潰瘍性大腸炎における粘膜細菌 *Fusobacterium varium* の病原性

F. varium is one of the elusive pathogenic factors in ulcerative colitis

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【目的】我々は、潰瘍性大腸炎（UC）の病変粘膜から分離された *Fusobacterium varium*（*F. varium*）が UC 患者の病変粘膜に特異的に付着していること、同菌が酪酸を産生し、その酪酸は Vero 細胞毒性を示し、その注腸により大腸潰瘍、大腸炎ができること。また、同菌に対する血清抗体価が UC 患者で有意に高値であることなどから、*F. varium* が UC の病原菌の一つではないかと報告してきた。今回、さらに病原性を明らかにするため、同菌の細胞侵入性や炎症性サイトカイン産生刺激作用を検討し、また、同菌を target とし、UC 患者に対して抗菌剤多剤併用療法を施行したので、その治療成績も含めて報告する。
【方法】1)大腸粘膜培養細胞 SW480 と *F. varium*(UC 粘膜分離株、ATCC8501), *E.coli, H.pylori(*ATCC43504), L.johnsonii(LC1), L.delbrueckii spp. bulgaricus(LB-021001)を $1.5X10^8$/well と共培養（1,3 時間）して、細胞接着能、侵入能（回収培養菌量／負荷菌量%）とサイトカイン産生刺激能（IL-8, IL-6, IL-18, MCP-1)を検討した。
2）同菌に感受性の抗菌剤 3 剤（AMPC, TC, metronidazole)を 2 週間投与して、RCT を行いその治療効果を検討した。
【結果】1) *F. varium* は粘膜分離株と ATCC8501 共に、$0.6\text{-}2.9X10^4$ CFU/mL と細胞侵入を認め、IL-8 も 54-146 pg/mL と産生されていた。また、IL-18 は UC 粘膜分離株でのみ 37.2-51.5 pg/mL と産生されていた。IL-6 と MCP-1 についてはほとんど検出限界以下であった。接着能では、$0.2\text{-}4.2X10^4$ と認められ、UC 粘膜分離株の 1 株で 34.5-35.4 pg/mL と IL-18 が検出された。一方、他の菌では、*H.pylori* で $1.0\text{-}2.3X10^4$ と細胞侵入性を認め IL-8 も 54-73.3 と産生されたが、IL-18 をはじめ、IL-6 と MCP-1 については検出限界以下であった。接着能も $1.5\text{-}4.2X10^4$ と見られたが、サイトカイン産生は見られなかった。E.coli と L.bulgaris については、接着能のみで、細胞侵入能は見られず、サイトカイン産生も認められなかった。LC-1 菌については、細胞侵入能も接着能も $0.6\text{-}3.9X10^4$ と認められたが、サイトカイン産生はなかった。
2）対照群と比較すると、症状では抗菌剤投与群で 3 ヵ月後に有意な改善が認められ、12 ヶ月後も改善が持続していた。また、内視鏡所見、病理所見でも投与群で投与 3 ヵ月後、12 ヶ月後と有意な改善を認めた。*F. varium* の血清抗体価と抗体染色による粘膜菌数についても投与群で 3 ヵ月後、12 ヵ月後と有意な低下が認められた。
【結論】*F. varium* が大腸粘膜に付着、侵入して炎症性サイトカインを産生させることが明らかとなった。また、同菌を target とした抗菌剤多剤併用療法が UC に対して有効であったことから、*F. varium* は UC の原因菌の１つであることが確認された。

F. varium is one of the elusive pathogenic factors in ulcerative colitis

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Background & Aim: Recently, we proposed that intestinal bacteria in the adjacent intestinal mucosa (mucosal bacteria), and in particular, *Fusobacterium varium* might be one of the elusive pathogenic factors in ulcerative colitis (UC). However, whether *F. varium* cause inflammation of the intestinal mucosa has not been determined. In this study, (1) we investigated bacterial penetration and the secretion of proinflammatory cytokines in human colon epithelial cells in response to *F.varium*. Also, (2) we conducted a randomized controlled trial using antibiotics combination therapy in order to confirm a causal relationship between *F. varium* and UC.
Methods: (1) Human colonic epithelial cells (SW480, ATCC) were cocultured with bacteria isolated from UC (*F.varium, E.coli*), *H.pylori* (ATCC 43504), and probiotics (*Lactobacillus johnsonii* (LC1), Nestle Japan Co., *L.delbrueckii subsp. bulgaricus* (LB-021001), Meiji Dairies Co., Japan). Cell cultures were infected with bacteria and resuspended in a 1:1 mixture of DMED and Ham's F-12 medium containing 10% FBS at a bacterium/cell ratio of 100:1. The co-cultures further incubated for 1 h to allow bacterial adhesion to cells, and the supernatants were collected for attachment assay. After removal supernatants, the monolayers were washed four times with PBS, lysed with water, and total cell-associated bacteria enumerated on agar plates. For invasion assays, the incubation was continued for 3h to allow bacterial entry to occur. After removal of the extracellular bacteria, the cultures were incubated for 1 h in the presence of 50 μ g/mL of imipenem to kill the remaining extracellular bacteria. Subsequently, the supernatants were removed and the cells were lysed with 0.1% SDS in isotonic saline, and the number of internalized bacteria released was determined by culturing for 3 days on agar plates. The IL-8, IL-6, IL-18 and MCP-1 concentrations in the supernatants were determined with ELISA kits. (2) Twenty chronic, active UC patients with *F. varium* infection were enrolled consecutively and were randomly assigned to receive either amoxicillin, tetracycline and metronidazole per os for two weeks (treatment group; n = 10), or no antibiotics (control group**;** n = 10). *F. varium* was sensitive to the antibiotics. Symptom assessment, endoscopic, and histological evaluations were performed blind before enrollment, at 3-5 months and 12-14 months after the treatment. Serum immunoglobulins to *F. varium* were measured using ELISA.

Immunohistochemical detection of *F. varium* in biopsy specimens was performed with the avidin-biotin complex method.

Results: (1) In invasion assays, *F. varium* was recovered from SW480 cells at 0.6-2.9×10^4 CFU/mL. IL-8 concentrations after exposure to *F.varium* were 54-146 pg/mL and IL-18 concentrations after exposure to two *F.varium* strains from UC were 37.2-51.5 pg/mL. IL-6 and MCP-1 concentrations after exposure to all bacteria strains were below 0.150 and 31.3 pg/mL. In attachment assays, *F. varium* was recovered at 0.2-4.2×10^4 CFU/mL. Only IL-18 was detected as 34.5-35.4 pg/mL in one *F.varium* strains from UC. In invasion assay, *H. pylori* were recovered from SW480 cells at 1.0-2.3×10^4 CFU/mL, and IL-8 concentrations after exposure to *H.pylori* were 54.0-73.3 pg/mL. In attachment assays, *H. pylori* were recovered at 1.5-4.2×10^4 CFU/mL, however the cytokines were not detected. *E.coli* and *L. delbrueckii subsp. bulgaricus* did not enter the SW480 cells, and no cytokines were detected in the supernatants. L.johnsonii was recovered at 0.6-3.9 ×10^4 CFU/mL, however the cytokines were not detected. (2) The clinical activity, endoscopic and histological scores in the treatment group decreased significantly at 3-5 and 12-14 months after the end of treatment compared with those in the control group (p=0.001-0.036). The remission rate in the treatment group was higher than in control group (p=0.037). In addition, the titers of antibody to *F. varium* and the *F. varium* density in the mucosa decreased at both the short and long-term follow-ups in the treatment group (p=0.0002-0.049).

Conclusion: *F.varium* isolated from UC invaded colonic epithelial cells and induced IL-8 and IL-18 secretion. Antibiotics combination therapy against *F. varium* was effective for ulcerative colitis. The number of mucosa-associated *F. varium* significantly decreased after the treatment. Therefore, the results support the hypothesis that *F. varium* is one of the causative agents of UC.