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Review

Mechanism for Maintaining Homeostasis in the Immune System of the Intestine

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Abstract. *Every organism possesses a mechanism for maintaining homeostasis. We have focused on the immune system as a system that helps maintain homeostasis of the body, and particularly on the intestine as the largest organ of immunity in the body. We have also focused our research on the mechanism that responds to foreign substances in the intestine, especially the toll-like receptors (TLR). The activation of myeloid differentiation marker (MyD88) signal transduction as a response to TLR in the intestine is believed to contribute to the maintenance of homeostasis of the body through the homeostasis of the intestine. Furthermore, significant findings were reported in which signal transduction from TLR4 was essential for the maintenance and regulation of the intestine. These results strongly suggest the possibility that homeostasis in the intestine is maintained by TLR4, and signaling by TLR4 after exposure to lipopolysaccharide (LPS) probably has a role in regulating homeostasis. It is expected that the prevention and treatment of various diseases using TLR4 will continue to develop. As LPS is a substance that enhances the activity of TLR4, it will also attract attention as a valuable substance in its own right.*

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The Immune System and Homeostasis

Every organism possesses a mechanism for maintaining homeostasis of the body. Homeostasis is the phenomenon by which an organism maintains the internal environment of the self, not as a closed system but through close interaction with the exterior world. The concept was proposed in 1929 by an American biologist, W.B. Cannon, as the general principle of life. This concept was developed from the idea of "maintenance of life by the homeostasis of interior environment" by C. Bernard (1854). Both the nervous system and endocrine system are well known to be parts of the mechanism that regulates homeostasis.

In addition, the immune system is a biophylactic system that protects an individual organism from invasion by foreign organisms such as bacteria. In other words, the immune system not only provides biophylaxis, but also functions as the system that maintains homeostasis. This is because it possesses the ability to respond to stimulation from the exterior environment. Barnet proposed the clonal selection theory in the book (cellular Immunology) and stated that "self defence that the process was initially concerned not with defence against infection but with the maintenance of cellular integrity of the body" (1). More recently, this concept has been

gradually clarified scientifically. For example, the secretion of preopiomelanocortin from the anterior lobe of the hypophysis in response to stress augments production of glucocorticoid and brings on potent immune suppression. These stress reactions promote infection. In addition, cytokines, in particular interleukin (IL)-1 and tumor necrosis factor (TNF), are endogenic pyrogenic substances that raise set points of body temperature. In this way, there is no doubt that molecules such as cytokines, for example, are closely associated with the nervous and endocrine systems in homeostasis.

The Intestine as an Immune Device

We have focused on the immune system as a system that helps maintain homeostasis of the body, and particularly on the intestine as the largest organ of immunity in the body. The intestine is not a simple tube. Embryologically, it is one of the oldest tissues and exists for digestion and adsorption of nutrients even in lower animals (2-4). It is also often described as being of both the 'inner and outside world' and is the organ that contacts most extensively with the exterior environment with surface area of 300 m^2 in humans. It has the longest contact with foreign substances such as viruses, microorganisms and antigens that are present in foods. Furthermore, it has been stated that the number of enterobacteria present in the intestine is about 10^{14}/ person and consists of 100 to 200 species (5, 6). While the intestine responds to the complicated task of recognizing self and non-self, it maintains homeostasis of the body without causing unnecessary responses by it (7, 8). The complex mechanism that maintains homeostasis is believed to depend on the immune system of the intestine. In fact, 60% of the immune cells in the body are said to be present in the intestine. Thus, the intestine is the largest organ of immunity in the body. It contains a variety of tissues that regulate the immune system (GALT), including Peyer's patches, mesenteric lymph nodes (MLN), the lamina propria, intraepithelial lymphocytes (IEL), and cryptopatches.

Whether or not the intestine maintains homeostasis through GALT has not been clarified. We have focused our research on the mechanism that responds to foreign substances in the intestine, especially that involving the toll-like receptors (TLR). These receptors recognize foreign substances and may be instrumental in helping to clarify the mechanism of homeostasis by the intestine and its role in immunity. TLRs belong to a family of pattern-recognition receptors (PRRs); these are present in macrophages of the lamina propria, dendritic cells, paneth cells in cryptopatches, and in intestinal and intestinal epithelial cells. Significant findings have recently been reported on the responses of the intestine to foreign substances through TLR signals, and these findings also suggest a relationship to homeostasis (7, 9-14).

Response to Foreign Substances by the Intestine

In a recent study on TLRs, Seth Rakoff-Nahoum *et al.* reported that the signal transmitted intracellularly by TLRs and MyD88 (myeloid differentiation marker), a signal transduction molecule of TLRs during the progression of tumors in the intestine, is essential for inducing the repair program of intestinal tissues (15). In an experiment with *IL-2*-knockout mice and in *IL-10*-knockout mice, animal models of inflammatory bowel disease exhibited a spontaneous onset of colitis. This indicated that IL-10 mediated a regulatory mechanism for homeostasis of the intestine after the recognition of microorganisms by MyD88 signal transduction-dependent TLRs (16). Thus, the activation of MyD88 signal transduction as a response to TLRs in the intestine is believed to contribute to the maintenance of homeostasis of the body through the homeostasis of the intestine (17-22).

Furthermore, significant findings were reported in which signal transduction from TLR4 was essential for the maintenance and regulation of the intestine (7, 22). It was reported that administration of dextran sodium sulfate (DSS) to *TLR2-, TLR4-* and *MyD88*-knockout mice aggravated ulcerative colitis (UC). In addition, administration of DSS after the elimination of enterobacteria within 4 weeks of administration of various antibiotics also aggravated UC. Conversely, by adding oral administration of LPS, a ligand of TLR4, when giving antibiotics, there was a suppression of the onset of UC (7).

These results support the concept that signals of TLR4 contribute to the maintenance of homeostasis in the intestine. This strongly suggests that enterobacteria play a role as a ligand to the receptors of foreign substances, such as TLR2 or TLR4, and contribute to homeostasis.

We studied the possibility that a component of such microorganisms were recognized by the TLRs because of the suppression of UC after TLR4 had come into contact with LPS. We focused on the relationship between TLR4 and LPS because as well as being a ligand of TLR4, LPS is also a cellular-wall component of Gram-negative bacteria and it is estimated that there are about 10^{11} of these bacteria present in the intestine.

TLR4 and LPS

LPS is an amphiphilic substance composed of a lipid portion called lipid A, which is a major cellular-wall component of Gram-negative bacteria, and several kinds of covalently-bonded sugars. A study on the bioactivity of LPS by Coley indicated that Gram-negative bacteria have an antitumor effect (23). Based on these findings, Coley accumulated much data on the antitumor effects by administering bacterial bodies or bacterial components to tumor patients (24). Currently, in the field of medical drugs, LPS is generally

considered to be an endotoxin. This is due to the fact that when LPS is administered intravenously, it takes only trace amounts (4 ng/kg of body weight) to induce cytokines and cause responses similar to sepsis (25). For this reason, contamination by LPS must be prevented during the intravenous administration of all medical drugs and products. However, Gram-negative bacteria are present not only in the soil and hydrosphere, but also persistently in the intestine, the oral cavity and on the skin (26).

These facts indicate that people normally live intimately with Gram-negative bacteria; they also consume LPS indirectly because certain bacterial species are used to produce particular foods (Table I). Of the substances known at present, LPS activates macrophages at a very low dose (about 100 pg/ml) (27). The mechanism of this activation is achieved by intracellular signal transduction by TLR4 (the main receptor of LPS) after forming a complex with myeloid differentiation protein 2 (MD2) and cluster of differentiation antigen 14 (CD14) (19-21, 28).

Recent findings have demonstrated that homeostasis of the body and the immune system balance is maintained by the transmission of signals related to TLR4 after contact with LPS (29-37). A new function was recently reported concerning the contact of LPS by TLR4. Methicillin-resistant *Staphylococcus aureus* (MRSA) is known to cause infections of patients in medical facilities, and this poses a major health problem. According to a 2007 report in the Journal of the American Medical Association (JAMA), the incidence of MRSA is 32 out of 100,000 cases, and the number of deaths has climbed to 19,000 (38). This is an important problem as the number of deaths is higher than for HIV, and 58% of the infections were observed to occur in medical facilities. Furthermore, bacterial strains have recently appeared that are resistant to vancomycin (an effective drug for MRSA). Thus, there is now a search for ways to suppress the appearance of resistant bacteria in medical facilities. Until now it has not been understood why MRSA or vancomycin-resistant *Enterococcus* (VRE) appeared after the administration of antibiotics but not in healthy individuals. Katharina Brandl *et al*. described the proliferative mechanism of VRE in 2008. According to this report, under normal conditions Reg-III-γ (lectin with antimicrobial activity from paneth cells) is secreted after stimulation with a component of enterobacteria, and this suppresses the proliferation of *Enterococcus* (39, 40). However, when the number of enterobacteria is reduced after administering antibiotics, the production of Reg-III-γ by paneth cells is suppressed, and *Enterococcus* proliferates (41). Furthermore, it was also noted that the production of Reg-III-γ was induced even in the presence of antibiotics if LPS derived from Gram-negative bacteria was also administered. This indicated that signals through TLR2 did not involve the induction of Reg-III-γ production by paneth cells (41). Surprisingly, another paper reported that *Bifidobacterium longum* and *Lactobacillus* (both Gram-positive bacteria used as probiotics) suppress Reg-III-γ, while *Bacteroides thetaiotaomicro* (a Gram-negative bacteria) and enterobacteria did not suppress Reg-III-γ (42).

Table I. *Species of Gram-negative bacteria used in food processing.*

Scientific name of bacteria	Name of food (producing district)
Acetobacter aceti	Vinegar (worldwide)
Zymomonas mobilis	Tequila (Mexico)
Xanthomonas campestris	Xanthan gum (worldwide)
Acetobacter xylinum	Nata de coco (Philippines)
Acetobacter orientalis	Caspian Sea yogurt (Caucasus)
Enterobacter cloacae	Sarapao (Thai)
Pantoea agglomerans	Fermented rye bread (Northern Europe)

Recently, there has been research on peptidoglycan derived from Gram-positive bacteria and β-1, 3-glucan derived from fungi (both ligands of TLR2). It was demonstrated that they have a role in maintaining homeostasis because of the intracellular signaling from TLR2 (43, 44). Many reports have discussed differences in the action between TLR2 and TLR4. These results strongly suggest the possibility that homeostasis in the intestine is maintained by TLR4, and signaling by TLR4 after exposure to LPS probably has a role in regulating homeostasis.

LPS in Food and in the Intestine

In 1991 while searching for macrophage-activating substances, we discovered a substance in a water extract of wheat flour that had a strong effect on the activation of macrophages. It was determined that the major component was LPS that had been derived from the cellular walls of a Gram-negative bacteria (*Pantoea agglomerans*, a bacterium symbiotic with wheat) (45). We named this LPS from IP-PA1. It was shown to provide a safe preventative or therapeutic effect after oral or percutaneous administration for various diseases including infectious diseases (30-37, 46). When people live in conditions with poor hygiene, they have a high intake of LPS from the environment. However, people living in hygienic conditions are exposed to LPS to a much lesser degree. Thus, it is believed that the influence of LPS on the body has decreased greatly as modernization of living conditions has increased.

LPS enters the body by oral or percutaneous means and is thought to be absorbed in the intestine. Currently, it is believed that it comes in contact with the TLR4 of immunity cells in the intestine. It has been demonstrated that LPS that has entered the body is present in the portal vein and liver (47), which indicates that LPS is absorbed in the intestine. From here onwards, the signals produced by LPS contact with TLR4 are suggested to contribute to maintenance of homeostasis.

Table II. *Comparison between the amount of LPS in the intestine and the dose of LPS by oral or percutaneous administration.*

	Number of bacteria	Amount of LPS released from of molecules	Relative quantity Gram-negative bacteria in intestines	Relative quantity
Intestinal bacteria	10^{14}			
Intestinal Gram-negative bacteria	10^{11}	0.1 mg	20,000 (*E. coli* LPS M.W.)	1
IP-PA1 by oral administration (20 μg/kg/day)		1.2 mg (human, BW 60 kg)	5,000 (IP-PA1 M.W.)	48

Prior to the current level of hygiene, humans ingested LPS from the environment. Now, however, people have fewer opportunities to come in contact with LPS, and the intake of LPS is thought to have decreased drastically (29, 48-50). From an extensive epidemiological investigation, Lauener *et al*. reported that there was a negative correlation between the degree of exposure to LPS and the frequency of the onset of asthma (51). After ingesting appropriate doses of LPS, humans are thought to maintain homeostasis partly by TLR4 signaling (52).

We determined from previous research that an effective dose of IP-PA1 is 10-20 μg/kg (body weight)/day. We compared the intake of LPS (IP-PA1) after oral administration (20 μg/kg body weight/day) to the amount of Gram-negative bacteria in the intestine (Table II). There are many species of Gram-positive bacteria in the intestine and they are a thousand times more abundant than Gram-negative bacteria. Thus, when the amount of Gram-negative bacteria in the intestine is converted to LPS, the weight is only 0.1 mg.

It is assumed that the molecular weight of LPS in the intestine is 20,000 Da (which is the molecular weight of *Escherichia coli* LPS). For the most part, Gram-negative bacteria in the intestine consist of *Bacteroides* and *Enterobacteriaceae*. By contrast, it can be assumed that the molecular weight of LPS (IP-PA1) that is taken in by oral administration is 5,000 Da. The intestine is the dominant location the intake of LPS as IP-PA1. It is believed that increasing the amount of the ligand as LPS enforces the contact with TLR4. Consequently contact between TLR4 and LPS that is taken in by oral administration, TLR4 signaling is activated more. Given the current level of hygiene, it is very important that TLR4 signaling be activated by ingesting LPS intentionally by oral or percutaneous administration.

Maintenance of Homeostasis by TLR4

To summarize the above results, it appears that homeostasis in the intestine is maintained by TLR4, and the signaling by TLR4 after exposure to LPS may regulate homeostasis of the whole body. Until recently, the ligands of TLR2 have received most attention; these are peptidoglycan derived from Gram-positive bacteria and a β-1, 3-glucan derived from fungi. However, when comparing the doses of the substances required to activate macrophages, the amount of ligand required for TLR4 signaling is of the order of Picograms while TLR2 signaling requires amounts of the order of micrograms (53). TLR4 promotes the formation of a receptor complex (CD14- MD2-TLR4) that recognizes ligands efficiently (19-21, 28) and activates macrophages. LPS is important because it is believed to be a ligand of TLR4.

Recently therapies that are based on innate immunity have received increasing amounts of attention. In particular, it has been reported that agonists of TLRs may be useful for the prevention and the treatment of tumors, allergies or viral infections. LPS is listed as the agonist of TLR4 (54). This suggests that LPS may be useful for preventing and treating various diseases. When used appropriately, LPS may be considered as a beneficial substance, not just as a toxic compound. In the future, it is expected that the prevention and treatment of various diseases using TLR4 will continue to develop. As LPS is a substance that enhances the activity of TLR4, it will also attract attention in its own right (26, 55-62).

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References

1 Burnet M: Cellular Immunology. Cambridge University Press 305, 1969.

2 Drasar BS: Human Intestinal Flora. London UK Academic Press, 1974.

3 Simon GL and Gorbach SL: Intestinal flora in health and disease. Gastroenterology *86*: 174-193, 1984.

4 Cummings JH, Antoine JM, Azpiroz F, Bourdet-Sicard R, Brandtzaeg P, Calder PC, Gibson GR, Guarner F, Isolauri E, Pannemans D, Shortt C, Tuijtelaars S and Watzl B: PASSCLAIM-gut health and immunity. Eur J Nutr *43(Suppl 2)*: II118-II173, 2004.

5 Cummings JH and Macfarlane GT: The control and consequences of bacterial fermentation in the human colon. J Appl Bacteriol *70*: 443-459, 1991.

6 Gibson GR and Roberfroid MB: Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr *125*: 1401-1412, 1995.
7 Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S and Medzhitov R: Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell *118*: 229-241, 2004.
8 Cebra JJ: Influences of microbiota on intestinal immune system development. Am J Clin Nutr *69*: 1046S-1051S, 1999.
9 Qureshi ST and Medzhitov R: Toll-like receptors and their role in experimental models of microbial infection. Genes Immun *4*: 87-94, 2003.
10 Kopp E and Medzhitov R: Recognition of microbial infection by toll-like receptors. Curr Opin Immunol *15*: 396-401, 2003.
11 Rakoff-Nahoum S and Medzhitov R: Role of the innate immune system and host-commensal mutualism. Curr Top Microbiol Immunol *308*: 1-18, 2006.
12 Harris G, KuoLee R and Chen W: Role of toll-like receptors in health and diseases of gastrointestinal tract. World J Gastroenterol *12*: 2149-2160, 2006.
13 Kabelitz D and Medzhitov R: Innate immunity-cross-talk with adaptive immunity through pattern recognition receptors and cytokines. Curr Opin Immunol *19*: 1-3, 2007.
14 Kelly D, Conway S and Aminov R: Commensal gut bacteria: mechanisms of immune modulation. Trends Immunol *26*: 326-333, 2005.
15 Rakoff-Nahoum S and Medzhitov R: Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. Science *317*: 124-127, 2007.
16 Rakoff-Nahoum S, Hao L and Medzhitov R: Role of toll-like receptors in spontaneous commensal-dependent colitis. Immunity *25*: 319-329, 2006.
17 Pull SL, Doherty JM, Mills JC, Gordon JI and Stappenbeck TS: Activated macrophages are an adaptive element of the colonic epithelial progenitor niche necessary for regenerative responses to injury. Proc Natl Acad Sci USA *102*: 99-104, 2005.
18 Jiang D, Liang J, Fan J, Yu S, Chen S, Luo Y, Prestwich GD, Mascarenhas MM, Garg HG, Quinn DA, Homer RJ, Goldstein DR, Bucala R, Lee PJ, Medzhitov R and Noble PW: Regulation of lung injury and repair by toll-like receptors and hyaluronan. Nat Med *11*: 1173-1179, 2005.
19 Takeda K, Kaisho T and Akira S: Toll-like receptors. Annu Rev Immunol *21*: 335-376, 2003.
20 Takeda K and Akira S: Toll receptors and pathogen resistance. Cell Microbiol 5: 143-153, 2003.
21 Takeda K and Akira S: Toll-like receptors in innate immunity. Int Immunol *17*: 1-14, 2005.
22 Bashir ME, Louie S, Shi HN and Nagler-Anderson C: Toll-like receptor 4 signaling by intestinal microbes influences susceptibility to food allergy. J Immunol *172*: 6978-6987, 2004.
23 Coley WB: Practitioner *83*: 589-613, 1909.
24 Nauts HC, Fowber GA and Bogatko FH: Acta Medica Scand *45*: 1-103, 1953.
25 Engelhardt R, Mackensen A and Galanos C: Phase I trial of intravenously administered endotoxin (*Salmonella abortus equi*) in cancer patients. Cancer Res *51*: 2524-2530, 1991.
26 Marshall JC: Lipopolysaccharide: an endotoxin or an exogenous hormone? Clin Infect Dis *41*: 470-480, 2005.
27 Raetz CR: Biochemistry of endotoxins. Annu Rev Biochem *59*: 129-170, 1990.
28 Akira S and Takeda K: Toll-like receptor signalling. Nat Rev Immunol *4*: 499-511, 2004.
29 Gehring U, Strikwold M, Schram-Bijkerk D, Weinmayr G, Genuneit J, Nagel G, Wickens K, Siebers R, Crane J, Doekes G, Di Domenicantonio R, Nilsson L, Priftanji A, Sandin A, El-Sharif N, Strachan D, van Hage M, von Mutius E and Brunekreef B: Asthma and allergic symptoms in relation to house dust endotoxin: Phase two of the International Study on Asthma and Allergies in Childhood (ISAAC II). Clin Exp Allergy *38*: 1911-1920, 2008.
30 Inagawa H, Nishizawa T, Yoshioka N, Taniguchi Y, Kohchi C and Soma GI: Preventative and therapeutic potential of lipopolysaccharide derived from edible Gram-negative bacteria to various diseases. Current Drug Therapy *3*: 26-32, 2008.
31 Okutomi T, Nishizawa T, Inagawa H, Takano T, Morikawa A, Soma GI and Mizuno D: Homeostasis as regulated by activated macrophage. VII. Suppression of serum cholesterol level by LPSw (a lipopolysaccharide from wheat flour) in WHHL (Watanabe heritable hyperlipidemic) rabbit. Chem Pharm Bull (Tokyo) *40*: 1268-1270, 1992.
32 Inagawa H, Saitoh F, Iguchi M, Nishizawa T, Okutomi T, Morikawa A, Soma GI and Mizuno D: Homeostasis as regulated by activated macrophage. III. Protective effect of LPSw (lipopolysaccharide (LPS) of wheat flour) on gastric ulcer in mice as compared with those of other LPS from various sources. Chem Pharm Bull (Tokyo) *40*: 998-1000, 1992.
33 Iguchi M, Inagawa H, Nishizawa T, Okutomi T, Morikawa A, Soma GI and Mizuno D: Homeostasis as regulated by activated macrophage. V. Suppression of diabetes mellitus in non-obese diabetic mice by LPSw (a lipopolysaccharide from wheat flour). Chem Pharm Bull (Tokyo) *40*: 1004-1006, 1992.
34 Inagawa H, Nishizawa T, Noguchi K, Minamimura M, Takagi K, Goto S, Soma GI and Mizuno D: Anti - tumor effect of lipopolysaccharide by intradermal administration as a novel drug delivery system. Anticancer Res *17*: 2153-2158, 1997.
35 Goto S, Sakai S, Kera J, Suma Y, Soma GI and Takeuchi S: Intradermal administration of lipopolysaccharide in treatment of human cancer. Cancer Immunol Immunother *42*: 255-261, 1996.
36 Pollard JW: Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer *4*: 71-78, 2004.
37 Ohno S, Inagawa H, Dhar DK, Fujii T, Ueda S, Tachibana M, Ohno Y, Suzuki N, Inoue M, Soma GI and Nagasue N: Role of tumor-associated macrophages (TAM) in advanced gastric carcinoma: the impact on FasL-mediated counterattack. Anticancer Res *25*: 463-470, 2005.
38 Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison HL, Lynfield R, Dumyati G, Townes JM, Craig AS, Zell ER, Fosheim GE, McDougal LK, Carey RB and Fridkin SK: Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA *298*: 1763-1771, 2007.
39 Cash HL, Whitham CV, Behrendt CL and Hooper LV: Symbiotic bacteria direct expression of an intestinal bactericidal lectin. Science *313*: 1126-1130, 2006.
40 Brandl K, Plitas G, Schnabl B, DeMatteo RP and Pamer EG: MyD88-mediated signals induce the bactericidal lectin RegIII gamma and protect mice against intestinal *Listeria monocytogenes* infection. J Exp Med *204*: 1891-1900, 2007.
41 Brandl K, Plitas G, Mihu CN, Ubeda C, Jia T, Fleisher M, Schnabl B, DeMatteo RP, Pamer EG: Vancomycin – resistant

enterococci exploit antibiotic-induced innate immune deficits. Nature *455*: 804-807, 2008.

42 Sonnenburg JL, Chen CT and Gordon JI: Genomic and metabolic studies of the impact of probiotics on a model gut symbiont and host. PLoS Biol *4*: 413, 2006.

43 Brodsky I and Medzhitov R: Two modes of ligand recognition by TLRs. Cell *130*: 979-981, 2007.

44 Dogi CA, Galdeano CM and Perdigon G: Gut immune stimulation by non pathogenic Gram (+) and Gram (–) bacteria. Comparison with a probiotic strain. Cytokine *41*: 223-231, 2008.

45 Nishizawa T, Inagawa H, Oshima H, Okutomi T, Tsukioka D, Iguchi M and Soma GI and Mizuno D: Homeostasis as regulated by activated macrophage. I. Lipopolysaccharide (LPS) from wheat flour: isolation, purification and some biological activities. Chem Pharm Bull (Tokyo) *40*: 479-483, 1992.

46 Mizuno D and Soma G: Oral or percutaneous administration of lipopolysaccharide of small molecular size may cure various intractable diseases: a new version of Coley's toxin. Mol Biother *4*: 166-169, 1992.

47 Lopez-Soriano FJ and Williamson DH: Acute effects of endotoxin (lipopolysaccharide) on tissue lipid metabolism in the lactating rat. The role of delivery of intestinal glucose. Mol Cell Biochem *141*: 113-120, 1994.

48 Romagnani S: The increased prevalence of allergy and the hygiene hypothesis: missing immune deviation, reduced immune suppression, or both? Immunology *112*: 352-363, 2004.

49 Wills-Karp M: Complement activation pathways: a bridge between innate and adaptive immune responses in asthma. Proc Am Thorac Soc *4*: 247-251, 2007.

50 von Mutius E: Asthma and allergies in rural areas of Europe. Proc Am Thorac Soc *4*: 212-216, 2007.

51 Lauener RP, Birchler T, Adamski J, Braun-Fahrlander C, Bufe A, Herz U, von Mutius E, Nowak D, Riedler J, Waser M and Sennhauser FH: Expression of CD14 and toll-like receptor 2 in farmers' and non-farmers' children. Lancet *360*: 465-466, 2002.

52 Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, Maisch S, Carr D, Gerlach F, Bufe A, Lauener RP, Schierl R, Renz H, Nowak D and von Mutius E: Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med *347*: 869-877, 2002.

53 Strachan DP: Hay fever, hygiene, and household size. BMJ *299*: 1259-1260, 1989.

54 Asong J, Wolfert MA, Maiti K, Miller D and Boons GJ: Binding and cellular activation studies reveal that toll-like receptor 2 can differentially recognize peptidoglycan from gram-positive and -negative bacteria. J Biol Chem *284*: 8643-8653, 2009.

55 Kanzler H, Barrat FJ, Hessel EM and Coffman RL: Therapeutic targeting of innate immunity with toll-like receptor agonists and antagonists. Nat Med *13*: 552-559, 2007.

56 Pamer EG: Immune responses to commensal and environmental microbes. Nat Immunol *8*: 1173-1178, 2007.

57 Ulevitch RJ: Therapeutics targeting the innate immune system. Nat Rev Immunol *4*: 512-520, 2004.

58 Miller SI, Ernst RK and Bader MW: LPS, TLR4 and infectious disease diversity. Nat Rev Microbiol *3*: 36-46, 2005.

59 van Duin D, Medzhitov R and Shaw AC: Triggering TLR signaling in vaccination. Trends Immunol *27*: 49-55, 2006.

60 Sansonetti PJ: War and peace at mucosal surfaces. Nat Rev Immunol *4*: 953-964, 2004.

61 Medzhitov R: Recognition of microorganisms and activation of the immune response. Nature *449*: 819-826, 2007.

62 Velasco G, Campo M, Manrique OJ, Bellou A, He H, Arestides RS, Schaub B, Perkins DL and Finn PW: Toll-like receptor 4 or 2 agonists decrease allergic inflammation. Am J Respir Cell Mol Biol *32*: 218-224, 2005.

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