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論文内容の要旨

The commensal gut micobiota confer health benefits to their host by helping dietary digestion, modulating gut immunity, maintaining the microbial balance, preventing pathogen colonization, and attenuation of gastrointestinal (GI) diseases. Recently many studies have reported that certain commensal bacteria may also be able to regulate immune responses outside of the GI tract. Among them, species of lactobacilli and bifidobacteria are prominent commensal bacteria with the anti-carcinogenic and anti-allergic properties, control of serum cholesterol levels, and improved lactose utilization in individuals with lactose intolerance. *Lactobacillus acidophilus* strain L-92 is an industrially important strain that have been used as a functional food and beverage. Some studies reported that heat-killed L-92 possesses anti-allergic properties, although its physiological function on allergic reaction has largely undefined. This study investigated the anti-allergic efficacy of heat-killed L-92 using four different murine models with the major features of allergic reaction including Th1 and Th2-associated immune responses and compared it to the effects of clinically active drugs. The anti-allergic mechanism of L-92 was also examined.

Anaphylaxis is a life-threatening immediate type of allergic reaction. Therefore, two murine models of skin anaphylaxis were used: the passive cutaneous anaphylaxis (PCA) and the active cutaneous anaphylaxis (ACA). In the PCA model, ICR mice were passively sensitized by anti-dinitrophenyl (DNP) mouse monoclonal IgE, and BALB/c mice were actively sensitized by ovalbumin (OVA) for the ACA model. Also, allergic contact dermatitis (ACD) and atopic dermatitis (AD) models were used based-on Th1 and Th2-associated immune responses, respectively. Allergic dermatitis in ACD and AD models was induced by repeated applications of 2,4-dinitrofluorobenzene (DNFB) and mite (*Dermatophagoides farinae*) fecal allergen, onto the BALB/c and NC/Nga mouse ear, respectively, causing ear swelling, severe itching, elevation of total serum IgE, increase in mast cell number, eosinophil infiltration, and increased Th1/Th2 cytokine expression. The results showed that orally administered L-92 significantly inhibited vascular permeability increase in both PCA and ACA, and elevation of OVA-specific IgE titer in ACA. In addition, L-92 treated mice exhibited lower levels of mast cell number, eosinophil infiltration, and Th1/Th2 cytokine expression in comparison with control group, while moderate suppression of ear swelling, scratching behavior and elevation of total serum IgE levels were also observed. However, despite some pre-clinical studies on animal

models as well as clinical trials have highlighted the beneficial roles of L-92, but the exact mechanisms behind the anti-allergic effects has not been investigated. Therefore, it has become increasingly important to delineate the anti-allergic mechanisms of L-92. Recently numerous studies have noticed that $CD4^+CD25^+Foxp3^+$ (forkhead box P3) T regulatory (Treg) cells play important roles in controlling allergic diseases. Hence, the effect of L-92 treatment on $CD4^+CD25^+Foxp3^+$ cell populations was examined. DNFB was used to induce ACD in BALB/c mice. Fluorescent-activated cell sorter (FACS) analysis was used to determine $CD4^+CD25^+Foxp3^+$ T cell populations in spleen and cervical lymph nodes (CLN). Interleukin-10 (IL-10), transforming growth factor-β (TGF-β), and Foxp3 mRNA expressions in mouse ear skin were investigated by real-time reverse transcription-polymerase chain reaction (RT-PCR). The percentage of $CD4^+CD25^+Foxp3^+$ T cell populations were significantly increased in both spleen and CLN of L-92-fed group than vehicle and control. In addition, L-92 produced higher levels of Foxp3, IL-10 and TGF-β compared to control mice.

In conclusion, L-92 exerts immunomodulatory effects by up-regulating the generation of Tregs, which prevents IgE-mediated hypersensitivity, modulates Th1/Th2 balance, and acts as an anti-allergic agent *in vivo*. But, the anti-allergic effects of L-92 were generally modest in comparison with clinically active drugs. Therefore, it might be useful as preventive food supplements for allergic patients rather than therapeutic entities

論文審査結果の要旨

本論文は乳酸菌の 1 種、*Lactobacillus acidophilus* strain L-92 (L-92、熱処理した死菌) のアレルギー疾患に対する有用性を、マウスアレルギー性皮膚炎モデルを用いて検討した成績をまとめたものである。ICR マウスの IgE 依存性受身皮膚アナフィラキシー (PCA)、卵白アルブミン (OVA) で免疫した BALB/c マウスの能動皮膚アナフィラキシー (ACA)、2,4-dinitrofluorobenzene (DNFB) を耳殻へ反復曝露して誘発する BALB/c マウスの皮膚炎モデル、およびダニ抗原を耳殻へ反復暴露して誘発する NC/Nga マウスの皮膚炎モデルを用い、L-92 の経口投与が、PCA および ACA による血管透過性亢進を抑制し、OVA 免疫による血中特異 IgE の上昇を抑制する傾向を示すこと、DNFB あるいはダニ抗原反復塗布によって誘発される耳殻腫脹、炎症細胞浸潤、血中 IgE の上昇、Th1 および Th2 サイトカインの発現、 および掻破行動に対して抑制あるいは抑制の傾向を示すことを明らかにした。 さらに、DNFB 反復塗布皮膚炎モデルを用い、L-92 投与が脾臓および頸部リンパ節中の $CD4^+CD25^+$ forkhead box P3 $(Foxp3)^+$ T 細胞 (制御性 T 細胞) の割合を増大させること、 耳殻 において Foxp3、interleukin-10 および transforming growth factor-β の mRNA 発現を増大させることを明らかにした。

以上の成績は、L-92 のアレルギー性皮膚炎に対する抑制効果は緩和であると推定されるが、制御性 T 細胞を誘導して抗アレルギー作用を発揮する可能性を示し、アレルギー症状の軽減に有用であることを示唆するものであり、博士論文として価値あるものと判断した。

最終試験結果の要旨

Shah 氏の学位論文の主要部分は審査付き学術雑誌に公表済みの 2 編の論文に基づくものであり、本論文が学位論文として完成された内容を有することを確認した。

また、公聴会において、学位論文の内容に関し、乳酸菌の抗アレルギー作用と作用機序、臨床応用の可能性などについて試問を行った。申請者から十分な内容の回答が得られたため、最終試験に合格したものと判定した。

論文リスト

1. Mohammad Monir Shah, Yoshihiro Miyamoto, Yoshihito Yamada, Hirotaka Yamashita, Hiroyuki Tanaka, Takayuki Ezaki, Hiroichi Nagai, Naoki Inagaki. Orally supplemented *Lactobacillus acidophilus* strain L-92 inhibits passive and active cutaneous anaphylaxis as well as 2,4-dinitrofluorobenzene and mite fecal antigen induced atopic dermatitis-like skin lesions in mice. *Microbiol Immunol* 54: 523-533, 2010. [IF: 1.227]
2. Mohammad Monir Shah, Masanao Saio, Hirotaka Yamashita, Hiroyuki Tanaka, Tsuyoshi Takami, Takayuki Ezaki, Naoki Inagaki. *Lactobacillus acidophilus* strain L-92 induces $CD4^+CD25^+Foxp3^+$ regulatory T cells and suppresses allergic contact dermatitis. *Biol Pharm Bull*, in press. [1.811]