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学位論文名	**食物アレルギーにおける腸内細菌叢と腸管上皮バリア機能：細胞間接着分子の解析**
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論文内容の要旨

1　研究目的

Food allergy is one of the serious public health problems and its incidence is increasing in children. The intestinal tract is considered to be a barrier to food allergens in cases of food allergy, thus increased intestinal permeability is thought to be associated with the pathogenesis of food allergy. The increase in intestinal permeability altered by internal and external environmental stimuli allows the regulations of microbiota and immune system dysfunctions. However, the mechanisms underlying the relationship between intestinal epithelial barrier function and regulation of gut microbiota in the context of food allergy have not been precisely clarified. The aim of the present study was to explore the mechanism responsible for changes in the morphology and function of the intestinal barrier using a juvenile rat model of food allergy, focusing on the contribution of intestinal microbiota and the effects of probiotics (*C. butyricum*, *L. reuteri*, and *B. breve*) and antibiotics.

2　研究方法

Juvenile rats were divided into an ovalbumin (OVA) sensitization group and a control group, and both were subdivided into those receiving added antibiotics or probiotics (*C. butyricum*, *L. reuteri* and *B. breve*). The serum OVA-IgE levels were determined, and histopathological features were studied using electron microscopy and immunofluorescence staining with antibodies against tight junction (TJ)-associated proteins. Intestinal permeability was assessed using a lactulose/mannitol assay kit, and expression of mRNAs for TJ molecules was analyzed by real-time PCR. Gut microbiota in feces was analyzed on the basis of 16S rRNA metagenomics sequences.

3　研究成果

OVA-IgE levels and intestinal permeability were significantly increased in the sensitized rats

relative to the controls. Although the gut mucosae were inflamed in the sensitized groups, those in rats that had received probiotics were only mildly affected. Expression of mRNAs for TJ molecules was down-regulated in rats given OVA with antibiotics, but not in those given probiotics. Immunofluorescence staining of TJ proteins was decreased in the OVA groups. TJs in rats treated with OVA and antibiotics were disrupted, but those in rats administered probiotics were not damaged. Clostridiaceae were increased in all the probiotics groups relative to the OVA-sensitized group.

4 考察

In the present study using juvenile rats with food allergy as a model, we found that not only antigen (OVA) sensitization but also antibiotic treatment induced leaky gut, causing allergen absorption and food allergy, presumably through significant expression of key TJ proteins. Moreover, administration of probiotics prevented this increase in intestinal permeability, presumably through an increase in Clostridiaceae and also a significant influence on the expression of TJ proteins. The results of our lactulose/mannitol assay showed that intestinal permeability was increased after OVA sensitization, and this was further confirmed by electron microscopy (shortened and widened intercellular TJs of intestinal villus epithelial cells). These changes in OVA-sensitized rats were not prevented by administration of antibiotics but were prevented by treatment with probiotics, as further confirmed by electron microscopy. These findings are in line with a previous report indicating that TJ and adherens junction microstructure is markedly disrupted and widened after OVA sensitization and treatment with antibiotics. TJ proteins play a pivotal role in the regulation of intestinal permeability and barrier function. Our real-time PCR study showed that OVA down-regulated the expression of ZO-1, occludin, and claudin-8, -9, and -15. These findings are in line with a previous similar study of OVA-sensitized rats, which demonstrated significant down-regulation of the TJ-mediating proteins ZO-1, and claudin -8 and -15. These results suggest that OVA induces damage to the intestinal barrier, and that TJ permeability is related to the expression and regulation of these proteins. On the other hand, treatment with *C. butyricum* and *B. breve* up-regulated the expression of both occludin and claudin-15. The microbiota of OVA-sensitized rats was significantly more diverse than that of each of the probiotic treatment groups. Clostridiaceae were also significantly enriched by treatment with *C. butyricum*, *L. reuteri*, and *B. breve*. *C.*

butyricum can produce butyrate, which is one of the SCFAs and the main energy source for enterocyte regeneration. We found that OVA sensitization alone changed the composition of gut microbiota in comparison to controls, in terms of the relative abundance of bacterial phyla. This is considered to indicate that the gut mucosal immune system can affect the composition of gut commensal bacteria. In other words, there is a bidirectional interaction between gut microbiota formation and the mucosal immune system.

5 結論

Eradication of commensal bacteria is able to promote OVA sensitization through an increase of intestinal permeability by influencing TJ protein regulation. On the other hand, administration of probiotics is able to suppress the level of sensitization by maintaining intestinal permeability and regulating the expression of TJ proteins. Our findings support the use of probiotic supplementation to prevent or treat food allergy. Those findings help to lay the groundwork for future research on food allergy and gut microbiome crosstalk, maintenance, function and development disease.

論文審査の結果の要旨

食物アレルギーはここ 20 年間で有病率が上昇しており、重要な公衆衛生分野の課題となっている。本学位論文は食物アレルギーのラットモデルを用いてその機序を明らかにする研究である。

4 週齢のオス SPF ラットをオボアルブミン（OVA）経口感作群と対照群（PBS）群に分け、それぞれを無処置群、抗菌薬投与群、プロバイオティクス（C.butyrium）投与群、プロバイオティクス（L. reuteri）投与群、プロバイオティクス（B. breve）投与群の 4 群の計 8 群を作製した。経口感作は 48 日間、低用量（1 mg）の抗原を投与し、第 49 日に高用量（100 mg）の抗原を投与した。第 50 日に組織などを回収した。第 0、14、28、50 日に採血し、血清を保存した。第 14、28、50 日に EnzyChrom intestinal permeability assay kit を用いて腸の物質透過性検査を施行した。ヘマトキシリン-エオジンによる組織染色、透過型電子顕微鏡による空腸 tight junction、Adherent junction の観察、抗 tight junction タンパク抗体による免疫染色、Real-time PCR による tight junction タンパク mRNA の解析、糞便 DNA を用いた細菌 16S rRNA の解析を行った。プロバイオティクス投与は IgE 抗 OVA 抗体の産生を抑制し、腸粘膜の炎症を改善し、tight junction の形態も保たれた。また、プロバイオティクス投与は OVA 感作群に比べて、糞便 Clostridiaceae が増加していた。

プロバイオティクスが炎症を抑制する機序として、プロバイオティクス投与が IgE 抗 OVA 抗体の産生を抑制している観点から腸管アレルギーの炎症そのものを抑制し、その結果として、tight junction の形態が保たれ、糞便 Clostridiaceae が増加した可能性と Dysbiosis（腸内菌共生

バランス失調）の改善が腸管上皮機能を回復させ、腸管から粘膜下への物質の移動を阻止し、アレルギー炎症が減弱する可能性が審査委員から指摘された。

食物アレルギーにおけるプロバイオティクスの役割を解明するために行われた膨大な量の研究とその成果は学位授与に十分値すると考えられた。プロバイオティクスの効果発現機序の考察、図の鮮明化などの委員から指摘された部分が修正され、本論文を全員一致で合格と判定した。

最終試験の結果の要旨

最終審査会において、論文内容について明快かつ時間内にプレゼンテーションがなされ、審査委員からの質問にも真摯に回答していた。研究内容に関してもよく理解し、研究者として充分な資質・能力を有することは明らかであった。