Oral or percutaneous administration of lipopolysaccharide of small molecular size may cure various intractable diseases: a new version of Coley's toxin

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Based on our new finding that an inflammation in which tumor necrosis factor (TNF) is primed or triggered (ontogenic inflammation) can regulate the homeostasis in ontogenesis, we have identified a new lipopolysaccharide from wheat flour (LPSw) that can induce ontogenic inflammation in adult mice. LPSw can prime adult mice to produce TNF when given orally or percutaneously, suggesting that it may maintain homeostasis in adults. LPSw can cure experimental animals of diabetes, hyperlipidemia, ulcer, and herpes. It can also stimulate bone resorption and egg-laying, and shows a strong analgesic effect that is blocked by naloxone. This effect even allows a release from drug addiction. Suppression of serum cholesterol level by oral uptake of LPSw in Watanabe heritable hyperlipidemic (WHHL) rabbit was also observed. Infection of toxoplasma was prevented by oral uptake of LPSw. The realization that a single oral or percutaneous administration of LPSw may be a cure for multiple intractable diseases may lead to the presentation of a nontoxic type of Coley's toxin, which is known to be an efficient cancer treatment, but has high toxicity.

Keywords: Ontogenic inflammation; tumor necrosis factor; lipopolysaccharide; bone resorption; diabetes; hyperlipidemia; arthritis; analgesic effect; ulcer; herpes.

Introduction

We have been studying production of what we call an ontogenic inflammation[1–3] in an adult to cure various intractable diseases. We found an inflammation in ontogenesis corresponding to Metchinikoff's phylogenic inflammation.[2] The two stages were identified in relation to endogenous production of tumor necrosis factor (TNF); one is the primed stage and the other is the triggered stage. Reproduction of the triggered stage in an adult was performed through the endogenous production of TNF and the results published using the term exogenous endogenous TNF (EET) therapy.[4,5] The present report concerns reproduction of the primed stage of ontogenic inflammation in an adult.

As previously reported,[6–17] this stage can be induced by various substances, such as Bacillus Calmette-Guerin (BCG), lentinan, OK-432, polystyrene latex, and various cytokines. Because we expected that the stage would reversibly and rapidly return to normal, we decided that the best induction would be by cytokines such as interferon (IFN)-γ, -α, and -β, interleukin-2, TNF, and others. However, all the samples known are available only for parenteral use and they invoke some degree of harm. We therefore sought a new sample among foodstuffs, especially plants, seeking a substance that could activate macrophages to the primed stage when administered orally[18] or percutaneously.[19] We found such a substance in wheat flour as a lipopolysaccharide (LPSw) of small molecular size (~5 kD)[20] and, as expected, it showed curative effect on various intractable diseases in experimental animals, as well as in some human clinical trials.[21] We also found that LPS derived from *Pantoea agglomerans* (gram-negative soil bacterium) of small molecular size (~5 kD) could substitute for the whole function of LPSw.[22]

Screening and identification of LPSw from wheat flour[20,22]

Water extracts of various foodstuffs were tested for priming activity[6] to produce TNF. Early in the sampling period, limulus reaction–positive substance was found, purified, and called LPSw. Its molecular size is ~5 kD (hexosamine, 4; phosphorus (P), 1; 2-keto-3-deoxy-D-mannooctonic acid (KDO), 1). LPSw can prime endogenous production of TNF in mice intravenously (1 ng/mouse), intradermally (1 mg/mouse), and orally (>100 mg/mouse).[20]

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Among 10 kinds of LPS of gram-negative soil bacteria, we selected an LPS of *P agglomerans* (~5 kD),[20] tested it similarly for the following activities, and obtained the same effects as described in the following sections.

Bone resorption, egg-laying, and thickness of eggshell[23,24]

Effect of oral uptake of LPSw on the bone resorption of 18-day-old chick embryonic calvaria (10–100 ng/ml)[25] was tested. The effect was comparable with that of parathyroid hormone (1 U/ml). When 60 mg/ml was given to a hen in drinking water *ad libitum*, the percentage of eggs with shell intensity of > 4 kg/cm^2 increased from 12 to 32% in a 1-month period. The same amount increased egg-laying by 28% in the same period. Increase in the number of eggs laid may suggest a stimulation of hormone excretion.[24]

Diabetes[26]

NOD mice were used. Intravenous or intradermal injection of LPSw (10 mg/mouse) was given once a week and observation was made for 35 weeks. Occurrence of the disease was delayed, and survival percentage was remarkably enhanced.

Analgesic effect[27] *and blocking of drug addiction*[28]

Adjuvant-induced arthritis in rats can be prevented by LPSw when given 4 days before the induction of this condition. This is comparable with the effect of phenylbutazone, but the analgesic effect is greater. The writhing syndrome was used for the test. Duration of the effect was far longer than that of phenylbutazone. Such analgesic effect is obtained due to the production of β-endorphin; the analgesic effect is blocked by naloxone. Endorphin can be transcribed, translated, and processed together with ACTH, which is known to be induced by endogenous TNF production. Actually, we were able to identify β-endorphin in mice by the administration of LPSw. Based on this result, a curative effect on drug addiction would be possible with LPSw or LPSp.[34]

Hyperlipidemia[29]

Hyperlipidemic (WHHL) rabbits, which were originally raised by Professor Watanabe and given to us by Professor Takano, were used. We gave them a crossover examination. They were given drinking water with or without LPSw. Water with LPSw was given to one rabbit until the 50th day and to the other rabbit from the 80th day. Cholesterol and LDL content in the serum decreased upon administration of LPSw, and the trace of cholesterol content in the serum of the two rabbits crossed over on about the 100th day.

Experimental ulcer[30]

LPSw provided fairly good protection from the occurrence of an experimental ulcer induced by indomethacin or water stress in mice. Before the stress, LPSw (1 mg or 100 ng/ml) in drinking water was given *ab libitum* for 4–6 days. The preventive effect as expressed on an ulcer index was marked, and this effect was also observed in indomethacin and water stress.

Preventive effect of toxoplasma[31]

A preventive effect of toxoplasma was observed even when an extraordinarily small amount of LPSw (20 ng/ml in drinking water *ad libitum*) was given to mice continuously.

Therapeutic effect of LPSw on viral infection

Because TNF at the molecular level can recognize virus-infected cells,[32–34] we applied 1–10 mg/m^2/day of LPSw liniment directly onto the lesion of a human herpes (type 1, 2, or zoster) patient.[27] The therapeutic effect was remarkable. Most of the 50 patients treated to date recovered within 1 to 2 weeks. The effect has not been restricted to acute infection, but has included chronic infection. A remarkable point is that pain occurring as a sequela can be overcome by percutaneous administration of LPSw. Mechanism of the analgesic effect may involve the endogenous production of endorphin as explained earlier.[27]

This result has encouraged us to try the therapy on other virus-infected diseases, including influenza, the common cold, and even acquired immunodeficiency syndrome. A small amount of TNF or precursor TNF in or on the surface of a primed macrophage can recognize even the slightest difference that exists between virus-infected and normal cells.[32–34]

Discussion

Our original starting point was in Metchinikoff's phylogenic concept of inflammation. We have developed a new concept, ontogenic inflammation, where TNF is automatically and endogenously produced as a self-regulatory system.[1–3] Reproduction of this ontogenic inflammation in an adult may benefit the body by regulating homeostasis. We have found two ways to reproduce the ontogenic inflammation in an adult: one is to induce the primed stage of endogenous TNF production, and the other is to induce the triggered stage. Used therapeutically, these two stages can be applied to cure or prevent various intractable diseases, by the primed stage as described in this article, and by the triggered stage for cancer in EET therapy.[4,5,19,21]

A single event, the activation of macrophages based on the endogenous production of TNF at the primed stage, can lead to a multiple curative effect in many intractable diseases. This is an innovative concept, because drugs have conventionally been developed aim-

ing at the relation of one drug versus one disease. Therefore, the activation of macrophages at this stage is very important. So far, we have found precursor TNF on the membrane surface of the macrophage with no apparent excretion of free TNF.[15]

Because TNF or its precursor is automatically and endogenously produced in a mouse fetus (i.e., ontogenic inflammation takes place in ontogenesis automatically), TNF may form a prototype of all inflammations. It obviously plays an important role in maintaining homeostasis in this stage with its own intrinsic factors. This suggests that a fetus may excrete an LPS-like substance intrinsically, a negatively charged amphiphilic substance (e.g., a ganglioside), to prime the entire regulatory process of ontogenesis.

That a single use of the LPS with small molecular size from wheat flour has curative impact on various intractable diseases opens an innovative gateway not only for therapeutic treatment but for fundamental biological sciences such as embryogenesis or morphogenesis, which clearly involves ontogenic inflammation.

As is well known, Coley's toxin[35] is applicable even now in cancer patients;[36,37] however, it is not so highly recommended because of its high toxicity when used parenterally. We have shown that LPS of small molecular size (~5 kD) can be active when administered orally or percutaneously, whereas conventional LPS from *Escherichia coli* (~50 kD) is active only in parenteral use and then with high toxicity. A trial on cancer therapy with LPS in percutaneous use is now underway, and we are very hopeful about the results. Aside from this, our therapy of oral or percutaneous administration of LPS of small molecular size may be viewed as a new version of Coley's toxin, but without toxicity. This new version of Coley's toxin appears to hold promise in a different way, far from those expected by Coley,[35] Starnes,[36] and Rook.[37]

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