム油と同様、特に大腸の免疫系を活性化することで IgA 産生を増強することが示された。興味深いこと

にその作用点はパーム油（パルミチン酸）と異なっており、抑制型サイトカインである IL-10 の産生阻害にあると思われる。この作用機序については今後の課題であるが、IL-10 の産生抑制は各種アレルギー炎症性疾患の発症リスクとなり得ることから、今後はパーム油の検討と同様、免疫疾患との関連を交えた研究が必要になると思われる。

これら本年度に得られた知見は、"脂質と腸管免疫"といった新視点から機能性健康食品の安全性に関する情報提供となる。これは国民の健康維持に直結する食品安全行政にとって重要な知見となると共に、これらの研究から得られる知見を応用することで、各種免疫疾患のリスク低減による国民の保健医療、ならびに免疫疾患等の患者数減少による医療経済の改善が期待され、今後さらに免疫疾患モデル等を用いた検討を進めることで、厚生労働行政に貢献できると期待される。

F. 健康危機情報

なし

G. 研究発表

1. 論文発表

J. Kunisawa and H. Kiyono, Aberrant interaction of gut immune system with environmental factors in the development of food allergy. ***Curr Aller Asthma Rep*** 10: 215-221, 2010

T. Obata, Y. Goto, J. Kunisawa, S. Sato, M. Sakamoto, H. Setoyama, T. Matsuki, K. Nonaka, N. Shibata, M. Gohda, Y. Kagiyama, T. Nochi, Y. Yuki, Y. Fukuyama, A. Mukai, S. Shinzaki, K. Fujihashi, C. Sasakawa, H. Iijima, M. Goto, Y. Umesaki, Y. Benno, and H. Kiyono, Indigenous opportunistic bacteria inhabit mammalian gut-associated lymphoid tissues and share a mucosal antibody-mediated symbiosis. ***Proc Natl Acad Sci USA*** 107:7419-24, 2010

J. Kunisawa, and H. Kiyono, Analysis of intestinal T cell populations and cytokine productions. ***Methods in Microbiology*** (Edited by Stefan H.E. Kaufmann and Dieter Kabelitz). Academic Press, Oxford, pp. 183-193, 2010

J. Kunisawa and H. Kiyono, Peaceful mutualism in the gut: Revealing key commensal bacteria for the creation and maintenance of immunological homeostasis. ***Cell Host Microbe*** 9: 83-84, 2011

D. Y., Kim, A. Sato, S. Fukuyama, H. Sagara, T. Nagatake, I. G. Kong, K. Goda, T. Nochi, J. Kunisawa, S. Sato, Y. Yokota, C. H. Lee, and H. Kiyono, The airway antigen sampling system: respiratory M cells as an alternative gateway for inhaled antigens. ***J Immunol*** 186: 4253-62,2011

J. Kunisawa*, Y. Kurashima, and H. Kiyono, Gut-associated lymphoid tissues for the development of oral vaccines. ***Adv Drug Deliv Rev*** (2011, in press)

國澤　純　粘膜免疫の新展開－生体最前線における腸内環境との調和と排除－　**無菌生物** 40, 25-28, 2010

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

倉島洋介、網谷岳朗、國澤　純、清野　宏　食物アレルギーの予防および治療的戦略の確立に向けた粘膜免疫研究の展開　**アレルギー免疫** 18: 66-77, 2011

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

2. 学会発表

國澤　純　粘膜組織における免疫学的普遍性と特殊性　**第 10 回鎌倉カンファレンス**（特別講演、2010 年 4 月、横浜）

Jun Kunisawa and Hiroshi Kiyono, MyD88 mediates intestinal IgA production in the maintenance of appropriate composition of commensal bacteria, **The 2nd International Conference on Modern Mucosal Vaccine, Adjuvants & Micrbicides**（2010 年 4 月、Dublin, Ireland）

Jun Kunisawa and Hiroshi Kiyono, Vitamin B6-Mediated Sphingosine 1-Phosphate Metabolism in the Immunological Homeostasis in the Gut, **ISSFAL2010**（2010 年 5 月、Maastricht, Netherland）

國澤　純　次世代ワクチンとしての粘膜免疫と DDS　**第 26 回日本 DDS 学会**（招待講演、2010 年 6 月、大阪）

Jun Kunisawa et al, MyD88-mediated high-IgA-secreting plasma cells for the effective mucosal immunity, **14th International Congress of Immunology**（2010 年 8 月、Kobe, Japan）

Yosuke Kurashima et al, Amelioration of gut inflammation by the regulation of P2X7 purinoceptor-mediated mast cell activation, **14th International Congress of Immunology**（2010 年 8 月、Kobe, Japan）

Naoko Shibata et al, Double negative dendritic cells retain naïve T cells in the interfollicular region of Peyer's patches, **14th International Congress of Immunology**（2010 年 8 月、Kobe, Japan）

Satoshi Tanaka et al, Development of functional human myeloid subsets in NOD/SCID/IL2rγKO mice, **14th International Congress of Immunology**（2010 年 8 月、Kobe, Japan）

Jun Kunisawa et al, New trend for oral vaccine development: Control of vitamin B9-mediated regulatory T cell function to enhance the vaccine antigen-specific IgA antibody responses, **4th Vaccine and ISV Annual Global Congress**（2010 年 10 月、

Vienna, Austria)

Jun Kunisawa, Regulation of IgA antibody responses by immunological crosstalk with intestinal environmental factors, **第5回千葉大学 G-COE シンポジウム**（招待講演、2010年12月、東京）

Jun Kunisawa, Immunological function of sphingosine 1-phosphate in the regulation of innate and acquired phases of intestinal IgA responses, **第33回日本分子生物学会年会・第83回日本生化学会大会 合同大会（BMB2010）**（招待講演、2010年12月、神戸）

Jun Kunisawa et al, The Unique pathway in the development and maintenance of mucosa-associated lymphoid tissues, **2nd Workshop of Synthetic Immunology**（招待講演、2010年12月、京都）

Jun Kunisawa, The uniqueness of mucosa-associated lymphoid tissues for the development of mucosal vaccine, **BIT Life Sciences' 3rd World Congress of Vaccine**（招待講演、2011年3月、北京、中国）

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
J. Kunisawa, and H. Kiyono	Populations and Cytokine Productions	Edited by Stefan H. E. Kaufmann and Dieter Kabelitz	Methods in Microbiology	Academic Press	Boston	2010	183-193

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
J. Kunisawa and H. Kiyono	Aberrant interaction of gut immune system with environmental factors in the development of food allergy	Curr Aller Asthma Rep	10	215-221	2010
T. Obata, Y. Goto, J. Kunisawa, S. Sato, M. Sakamoto, H. Setoyama, T. Matsuki, K. Nonaka, N. Shibata, M. Gohda, Y. Kagiyama, T. Nochi, Y. Yuki, Y. Fukuyama, A. Mukai, S. Shinzaki, K. Fujihashi, C. Sasakawa, H. Iijima, M. Goto, Y. Umesaki, Y. Benno, and H. Kiyono	Indigenous opportunistic bacteria inhabit mammalian gut-associated lymphoid tissues and share a mucosal antibody-mediated symbiosis	Proc Natl Acad Sci USA	107	7419-7424	2010
J. Kunisawa, and H. Kiyono	Peaceful mutualism in the gut: Revealing key commensal bacteria for the creation and maintenance of immunological homeostasis	Cell Host Microbe	9	83-84	2011
D. Y., Kim, A. Sato, S. Fukuyama, H. Sagara, T. Nagatake, I. G. Kong, K. Goda, T. Nochi, J. Kunisawa, S. Sato, Y. Yokota, C. H. Lee, and H. Kiyono	The airway antigen sampling system: respiratory M cells as an alternative gateway for inhaled antigens	J Immunol	186	4253-4263	2011
J. Kunisawa, Y. Kurashima, and H. Kiyono	Gut-associated lymphoid tissues for the development of oral vaccines	Adv Drug Deliv Rev			In press
國澤　純	粘膜免疫の新展開－生体最前線における腸内環境との調和と排除－	無菌生物	40	25-28	2010
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

倉島洋介、網谷岳朗、 國澤　純、清野　宏	食物アレルギーの予防および治療的戦略の確立に向けた粘膜免疫研究の展開	アレルギー免疫	18	66-77	2011
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

Published online: 6 April 2010

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

J. Kunisawa · H. Kiyono (✉)
Division of Mucosal Immunology, Institute of Medical Science,
The University of Tokyo,
Tokyo 108-8639, Japan
e-mail: kiyono@ims.u-tokyo.ac.jp

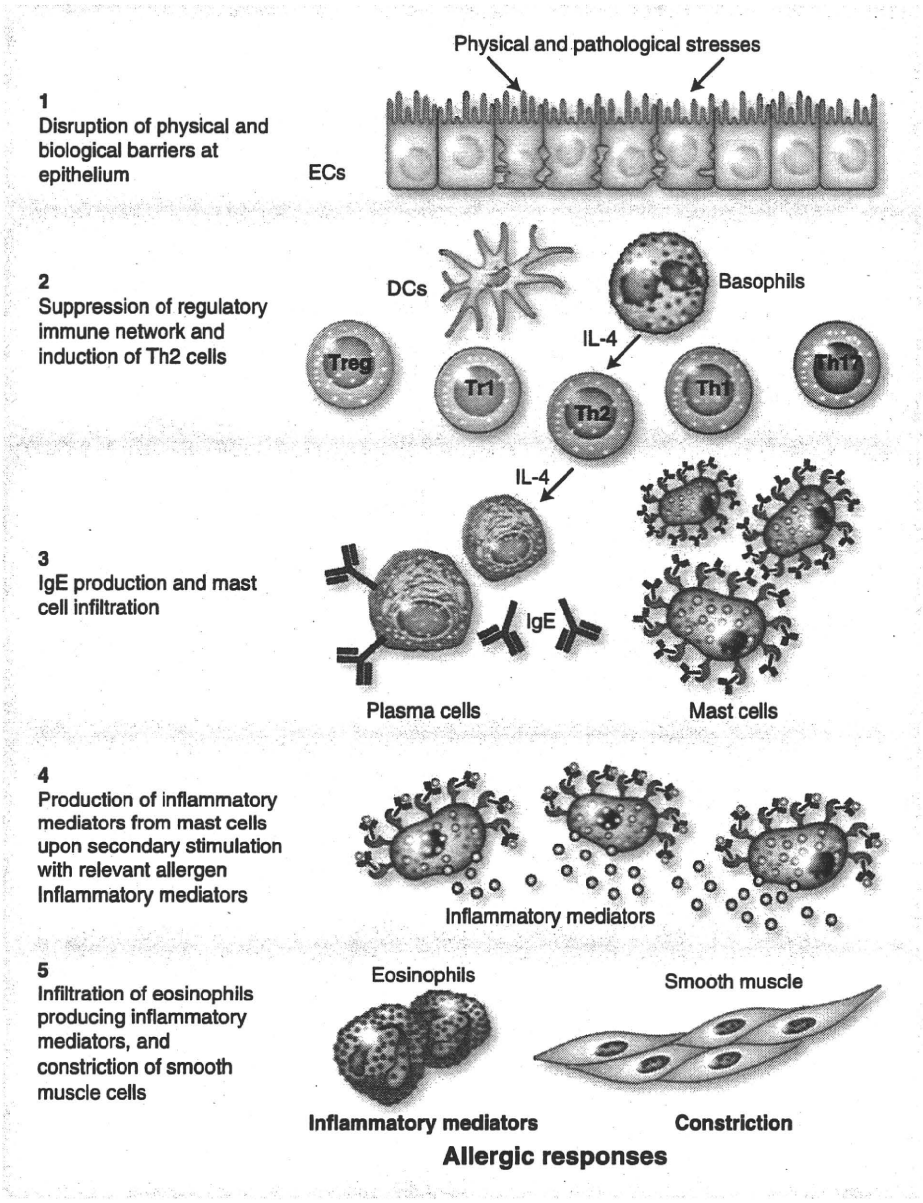

Fig. 1 Multiple steps in the development of allergic responses. **1**, Several stresses, including psychological, bacterial, and cytokine stimulation, disrupt the epithelial barrier, permitting the penetration of allergens. **2**, The immunologic environment mediated by dendritic cells (DCs) and presumably 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 CD8αα 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

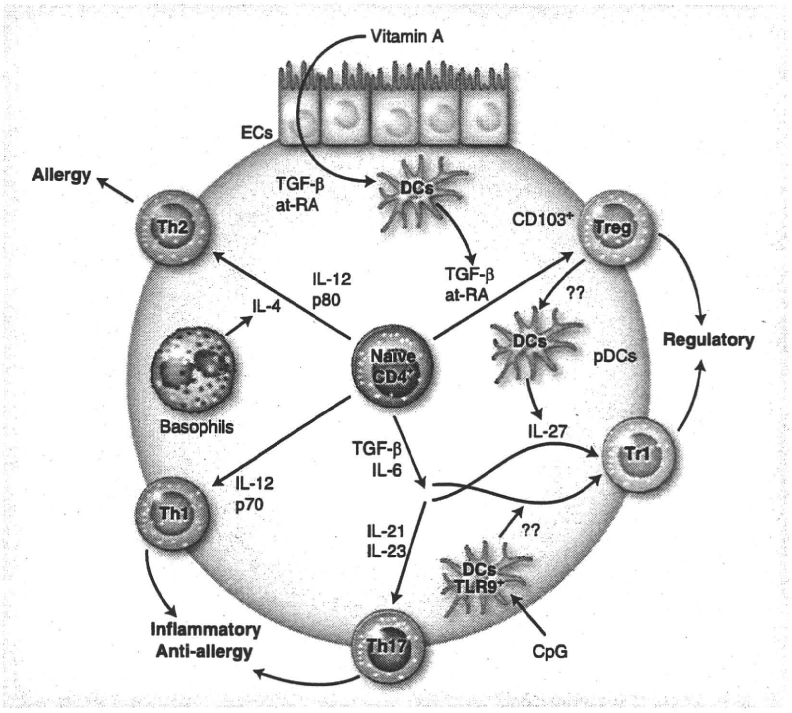

Fig. 2 Versatile pathways for the induction of regulatory and pathological T-cell network. Epithelial cells (ECs) produce transforming growth factor (TGF)-β and all-trans retinoic acid (at-RA), which make $CD103^+$ dendritic cells (DCs) tolerogenic. Naïve $CD4^+$ T cells activated by $CD103^+$ DCs differentiate 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.

Disclosure No potential conflicts of interest relevant to this article were reported.

References

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Devereux G: The increase in the prevalence of asthma and allergy: food for thought. Nat Rev Immunol 2006, 6:869–874.
2. Theoharides TC, Kempuraj D, Tagen M, et al.: Differential release of mast cell mediators and the pathogenesis of inflammation. Immunol Rev 2007, 217:65–78.
3. Kweon MN, Kiyono H: Eosinophilic gastroenteritis: a problem of the mucosal immune system? Curr Allergy Asthma Rep 2003, 3:79–85.
4. Chehade M, Mayer L: Oral tolerance and its relation to food hypersensitivities. J Allergy Clin Immunol 2005, 115:3–12; quiz 13.
5. Faria AM, Weiner HL: Oral tolerance. Immunol Rev 2005, 206:232–259.
6. • Berin MC, Mayer L: Immunophysiology of experimental food allergy. Mucosal Immunol 2009, 2:24–32. *This review nicely summarizes the immunologic features of an updated experimental food allergy model.*
7. Romagnani S: Coming back to a missing immune deviation as the main explanatory mechanism for the hygiene hypothesis. J Allergy Clin Immunol 2007, 119:1511–1513.
8. Wannemuehler MJ, Kiyono H, Babb JL, et al.: Lipopolysaccharide (LPS) regulation of the immune response: LPS converts germfree mice to sensitivity to oral tolerance induction. J Immunol 1982, 129:959–965.
9. Iweala OI, Nagler CR: Immune privilege in the gut: the establishment and maintenance of non-responsiveness to dietary antigens and commensal flora. Immunol Rev 2006, 213:82–100.
10. Kiyono H, Kunisawa J, McGhee JR, et al.: The mucosal immune system. In Fundamental Immunology. Edited by Paul WE. Philadelphia, PA: Lippincott-Raven; 2008:983-1030.
11. Yang PC, Jury J, Soderholm JD, et al.: Chronic psychological stress in rats induces intestinal sensitization to luminal antigens. Am J Pathol 2006, 168:104–114; quiz 363.
12. Yamaguchi N, Sugita R, Miki A, et al.: Gastrointestinal Candida colonisation promotes sensitisation against food antigens by affecting the mucosal barrier in mice. Gut 2006, 55:954–960.
13. •• Forbes EE, Groschwitz K, Abonia JP, et al.: IL-9- and mast cell-mediated intestinal permeability predisposes to oral antigen hypersensitivity. J Exp Med 2008, 205:897–913. *This article shows that IL-9 plays a major role in the mast cell–mediated increase of epithelial permeability.*
14. Romagnani S: Regulation of the development of type 2 T-helper cells in allergy. Curr Opin Immunol 1994, 6:838–846.
15. Hino A, Kweon MN, Fujihashi K, et al.: Pathological role of large intestinal IL-12p40 for the induction of Th2-type allergic diarrhea. Am J Pathol 2004, 164:1327–1335.
16. •• Perrigoue JG, Saenz SA, Siracusa MC, et al.: MHC class II-dependent basophil-CD4+ T cell interactions promote Th2 cytokine-dependent immunity. Nat Immunol 2009, 10: 697–705. *Together with those by Yoshimoto et al.* [17] *and Sokol et al.* [18], *this study demonstrates the importance of basophils as antigen-presenting cells in the induction of allergic responses.*
17. •• Yoshimoto T, Yasuda K, Tanaka H, et al.: Basophils contribute to Th2-IgE responses in vivo via IL-4 production and presentation of peptide-MHC class II complexes to CD4+ T cells. Nat Immunol 2009, 10:706–712. *Refer annotations on reference 16.*
18. •• Sokol CL, Chu NQ, Yu S, et al.: Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. Nat Immunol 2009, 10:713–720. *Refer annotations on reference 16.*
19. Saurer L, Mueller C: T cell-mediated immunoregulation in the gastrointestinal tract. Allergy 2009, 64:505–519.
20. Strober W: Vitamin A rewrites the ABCs of oral tolerance. Mucosal Immunol 2008, 1:92–95.
21. Takayama N, Igarashi O, Kweon MN, et al.: Regulatory role of Peyer's patches for the inhibition of OVA-induced allergic diarrhea. Clin Immunol 2007, 123:199–208.
22. • Shreffler WG, Wanich N, Moloney M, et al.: Association of allergen-specific regulatory T cells with the onset of clinical tolerance to milk protein. J Allergy Clin Immunol 2009, 123:43–52. *This article describes the clinical involvement of allergen-specific Tregs in tolerance to milk protein.*
23. Harrington LE, Hatton RD, Mangan PR, et al.: Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 2005, 6:1123–1132.
24. Park H, Li Z, Yang XO, et al.: A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol 2005, 6:1133–1141.
25. Schnyder-Candrian S, Togbe D, Couillin I, et al.: Interleukin-17 is a negative regulator of established allergic asthma. J Exp Med 2006, 203:2715–2725.
26. •• Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, et al.: A functionally specialized population of mucosal CD103+ DCs induces FoxP3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. J Exp Med 2007, 204:1757–1764. *Together with those by Sun et al.* [27] *and Benson et al.* [28], *this study indicates that gut DCs generate retinoic acid from vitamin A, which induces de novo differentiation of Tregs and simultaneously inhibits Th17 cell induction.*
27. •• Sun CM, Hall JA, Blank RB, et al.: Small intestine lamina propria dendritic cells promote de novo generation of FoxP3 Treg cells via retinoic acid. J Exp Med 2007, 204:1775–1785. *Refer annotations on reference 26.*
28. •• Benson MJ, Pino-Lagos K, Rosemblatt M, et al.: All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. J Exp Med 2007, 204:1765–1774. *Refer annotations on reference 26.*
29. •• Mucida D, Park Y, Kim G, et al.: Reciprocal Th17 and regulatory T cell differentiation mediated by retinoic acid. Science 2007, 317:256–260. *Refer annotations on reference 26.*
30. • Iliev ID, Mileti E, Matteoli G, et al.: Intestinal epithelial cells promote colitis-protective regulatory T-cell differentiation through dendritic cell conditioning. Mucosal Immunol 2009, 2:340–350. *This article demonstrates immunologic cross-talk between ECs and DCs in the gut for the induction of Tregs.*
31. •• Awasthi A, Carrier Y, Peron JP, et al.: A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. Nat Immunol 2007, 8:1380–1389. *This article shows that IL-10–producing Tr1 cells are generated by IL-27.*
32. Edelman SM, Kasper DL: Symbiotic commensal bacteria direct maturation of the host immune system. Curr Opin Gastroenterol 2008, 24:720–724.
33. Bashir ME, Louie S, Shi HN, et al.: Toll-like receptor 4 signaling by intestinal microbes influences susceptibility to food allergy. J Immunol 2004, 172:6978–6987.
34. Schnare M, Barton GM, Holt AC, et al.: Toll-like receptors control activation of adaptive immune responses. Nat Immunol 2001, 2:947–950.

35. •• Maynard CL, Hatton RD, Helms WS, et al.: Contrasting roles for all-trans retinoic acid in TGF-beta-mediated induction of FoxP3 and IL10 genes in developing regulatory T cells. J Exp Med 2009, 206:343–357. *This article describes the contrasting relationship between retinoic acid– and TLR-mediated stimulation in the induction of Tregs and Tr1 cells.*
36. Lee J, Gonzales-Navajas JM, Raz E: The "polarizing-tolerizing" mechanism of intestinal epithelium: its relevance to colonic homeostasis. Semin Immunopathol 2008, 30:3–9.
37. Cario E, Gerken G, Podolsky DK: Toll-like receptor 2 controls mucosal inflammation by regulating epithelial barrier function. Gastroenterology 2007, 132:1359–1374.
38. Lee J, Mo JH, Katakura K, et al.: Maintenance of colonic homeostasis by distinctive apical TLR9 signalling in intestinal epithelial cells. Nat Cell Biol 2006, 8:1327–1336.
39. Trebichavsky I, Rada V, Splichalova A, et al.: Cross-talk of human gut with bifidobacteria. Nutr Rev 2009, 67:77–82.
40. Savilahti E, Kuitunen M, Vaarala O: Pre and probiotics in the prevention and treatment of food allergy. Curr Opin Allergy Clin Immunol 2008, 8:243–248.
41. • Maslowski KM, Vieira AT, Ng A, et al.: Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature 2009, 461:1282–1286. *This study provides a novel cross-talk pathway among diet, intestinal microbiota, and host immune system by showing that short-chain fatty acids produced by dietary fiber by intestinal microbiota regulate gut inflammation through the interaction with G-protein-coupled receptor 43.*
42. Ivanov II, Atarashi K, Manel N, et al.: Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 2009, 139:485–498.
43. Gaboriau-Routhiau V, Rakotobe S, Lecuyer E, et al.: The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. Immunity 2009, 31: 677–689.
44. Atarashi K, Nishimura J, Shima T, et al.: ATP drives lamina propria Th17 cell differentiation. Nature 2008, 455:808–812.
45. Heine RG, Tang ML: Dietary approaches to the prevention of food allergy. Curr Opin Clin Nutr Metab Care 2008, 11:320–328.
46. Hodge L, Salome CM, Peat JK, et al.: Consumption of oily fish and childhood asthma risk. Med J Aust 1996, 164:137–140.
47. Kurashima Y, Kunisawa J, Higuchi M, et al.: Sphingosine 1-phosphate-mediated trafficking of pathogenic Th2 and mast cells for the control of food allergy. J Immunol 2007, 179: 1577–1585.
48. Schwab SR, Cyster JG: Finding a way out: lymphocyte egress from lymphoid organs. Nat Immunol 2007, 8:1295–1301.
49. Kunisawa J, Kurashima Y, Gohda M, et al.: Sphingosine 1-phosphate regulates peritoneal B-cell trafficking for subsequent intestinal IgA production. Blood 2007, 109:3749–3756.
50. Kunisawa J, Kurashima Y, Higuchi M, et al.: Sphingosine 1-phosphate dependence in the regulation of lymphocyte trafficking to the gut epithelium. J Exp Med 2007, 204:2335–2348.
51. Greer FR, Sicherer SH, Burks AW: Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. Pediatrics 2008, 121:183–191.
52. •• Verhasselt V, Milcent V, Cazareth J, et al.: Breast milk-mediated transfer of an antigen induces tolerance and protection from allergic asthma. Nat Med 2008, 14:170–175. *This article shows the immunologic importance of breast milk in the induction of tolerance to dietary allergens in neonates.*
53. Matson AP, Zhu L, Lingenheld EG, et al.: Maternal transmission of resistance to development of allergic airway disease. J Immunol 2007, 179:1282–1291.
54. Broide DH: Immunomodulation of allergic disease. Annu Rev Med 2009, 60:279–291.