Regulation of allergic reactions of mast cells by intestinal bacteria and probiotics

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Research aims

People suffering from allergic diseases have dramatically increased in recent years. Mast cells, which are responsible for allergic inflammation, express the high affinity IgE receptor (FcεRI). Crosslinking of FcεRI with IgE and allergen induces degranulation, which is the release of inflammatory mediators such as histamine stored in intracellular granules.

Mast cells are differentiated in the bone marrow, released into blood as immature cells, and finally differentiated into mature cells after reaching peripheral tissues such as the mucous membrane and skin. The intestinal tract, which has a vast mucosal area and functions as a reservoir of mast cells *in vivo*, is inhabited by an enormous number of commensal bacteria. This study aimed to clarify novel mechanisms of the anti-allergic action of intestinal bacteria on mast cells for application to preventing or improving allergic symptoms through diet.

Methods

①*Regulation of granule formation in mast cells by intestinal bacteria*

We have reported that *Lactobacillus casei* JCM[1134] (LC) suppresses granule formation in mast cells through increased expression of the transcription factor C/EBPα.[1] To clarify the underlying molecular mechanisms, following experiments were performed. Bone marrow cells from femurs of female C57BL/6 mice at 6 weeks of age were cultured for 4 to 8 weeks in medium supplemented with IL-3 to obtain bone marrow-derived mast cells (BMMCs). BMMCs were stimulated with LC, and the expression of molecules involved in granule formation was analyzed by qRT-PCR. Alternatively, an expression plasmid for C/EBPα was introduced into BMMCs by electroporation and similar analyses were performed. In addition, BMMCs were cultured in the presence of fractionated components of LC for 24 h, followed by measurement of C/EBPα expression by qRT-PCR. The fractionation method is shown in Fig. 1

②*Regulation of IgE receptor expression on mast cells by intestinal bacteria*

We have found that *Bacteroides acidifaciens* type A43 (BA) suppresses the cell surface expression of FcεRI on BMMCs among a variety of heat-killed intestinal bacteria. To clarify the underlying mechanism, the effects of BA on mRNA and total protein expression of each subunit of FcεRI and the cell surface expression of FcεRI were analyzed in BMMCs by qRT-PCR, western blotting, and flow cytometry, respectively. The influence of BA on activation of signal molecules such as MAPK in BMMCs was also analyzed by western blotting.

Results

①*Regulation of granule formation in mast cells by intestinal bacteria*

The expression of HDC, serglycin, and Ctr2 was decreased in BMMCs treated with LC. Overexpression of C/EBPα in BMMCs resulted in decreased expression of serglycin, but not that of HDC and Ctr2. Therefore, serglycin expression was suppressed depending on C/EBPα, whereas suppression of HDC and Ctr2 expression was independent of C/EBPα. These results are summarized in Table 1. It was suggested that suppression of granule formation by bacterial stimulation is achieved by reduction in proteoglycans such as chondroitin sulfate and heparin that constitute the granules. In addition, the cell wall fraction of LC enhanced the C/EBPα expression, and teichoic acid particularly showed a strong enhancing activity in the cell wall fraction.

②*Regulation of IgE receptor expression on mast cells by intestinal bacteria*

Lasting stimulation of BMMCs with BA decreased the cell surface expression of FcεRI on BMMCs. On the other hand, mRNA expression of FcεRI α and β chains decreased transiently at the early stage of stimulation, although a statistically significant difference was not observed. In addition, during the stimulation period, there was no change in the total protein expression level including the intracellular content of the β chain. Furthermore, Erk phosphorylation tended to be suppressed at the early stage of stimulation. Addition of an Erk inhibitor to BMMCs also decreased the expression of FcεRI on the cell surface. Collectively, it was thought that BA decreases the expression of FcεRI on mast cells mainly by posttranslational control via inhibition of Erk activation.

Conclusion

The following regulatory mechanisms of mast cells by intestinal bacteria were shown. (1) Specific bacterial components suppress granule formation in mast cells by reducing the proteoglycans constituting the granules in a C/EBPα-dependent manner. (2) Specific intestinal bacteria suppress the cell surface expression of FcεRI on mast cells mainly at the post-translational stage through inhibition of Erk activation. These mechanisms will provide novel targets for evaluating the anti-allergic activity of probiotics.

References

1. Kasakura, K. *et al.* (2014) C/EBPα controls mast cell function. *FEBS Lett.* **588**: 4645-4653.

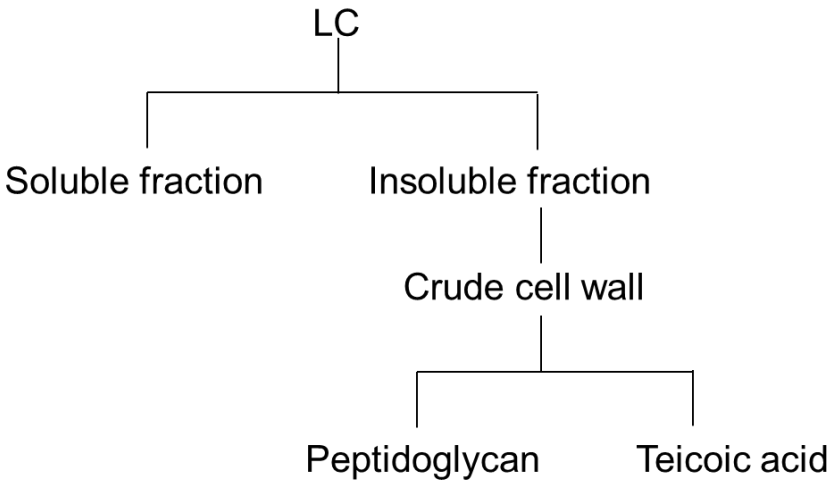

Fig. 1 Fractionation of LC

LC cells were disrupted by sonication and centrifuged to obtain soluble and insoluble fractions. Crude cell wall was prepared from the insoluble fraction by removal of lipids and treatment with pronase and nuclease. Peptidoglycan and teicoic acid were purified from the crude cell wall.

	LC stimulation	C/EBPα overexpression	C/EBPα dependency
HDC	↓	→	-
serglycin	↓	↓	+
Ctr2	↓	→	-

Table 1 Effect of LC stimulation and C/EBPα overexpression on the expression of molecules involved in granule formation

BMMCs were simulated with LC, or C/EBPα expression plasmid was introduced into BMMCs. Expression of HDC, serglycin and Ctr2 was measured by qRT-PCR.

↓: Decreased expression
→: no change
- : independent
＋: dependent