Mechanism for Maintaining Homeostasis in the Immune System of the Intestine

YOSHIE TANIGUCHI\textsuperscript{1,2,3}, NORIKO YOSHIOKA\textsuperscript{1,2,3}, KAZUE NAKATA\textsuperscript{2,4}, TAKASHI NISHIZAWA\textsuperscript{1,2,5}, HIROYUKI INAGAWA\textsuperscript{1,2,5,6,7}, CHIE KOCHI\textsuperscript{1,2,3,5,7} and GEN-ICHIRO SOMA\textsuperscript{1,2,3,5,7}

\textsuperscript{1}Institute for Health Science, Tokushima Bunri University, Nishihama, Yamashiro-cho, Tokushima-shi, Tokushima, 770-8514;
\textsuperscript{2}Nonprofit Organization Linking Setouchi Innate Immune Network (LSIN), Kamiita-cho, Itano-gun, Tokushima, 771-1342;
\textsuperscript{3}Department of Integrated and Holistic Immunology, Faculty of Medicine, Kagawa University, Miki-cho, Kida-gun, Kagawa, 761-0793;
\textsuperscript{4}Department of Nutrition, Okayama Prefectural University, Souji-cho, Okayama, 719-1197;
\textsuperscript{5}Macroph Incorporation, Hayashi-cho, Takamatsu-shi, Kagawa, 761-0301;
\textsuperscript{6}Department of Applied Aquabiology, National Fisheries University, Nagatahon-machi, Shimonoseki-shi, Yamaguchi, 759-6595;
\textsuperscript{7}Institute for Drug Delivery Systems, Tokyo University of Science, Yamazaki, Noda-shi, Chiba, 278-8510, Japan

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 primary response gene 88 (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.

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 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
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. Phylogenetically, 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 m² in humans. It has the greatest 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, gut-associated lymphoid tissue (GALT), including Peyer’s patches, mesenteric lymph nodes (MLN), the lamina propria, intraepithelial lymphocytes (IEL), and crypto patches.

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 (TLRs). 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 inintestinal 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 myeloid differentiation primary response gene 88 (MyD88), 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, 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 (39). 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* (40, 41). 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 (39). 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 (39). Surprisingly, another paper reported that *Bifidobacterium longum* and *Lactobacillus* (both Gram-positive bacteria used as probiotics) suppress Reg-III-γ, while *Bacteroides thetaiotaomicron* (a Gram-negative bacteria) and enterobacteria did not suppress Reg-III-γ (42).

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 greatly as modernization of living conditions has increased. Thus, it is thought that the inflow 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 I. Species of Gram-negative bacteria used in food processing.**

<table>
<thead>
<tr>
<th>Scientific name of bacteria</th>
<th>Name of food (producing district)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acetobacter aceti</em></td>
<td>Vinegar (worldwide)</td>
</tr>
<tr>
<td><em>Zymomonas mobilis</em></td>
<td>Tequila (Mexico)</td>
</tr>
<tr>
<td><em>Xanthomonas campestris</em></td>
<td>Xanthan gum (worldwide)</td>
</tr>
<tr>
<td><em>Acetobacter xylinum</em></td>
<td>Nata de coco (Philippines)</td>
</tr>
<tr>
<td><em>Acetobacter orientalis</em></td>
<td>Caspian Sea yoghurt (Caucasus)</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>Sarapao (Thai)</td>
</tr>
<tr>
<td><em>Pantoea agglomerans</em></td>
<td>Fermented rye bread (Northern Europe)</td>
</tr>
</tbody>
</table>
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).

**Acknowledgements**

This work was supported in part by grants-in-aid for “City Area Program (Development Stage, Takamatsu Area)” from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

**References**


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

<table>
<thead>
<tr>
<th>Number of bacteria</th>
<th>Amount of LPS released from of molecules</th>
<th>Relative quantity Gram-negative bacteria in intestines</th>
<th>Relative quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal bacteria</td>
<td>10&lt;sup&gt;14&lt;/sup&gt;</td>
<td>0.1 mg</td>
<td>20,000 (E. coli LPS M.W.)</td>
</tr>
<tr>
<td>Intestinal Gram-negative bacteria</td>
<td>10&lt;sup&gt;11&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IP-PA1 by oral administration (20 μg/kg/day)</td>
<td>1.2 mg (human, BW 60 kg)</td>
<td>5,000 (IP-PA1 M.W.)</td>
<td>48</td>
</tr>
</tbody>
</table>


Nauts HC, Fowber GA and Bogatko FH: A review of the influence of bacterial infection and of bacterial products (Coley’s toxins) on malignant tumors in man; a critical analysis of 30 inoperable cases treated by Coley’s mixed toxins, in which diagnosis was confirmed by microscopic examination selected for special study. Acta Medica Scand 45: 1-103, 1953.


Received April 28, 2009
Revised July 22, 2009
Accepted August 31, 2009