Effects of endotoxin on pathology of rheumatoid arthritis

KITAMURA Kaori

Microflora composition of rheumatoid arthritis (RA) patients is different from that of healthy people, the increase of *Prevotella* and the decrease of *Bacteroides* species. Dysbiosis, which occurs when the microbiota is imbalanced, causes gut barrier damage resulting in a leaky gut. The leaky gut results in bacterial translocation, which is the passage of bacteria and its toxic components (such as LPS) from the gastrointestinal tract to inner sites such as the mesenteric lymph node and blood, and then triggers systemic inflammation. However, there are few researches how the bacterial substances have effects on the RA pathology. The purpose of this study is to reveal the involvement of excess inflammation induced by LPS in the onset and development of RA. The correlation between RA pathological scores and gut bacterial substances was comprehensively examined. In addition, I focused on the lipopolysaccharide (LPS), the component of the gram-negative cell wall, and examined the effects of different kinds of LPS on the inflammation in the intestinal tract and in the body *in vitro*.

In Study 1, the correlation of the intestinal bacteria and LPS-related markers with the pathology of RA (disease activity and disease biomarkers) was examined in 87 RA patients. There was no correlation between intestinal bacteria and RA pathological scores. In contrast, serum LPS binding protein (LBP) levels were positively correlated with disease biomarkers, and anti-*Porphyromonas gingivalis* (Pg) LPS IgG antibody inversely correlated with disease activity indices. Because LBP increases LPS affinity to the cells, the serum levels of LBP could reflect the status of inflammation induced by LPS. Therefore, I speculated that the increment of LPS inflow from gastrointestinal tract might be involved in increase in the RA pathological scores. In addition, Pg LPS might also affect the activity of RA in the patients.

There are two kinds of LPSs, having strong or weak proinflammatory properties, arising from its structural difference of lipid A. Thus, in Study 2, I examined the effects of *Bacteroides fragilis* LPS, a weak proinflammatory LPS, on *Escherichia coli* LPS, a strong one, using the heat-damaged human intestinal Caco-2 cell line. Treatment with *E. coli* LPS led to the decrease in trans-epithelial electrical resistance (TEER) level. In contrast, treatment with *B. fragilis* LPS inhibited cell damage and significantly recovered the TEER level compared with *E. coli* LPS. Furthermore, TEER level of the Caco-2 cells treated with both *B. fragilis* LPS and *E. coli* LPS was significantly increased compared with that of cells treated with *E. coli* LPS alone.

Furthermore, I investigated the effect of *B. fragilis* LPS on *E. coli* LPS-induced cytokine production using human THP-1 monocytic cell line and human peripheral blood mononuclear cells (PBMC). *E. coli* LPS significantly induced cytokines production from both cells. Simultaneous administration of *B. fragilis* LPS and *E. coli* LPS, or administration of *B. fragilis* LPS prior to *E. coli* LPS, significantly

suppressed cytokines production from THP-1 cells and from PBMC compared with administration of *E. coli* LPS alone. Similar results were obtained when autoclaved feces were used instead of *E. coli* LPS to induce cytokine production.

These results show that *B. fragilis* LPS may promote cell proliferation, reduce the cell damage by heating, and alleviate inflammation induced by proinflammatory LPS such as *E. coli* LPS. In previous study using a murine model of RA, we found that simultaneous administration of high dose *B. fragilis* LPS or prior administration of *B. fragilis* LPS suppressed *E. coli* LPS-induced arthritis onset. Concomitant with our previous findings, present study suggests that the influx of strong proinflammatory LPS in the body could be one of the causes of aggravation of RA, and that increasing the weak proinflammatory LPS in the gut could be a therapeutic way to control RA.