Short Review

Lipopolysaccharide IP-PA1 from *Pantoea agglomerans* Prevents Suppression of Macrophage Function in Stress-induced Diseases

KAZUE NAKATA[1], HIROYUKI INAGAWA[2,3] and GEN-ICHIRO SOMA[2,3,4]

[1]*Department of Nutritional Science, Okayama Prefectural University, Kuboki, Soja, Okayama 719-1197, Japan;*
[2]*Macrophi Inc., 2217-44 Hayashi-cho, Takamatsui, Kagawa 761-0301, Japan;*
[3]*Department of Integrated and Holistic Immunology, Faculty of Medicine, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kida-gun, Kagawa 761-0793, Japan;*
[4]*Institute for Health Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan*

Abstract. *Chronic psychological stress impairs health and induces various diseases by causing an imbalance in the immune, neuropsychiatric and endocrine systems. The primary reason for the development of stress-induced disease is suppression of macrophage function, which plays a pivotal role in innate immunity. In fact, surgical stress has been shown to exacerbate opportunistic infections by significantly suppressing macrophage function. Conversely, administration of macrophage activating substances before surgery, such as tumor necrosis factor (TNF)-α or Picibanil (OK-432), has been shown to protect against macrophage suppression and the resulting exacerbation of infectious diseases, and against tumor metastasis in the lungs. Thus, if suppression of macrophage function by stress could be safely prevented by use of a macrophage activating substance, the detrimental side effects of stress could be reduced. Recently, we identified a lipopolysaccharide, IP-PA1, derived from Pantoea agglomerans, a symbiotic Gram-negative bacteria found in wheat and other food plants. Oral administration of IP-PA1 demonstrated macrophage activation (priming) and protective effects against infection, allergy and cancer, without any side-effects. In this review, the possibility of using IP-PA1 as a safe, macrophage activating substance for prevention of stress-induced impairments is discussed.*

Correspondence to: Kazue Nakata, Department of Nutritional Science, Okayama Prefectural University, Kuboki, Soja, Okayama 719-1197, Japan. Tel/Fax: +81 866942146, e-mail: nakata@fhw.oka-pu.ac.jp

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Increasing Psychiatric Disorders and Allergic Diseases Caused by Psychological Stress

In the Japanese National Health and Nutrition Survey of 2007, more than 70% of the population aged 20-40 years reported stress on a daily basis (1). Recently, stress has been linked to an increasing number of health problems in Japan including psychiatric disorders (depression and psychophysiological disorders) (2) and allergic diseases (atopy and pollinosis) (3). Stress is generally defined as a state of altered homeostasis resulting from an external or an internal change (4). There appear to be individual differences in the response to stress, which are attributable to different kinds of stressors (chemical, physical, biological and mental stimulation), genetic factors, environmental factors and age (5). Thus, health problems caused by stress are idiopathic and difficult to prevent.

Stress and Mechanism of Immunosuppression

Health is a maintained balance of immune, neuropsychiatric and endocrine systems. Health problems that cause an imbalance can be induced by chronic psychological stress at work or in personal relationships. Immunosuppression causes infection, autoimmune diseases and cancer (6, 7). Stress induces immunosuppression, which in turn affects the nervous system. Psychological stress is associated with activation of the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic and adrenomedullary systems, resulting in an enhanced secretion of cortisol and catecholamines, respectively (8).

In the HPA axis, stress stimulates activation of corticotropin-releasing hormone (CRH) neurons in the hypothalamus and induces the release of adrenocorticotropic hormone (ACTH)

from ACTH cells in the pituitary gland and glucocorticoids from the adrenal cortex (9). Glucocorticoids, in particular, are thought to play a critical role in stress-induced immunosuppression. Exposure to excessive stress results in excess production of glucocorticoids, inducing thymic atrophy and reducing lymphocyte function (10, 11). Glucocorticoids are thought not only to affect immunosuppression but also to reduce initiation of the inflammatory response.

In the autonomic nervous system, receptors of norepinephrine, which is released from the sympathetic nerve, are found in lymphocytes and macrophages. Norepinephrine attenuates intracellular levels of tumor necrosis factor (TNF)-α protein in T-cells and macrophages (12). Since the autonomic nervous system is also present in the hypothalamus, it is thought that stress might affect immune function *via* the autonomic nervous system.

Maintenance of Homeostasis by Macrophages and the Influence of Stress

Of all the immune cells, macrophages are the most important as they play a central role in innate immunity and also induce adaptive immunity. Macrophages are not only part of the defence mechanism against infections but also maintain homeostasis (including aspects such as tissue regeneration and metabolic control) (13).

Previously, we demonstrated the exacerbation of an opportunistic infection due to surgical stress-induced suppression of macrophage function (14). Stress has also been reported to suppress phagocytosis (15) and the release of nitric oxide and TNF-α in macrophages (16). It is reported that in rats the suppression of macrophage function by glucocorticoids induces differentiation of a specifically activated anti-inflammatory subtype of monocytes (17), and corticosterone and epinephrine induce reduction of toll-like receptor (TLR)-2 and TLR-4 expression (18). Because macrophage suppression occurs during the 6-h period directly after surgery, which is known to be associated with increased cortisol levels (19, 20), it is believed that increased cortisol is one of the factors leading to the suppression of macrophage function.

Nakamoto *et al*. showed that a reduction in TNF-α production after laparotomy in mice could be prevented by intravenous administration of OK-432 (dead bacterial bodies of *Streptococcus pyogenes*) or TNF-α before laparotomy, and this also reduced mortality due to infectious disease and suppressed tumor metastases (14). These results suggest that appropriate activation of macrophages (namely primed stage) prior to surgery is useful for preventing the detrimental effects caused by surgical operations. A priming stage is insufficinet to stimulate macrophages to release proinflammatory cytokines, although priming sensitizes macrophages, resulting in enhanced secretion of proinflammatory cytokines in response to subsequent activator such as LPS (21). Thus, in the priming stage, up-regulation of macrophage recognition of foreign substances (bacteria, viruses, and apoptotic cells) occurs without side-effects.

Macrophage-activating Food Component (IP-PA1)

We discovered IP-PA1, a low molecular weight (5 kDa) lipopolysaccharide (LPS) derived from symbiotic Gram-negative bacteria in wheat. IP-PA1 induces activation of macrophages *in vitro* (22). IP-PA1 activates macrophages and dendritic cells (DC), mainly *via* TLR-4 (23). In certain situations, LPS can behave as an endotoxin, causing severe systemic inflammation, but more commonly it has beneficial effects. LPS functions that have recently been discovered include the regulation of intestinal homeostasis, such as prevention of enteric infectious diseases or enteric immunity regulation through interaction with gut-associated lymphoid tissues (24, 25), modulation of proliferation and differentiation of the epithelial lineages (26), and regulation of angiogenesis (27) following oral administration. Thus, LPS is now recognized as an exohormone rather than simply an endotoxin (28). *P. agglomerans* is found not only in wheat, but also in other food plants, such as rice (29), sweet potatoes (30), apples and pears (31). Previous studies have suggested that IP-PA1 is a safe substance, which has been confirmed with a conventional oral administration safety test (13). We have previously shown that IP-PA1 can suppress progression of type 1 diabetes in non-obese diabetic mice (32), improve plasma glucose levels in type 2 diabetic mouse model, decrease the LDL level in WHHL rabbits (with familial hyperlipidemia) (33), improve atopy, prevent infection, decrease pain and increase bone mineralization. Onset of type 1 and type 2 diabetes are inflammatory diseases which are associated with macrophages (34-37). Macrophages are also known to phagocytose oxidized LDL, inducing hyperlipidaemia. In the case of atopy, it is believed that the immune balance (Th1/Th2) shifts to the Th2 type (38) and macrophages are associated with the correct regulation of this balance.

Potential Anti-stress Effect of IP-PA1

Intravenous and intradermal administration of IP-PA1 demonstrated analgesic effect in mice. This effect is related to β-endorphin (an endogenous opioid) because it can be inhibited by naloxone, an antagonist of opioids (39, 40). Similarly, oral administration of IP-PA1 mitigates pain after surgery and promotes an increase in the β-endorphin concentration in the plasma of patients (data not shown). Moreover, β-endorphin activates macrophage functions (41, 42). These effects of IP-PA1 suggest that homeostasis is improved by appropriate priming of macrophages.

Dexamethasone is a synthetic glucocorticoid used to induce stress in animal models (43, 44). Dexamethasone-induced immunosuppression prevented a reduction in the production of anti-*Salmonella enteritidis* (SE) and anti-sheep red blood cell (SRBC)-specific antibodies in response to vaccination, and IP-PA1 administration suppressed excessive apoptosis in lymphocytes induced by dexamethasone. IP-PA1 is thought to indirectly inhibit dexamethasone-induced lymphocytic apoptosis because pre-treatment with IP-PA1 does not protect cultured bursal and splenic lymphocytes from dexamethasone-induced cell death in chicken (45). Oral administration of IP-PA1 in mice increased serum levels of interleukin (IL)-12 and interferon (IFN)-γ, typical cytokines produced by activated macrophages and DCs. IL-12 induces proliferation of natural killer cells and T-cells, and production of cytokines, particularly of IFN-γ. In addition, it enhances the activity of cytotoxic T lymphocytes (46), and suppresses excessive apoptosis in lymphocytes. The improvement of stress-induced immunosuppression by IP-PA1 administration suggests that IL-12 is the effector molecule produced by activated macrophages.

Conclusion

Health problems are increasing due to psychological stress caused by social anxiety and stressful personal relationships. Although a countermeasure for reducing stress is required, the degree of psychological stress differs in each individual. Therefore, it is difficult to find a drug-based therapy for avoiding the effects of stress. In this review, we focused on the influence of stress on immune, neuropsychiatric and endocrine systems, which are important for maintaining health. In addition, we reviewed the factors that prevent suppression of macrophage function, which plays a pivotal role in the maintenance of homeostasis. Appropriate activation of macrophages prior to surgery is useful for the prevention of infectious diseases and tumor metastasis. IP-PA1 (a macrophage-priming agent) is not only effective in preventing and/or improving immunity against various diseases, but also in preventing immunosuppression due to stress. Priming macrophages with IP-PA1 is believed to find application in the prevention and treatment of a wide range of disorders, including stress-induced diseases.

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