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Review

Intestinal Macrophages Involved in the Homeostasis of the Intestine Have the Potential for Responding to LPS

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Abstract. *Recently, there has been interest in the tertiary functions of food, those that maintain human health. Moreover, lipopolysaccharides (LPS), which are components of Gram-negative bacteria, have been found to be highly effective in activating innate immunity and have been rediscovered as new functional food materials. In this review, we discuss the significance of LPS as a food component with reference to these tertiary functions based on recent findings. There is special emphasis on the plasticity of responses to LPS by intestinal macrophages. According to the macrophage-network theory, local macrophages cooperate with other tissue macrophages. For this reason, this review also discusses the possibility that information is transferred throughout the body from intestinal macrophages.*

Food supplies both nutrients and may provide a tertiary function in regulating health. Immune regulation of these tertiary functions may prevent certain diseases or maintain and augment health. In advanced countries, it is hoped that these tertiary functions can be developed to counteract problems of lifestyle-related diseases such as hyperlipidemia, diabetes and cancer, and problems related to the transition to an aging society.

LPS derived from Gram-negative bacteria contained in food can regulate immunity. LPS in food is considered to show its function through residential macrophage in the intestine.

Macrophages are present in every tissue of the human body where they are referred to as tissue macrophages. When resident tissue macrophages encounter foreign substances, they produce cytokines and present antigens for transfer signals to neighboring macrophages (1). Intestinal macrophages are found in the lamina propria of the intestinal mucosa (2) where they are in close contact with the external environmental. They make up the largest pool of macrophages present in manuals (3) and are thought to be able to recognize functional foods. Therefore, we believe homeostasis is regulated in part by this response of intestinal macrophages.

LPS is present in some foods and plays a role in regulating immunity. The LPS in foods functions through the actions of the resident intestinal macrophage in the intestine. LPS is the strongest macrophage-activating substance of known immunopotentiators. Food contains about 0.16 to 600 ng/g of LPS (4). We believe that these LPS function to regulate immunity after recognition by intestinal macrophages.

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In this review, we discuss the significance of LPS as a food component that has the tertiary function of regulation. Recent findings indicate that there is plasticity in the responses to LPS by intestinal macrophages. Based on the macrophage network theory, local macrophages cooperate with other tissue macrophages. This may allow information transfer to the whole body from intestinal macrophages

Characteristics of the Intestinal Macrophages Present in the Lamina Propria Mucosa of the Digestive Tract

It is well known that cytokines such as interferon (IFN) α, tumor necrosis factor (TNF), and interleukin (IL)-1 are secreted in response to LPS stimulation by tissue macrophages in the abdominal cavity, spleen, alveolus, *etc.* CD14 and toll-like receptor (TLR) 4 exist on the cellular membrane of macrophages and recognize LPS (5). Signals are transmitted to adapter molecules such as MyD88 to activate transcription factors (6, 7). This characteristic is considered to be common in macrophages. However, it is now clear that intestinal macrophages, have different characteristics.

Intestinal macrophages express markers for CD33, CD68, and CD13 in the same way as monocytes and other tissue macrophages (8, 9). However, unlike other tissue macrophages, intestinal macrophages do not express CD14 or TLR4 on their membranes (9). It has been reported that intestinal macrophages do not secrete cytokines after LPS stimulation (10). For this reason they are considered to be non-responsive to LPS. It has also been reported that intestinal macrophages do not express either the Fc receptor for IgA (CD89) or IgG (CD16, CD32, CD42), or receptor complexes such as CR3 (CD11b/CD18) or CR4 (CD11c/CD18) other than LPS receptors (9, 11-14). In addition to the suppression of the expression of receptors, it was also reported that intestinal macrophages do not produce IL-12, which is an inflammatory cytokine. Instead they produce IL-10, which exhibits anti-inflammatory properties (15). We compared the expression of mRNA between intestinal macrophages and peritoneal macrophages using a DNA array. The expression of ficolin- and mannose-binding lectins (MBL) (which are known as defense lectins) (16) recognizes pathogen-associated molecular patterns (PAMPs); this is less than in intestinal macrophages (Table I). From these results, intestinal macrophages are believed to possess the ability to eliminate foreign substances and to regulate the mechanism of inflammation. Their responses are different from those of other tissue macrophages.

However, CD14, which was not observed as being expressed on the membrane of resident intestinal macrophages, recently was recently reported to exist intracellularly as a protein (17). In culture of intestinal macrophage on IgA-coated wells, expression of CD14 was induced on the cellular membranes (18). Furthermore, the induction of TNF occurred when these cells were stimulated with LPS (10). This suggests the possibility of plasticity: under certain conditions when CD14 in intestinal macrophage is expressed on the membrane, intestinal macrophages show LPS responsiveness.

Table I. *Ratio of mRNA expression by mouse intestinal macrophages when compared to mouse peritoneal macrophages.*

Gene	Normalized
Ficolin-A	0.02
Ficolin-B	0.92
MBL-A	0.44
MBL-C	0.20
RegIIIγ	132.99
pIgR	1,165.09
β-Actin	1.00

From the above, we believe that although intestinal macrophages appear to be non-responsive to LPS, they are capable of recognizing the LPS. This is the way that LPS leads to maintaining homeostasis of the intestine through intestinal macrophages.

LPS as a Component of Functional Foods

LPS as a component of the cellular wall of Gram-negative bacteria is commonly referred to as an endotoxin. Currently, there are only a few reports that describe the usefulness of LPS including adding it to food. Nevertheless, it is present in many foods and is reported to be contained in significant quantities in wheat bran (132 μg/human) and malt (180 μg/human), both known as health foods (4). Gram-negative bacteria are known to be symbiotic on many food crops such as rice, sweet potatoes, apples and pears (19). Furthermore, fermentation by Gram-negative bacteria is necessary for the production of Nata de Coco and Caspian Sea yoghurt, so LPS is contained in these foods. Thus, people have been unknowingly consuming LPS and may have benefited from it. These are several reports that provide concrete examples of LPS as an important molecule for homeostasis of the intestine. First there is a study on the survival rate and the pathology of mice with ulcerative colitis caused by sodium dextran sulfate (DSS) administered after the elimination of enterobacteria by preadministration of antibiotics (20). The results showed there was no survival 12 days after DSS was administered alone. By contrast, in the group of mice administered LPS in addition to DSS, the survival rate was 100%. Significantly favorable results were also obtained related to the pathology of the ulcerative colitis (in loss in body weight and bloody stool).

RegIIIγ is a lectin which has the ability to suppress the activity of Gram-positive bacteria (21). It is secreted from

intestinal epithelial or paneth cells and is induced by TLR4 MyD88-mediated signals from intestinal symbiotic bacteria (21, 22). It is reported that the production of RegIIIγ was inhibited after the administration of antibiotics and that vancomycin-resistant bacteria proliferated. But this proliferation of vancomysin-resistant bacteria was suppressed when LPS was administered in addition to the antibiotics (23). Accordingly, in our comparative analysis between intestinal macrophages and peritoneal macrophages using DNA array analysis, RegIIIγ was observed to have higher expression in intestinal macrophages than in peritoneal macrophages (Table I). In other words, our experimental results suggested that intestinal macrophages may produce RegIIIγ. We presume that intestinal macrophages produce RegIIIγ after recognizing LPS through TLR4.

Recognition of LPS in Intestinal Macrophage

Current findings indicate that resident intestinal macrophages, which do not respond to LPS, cannot acquire responsiveness to LPS without contact with IgA in the intestinal tissue. Normally, resident intestinal macrophages are present in lamina propria mucosa (2), while IgA is produced and secreted as a dimer by IgA plasma cells in the lamina propria mucosa of the intestine (14). This dimeric IgA is formed by combining with secretory component (SC) in the epithelial cells in the intestinal mucosa; it is secreted in the mucus of the surface layer of mucosa and contributes to immunity on the mucosa (24). Thus, intestinal macrophages and IgA are separated by intestinal epithelial cells and are not in direct contact with each other.

The intestine is always exposed to antigens derived from the diet and resident enterobacteria. While mucosal epithelial cells do not cause severe inflammation by these antigens, they produce antimicrobial peptides or chemokines to protect the intestine from infection. This phylaxis requires that antigens be moved effectively in the lymphatic tissues related to the mucosa such as Peyer's patches (25). For this purpose, M cells actively take in antigens on the mucosa by transcytosis and deliver them to antigen-presenting cells that are present immediately under the epithelium (26). If this antigen is opsonized by IgA, it is possible that responsiveness to LPS is obtained by the induction of expression of CD14 or TLR4 on the membrane in macrophages that are present directly under the epithelium and that recognize the IgA.

However, it has already been reported that CD89, a molecule that recognizes IgA, was not expressed in intestinal macrophages (9, 11, 27). For this reason, it has been suggested that intestinal macrophages may express IgA receptors other than CD89, such as the Fc receptor to IgA or IgM (Fcα/μR) (28, 29), polymeric Ig receptor (pIgR) (30, 31), CD71 (32), and asialoglycoprotein receptor (ASGPR).

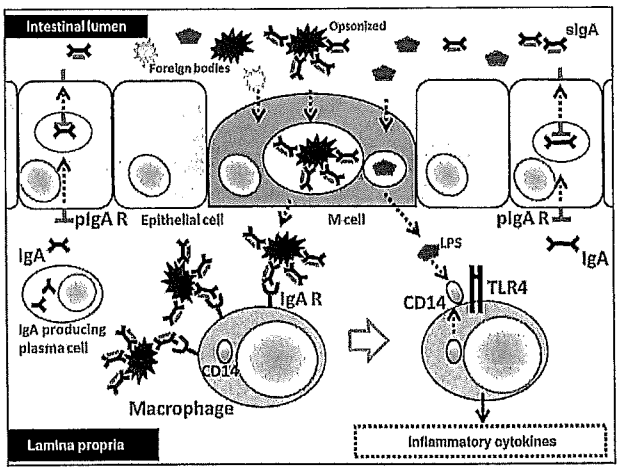

Figure 1. *Hypothetical process responsible for intestinal macrophage contact with LPS. IgA-producing plasma cells produce IgA in the intestinal lamina propria. Secretory IgA (sIgA) is secreted in the lumen (sIgA-opsonized antigens). Transcytosis of opsonized antigens occurs in M cells. Intestinal macrophages then recognize opsonized antigens from the IgA and are induced to express CD14. Finally, the intestinal macrophages recognize the presence of invasive LPS in the intestinal lamina propria.*

In fact, pIgR was observed to have higher expression in intestinal macrophages than in peritoneal macrophages (Table I). Furthermore, the neonatal immunoglobulin receptor (FcRn) is reported to be expressed in intestinal macrophages (33-35). It is not yet clear which receptor recognizes IgA and induces membrane expression of CD14, but it is believed that there are receptors in the intestinal macrophages that are involved in membrane expression of CD14 (Figure 1).

Systemic Effect Provoked by Intestinal Macrophages in the Digestive Tract

As mentioned above, LPS recognition by intestinal macrophages does more than just maintain homeostasis of the intestine. There is a report of a signal-transfer system from local activated macrophages to other macrophages by cell-to-cell contact. This is called a 'macrophage network' (36). It is thought that the macrophage network is the control system for homeostasis.

It is well known that intestinal macrophages are not activated by LPS. But they are in close contact with the external environmental. Moreover, the lamina propria contains the largest reservoir of macrophages in the body. Under these circumstances, it is thought that the intestinal macrophages become the cells that dispatch information through the macrophage network.

LPS recognition by intestinal macrophages is believed to have an effect that extends beyond the maintenance of

homeostasis of the intestine. Further study is required to clarify the molecular evidence for interaction of LPS with intestinal macrophages.

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