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Review

Improvement of Allergic Dermatitis *via* Regulation of the Th1/Th2 Immune System Balance by Macrophages Activated with Lipopolysaccharide Derived from *Pantoea agglomerans* (IP-PA1)

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Abstract. *Recently, the incidence of allergies has been increasing especially in advanced countries. The cause of these allergies is believed to be a failure in the immune system balance that has been caused by changes in the living environment. The incidence of allergy shows a negative correlation with the decrease of infectious diseases in childhood. It has been suggested that the key to alleviating allergies is to activate innate immunity by exposure to microbial components such as lipopolysaccharides (LPS). The activation of innate immunity is expected to normalize the T-helper type 1 and 2 (Th1/Th2) immune system balance and to suppress the excessive reaction of Th2 type responses that cause immunoglobulin (Ig) E-dependent allergies. This study introduces information on how the activation of macrophages, which are important in innate immunity, by LPS derived from Pantoea agglomerans (IP-PA1) caused suppressive effects on type I allergic reactions and improved allergic dermatitis. We also summarize our hypothesis that regulating the immune system balance using LPS to stimulate macrophages may be an important procedure for preventing and improving allergic dermatitis.*

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Recently, the incidence of allergic dermatitis, such as atopic dermatitis, has increased markedly, especially in advanced countries. The symptoms of allergic dermatitis are believed to appear as a result of a combination of hereditary and environmental causes. The increase in this disease is considered to depend largely on environmental changes (aggravation by environmental pollution) and changes in life style (improvement in hygienic conditions). The modernization of the environment as seen in advanced countries is believed to result in a disruption of the immune system balance in the body. This is now being considered as a main cause of recent allergic diseases.

The human immune system composes acquired immunity, which is only developed in vertebrates, and innate immunity, which is a self defense system possessed by all multicellular organisms. In acquired immunity the functionally important cells are T-cells and B-cells. These cells produce and memorize specific antibodies or receptors against molecular structures of foreign body (non self) antigens. In innate immunity, macrophages are the most important cells as they have constitutional foreign body receptors that recognize non self. Until recently, immunity studies have tended to focus on acquired immunity. This is because specificity can be clearly observed, and this information has been highly useful in applicable fields such as the prevention of infection by

vaccinations. However, the cells involved in innate immunity are indispensable for initiating the acquired immunity mechanism. As an example, the presentation of antibodies by innate immunity cells is indispensable to the production of antibodies. For this reason, the recognition of the importance of innate immunity has been increasing rapidly. The innate immune system plays the key role in biophylaxis by its direct function of eliminating foreign bodies, such as invading pathogens, or removing unnecessary self cells. It also supports the whole system of immunity and maintains homeostasis. From this viewpoint, we consider that the innate immune system, especially the function of macrophages, is the key to improving or preventing allergic dermatitis that is caused by a failure in the immune system balance.

In this paper, we summarize the suppressive effects of a lipopolysaccharide (LPS) on type I allergic responses, the improvement in allergic dermatitis, and the usefulness of regulating immune balance by activating the innate immune system mediated by LPS for allergic dermatitis. The LPS was derived from an edible Gram-negative bacteria discovered by us.

Maintenance of Homeostasis by Macrophages

Macrophages are a group of cells bearing the important function of being the front line responders of the innate immune system. They are called microglia in the brain, kupffer cells in the liver, alveolar macrophages in the lung, and intestinal macrophages in the intestine. Precursor cells called monocytes are also present in the blood. Thus, macrophages in a variety of forms keep guard over the whole body. The main function of macrophages includes elimination of foreign substances or dead cells, antigen presentation, and regulation of other immune cells by cytokine production. Via these functions, macrophages play a role in preventing infection of an organism, in metabolic regulation, in metabolism, and in wound healing (1). For example, when neuronal cells die after damage to the brain, microglia present in the brain eliminate the dead cells. It has been reported that the activation of microglia promotes phagocytosis, which is necessary for the generation of neuronal cells (2, 3). This indicates that tissue macrophages bear a regulatory role in maintaining the function of the body in the normal condition. It also suggests that an appropriate activation of macrophages can possibly lead weakened functions of the body to a normal state.

From this viewpoint, we considered that it was possible to maintain homeostasis by activating appropriate regulation of macrophages so that they would support or recover a healthy state. With this in mind, we searched for substances that would suitably activate macrophages after oral or transdermal administration.

The Characteristics of IP-PA1 a Functional Lipopolysaccharide

We preferred a macrophage-activating substance that could be administered in a non-invasive way, *i.e.* by oral or transdermal administration. For this reason, we sought macrophage-activating substances that could be derived from foods that have a long history of human consumption. We found a substance that strongly activated macrophages in a water extract of wheat flour in 1991 (4). After analyzing the main substance of activation, we discovered a LPS derived from *Pantoea agglomerans*, a Gram-negative bacteria symbiotic with wheat. This LPS we named IP-PA1, which stands for immune potentiator from *P. agglomerans* 1. This species is known to be a symbiotic bacteria that stimulates growth in many plants because it fixes nitrogen or phosphorus. In Europe, living *P. agglomerans* is used as a biocontrol agent to prevent decomposition of fruit by fungus (5-7). Moreover, *P. agglomerans* is a necessary component during fermentation of rye sourdough because it produces and provides folate for *Lactobacillus* (8). Structural analysis of IP-PA1 shows that although part of lipid A has the same structure as bacteria such as *Escherichia coli* or salmonellae LPS, the constitution of the sugar (rhamnose and glucose units) and its molecular weight (5 kDa) are different (4).

It has been reported that IP-PA1 activates macrophages *via* toll-like receptor-4 (TLR4) (9). The initial repair mechanism of activated macrophages may be to act on dermal production of cytokines such as β-fibroblast growth factor (β-FGF), vascular endothelium growth factor (VEGF), or granulocyte colony-stimulating factor (GM-CSF) (10, 11). Because activation of macrophages with IP-PA1 as part of homeostasis is controlled by a feedback mechanism to suppress excessive activation, it is possible to use IP-PA1 routinely on a continuing basis to prevent skin problems. This is because the functional mechanism for IP-PA1 is completely different from that of immune suppression drugs, such as steroids or anti-inflammatory agents. n

Antiallergic Effect of IP-PA1

Transdermal administration of IP-PA1 has been shown to improve burn injuries, decubitus and allergic dermatitis (12). An antiallergic effect is the most prominent and characteristic effect of IP-PA1 on dermatosis.

When mice are injected with anti-dinitrophenol IgE monoclonal antibodies (1 μg/mouse), following application of acetone including 0.25% dinitrofluorbenzene-olive oil (1:4) as an antigen on the ear, the ear becomes swollen (this is one of the animal models for IgE-dependent allergy). If IP-PA1 was intradermally administrated before antigen application, the swelling was suppressed to the same degree as the negative control mice which were not given IgE antibodies

(13). IP-PA1 also exhibited a curative effect in a clinical trial (12). After receiving informed consent, five patients with grave atopic dermatitis were tested. A curative effect was observed after oral administration of IP-PA1 (10 μg/ml/50% glycerol solution, 1-3 ml/day) after only two to three days, and in four patients there was an improvement of atopic dermatitis after two months. Furthermore, the same effect was demonstrated using skincare agents that included IP-PA1. The improvement effect of moisturizing cream containing IP-PA1 (1 μg/g) was investigated for two weeks in 100 patients who had mild atopic dermatitis. Although these research results were subjective data by patients, the number of patients with an improvement in symptoms increased significantly (12). In addition, a double-blind test (50 persons each group) was performed using a bath agent containing IP-PA1 (final concentration 0.1 μg/l) (12). When compared to a control bath agent without IP-PA1, all measured parameters had higher point values (heat retention, backache, athlete's foot, rough skin and atopic dermatitis).

The antiallergic effect of IP-PA1 suggested that LPS suppressed allergic responses *via* an as yet unknown anti-inflammatory mechanism. This type of LPS can be used in new products for suppressing atopic dermatitis symptoms.

Normalization in Skin of the Th1/Th2 Immune Balance by Macrophages Stimulated with LPS

Recently, there has been an accumulation of interesting findings about the suppressive effect of LPS on allergies. There was an epidemiological investigation of 812 children aged 6-13 years who lived in households of farmers and in households with parents having other professions in Germany, Austria and Switzerland. There was a negative correlation between the amount of LPS in the mattress used by the child and the sensitization to antigens, pollinosis, and atopic asthma of the child (14). These results indicated that the greater the exposure to LPS, the smaller the probability that a child would have allergic diseases. Exposure to LPS, which activates innate immunity, had an important regulatory function in the expression of allergic disease. It was also reported that tuberculin test-positive children (12-13 years of age) had a lower prevalence of asthma, allergic rhinitis, eczema, and a lower allergic response (total IgE value) compared to tuberculin-negative children (15). Another report indicates that a history of oral administration of antibiotics before two years of age is a risk factor for allergic diseases (16). Shirakawa *et al*. proposed a 'hygiene hypothesis' that the increase of allergic diseases in recent years relates to the decrease of infectious diseases in childhood because of the improvement of hygienic conditions, a change in diet, and the overuse of antibiotics.

The shift in the Th1/Th2 immune system balance is one way to explain the 'hygiene hypothesis'. The T-cells of the acquired immune system are classified according to differences in cytokine production into a T-helper type 1 (Th1) system and a Th2 system. In the immune responses of the Th1 system, cytokines such as interferon–γ (IFN-γ) and interleukin 12 (IL-12) are produced, which induce activation of natural killer (NK) cells and cytotoxic T lymphocytes (CTL). By contrast, in the immune responses of the Th2 type, cytokines such as IL-4 or IL-5 are produced, which promote maturation of mast cells or B-cells; these produce histamine and antibodies, respectively, that play the central role in allergic reactions. It is believed that once mast cells or B-cells are activated, the immune system is inclined towards Th2-type responses which results in allergic diseases such as allergic dermatitis. In fact, it has been reported that the level of the cytokines of the Th2 system, such as IL-4, IL-13 and IL-10, is higher and that the level of cytokines of Th1 system (IFN-γ) is lower in tuberculin test-negative children than in test-positive children (15). Furthermore, gene expressions of CD14 and TLR2 in the blood were found to be significantly higher in the children of farmers' households than in non-farmers' households; this fact shows that there is activation of innate immunity and provides strong evidence to support the hygiene hypothesis (17).

Another characteristic of LPS is that its effects are mediated by macrophages. Langerhans cells, which are macrophage-like cells, are present in the epidermis. Langerhans cells phagocytose antigens and present them to dendritic cells (18). It has been recently reported that dendritic cells extend pseudopods and receive external information (19). Langerhans cells are also reported to move from the epidermis to lymph nodes after receiving antigens (12). These results strongly suggest that LPS applied on the skin is received by the Langerhans cells present in the epidermis. These cells then move to the dermis and transmit information to histiocytes. We showed that tumor necrosis factor α (TNF-α) expressed on the membrane surface of activated macrophages gives information to adjacent macrophages. Membrane-bound TNF also functions at the same time as the receptor and them transmits information in both directions. We hypothesize that there is a mechanism by which the macrophages that are present in every tissue can systemically transmit and correlate information on external stimulation. We have named this mechanism the 'macrophage network'. We believe that Langerhans cells receive and transmit information of the local site in the skin; this plays an important role in the elimination of foreign substances or dead cells, and in the generation of skin tissue.

As described above, the improvement effect brought about by IP-PA1 is believed to be a result of the shift induced in the balance of immune system. The balance shifts from the Th2 type to the Th1 type after appropriate activation of macrophages in the epidermis by LPS. The macrophages are then induced to produce cytokines of the Th1 type, such as IL-12 or TNF (12).

Conclusion

LPS is a novel material which mediates the activation of macrophage cells that play a central role in innate immunity. Recently, signal transduction *via* TLR4 by LPS was reported to play an important role in the maintenance of homeostasis in the intestine (20). This shows that mammals use LPS advantageously as a molecule for homeostasis and suggests that IP-PA1 may also be used for the maintenance of homeostasis in the skin. IP-PA1 is believed to cause an improvement of allergic dermatitis by normalizing the immune system balance. The maintenance of homeostasis in the skin using activated macrophages present in the skin promotes the regeneration of the skin tissue, and it can be expected that this will lead to improvement of various types of dermatitis. Drugs to cure allergies that target TLR4 are currently being developed (21). This shows that there is current interest in methods to activate innate immunity for the improvement of allergies. We believe that LPS has great potential as a novel material for macrophage activation mediated by the skin. In addition, this substance can possibly extend its effects to the whole body *via* the macrophage network.

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