The efficacy of an anti-inflammatory peptide in a mouse model of Behçet's disease induced by herpes simplex virus 1

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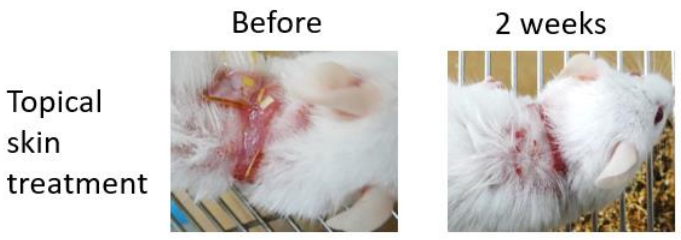
Abstract Behçet's disease (BD) is a chronic, recurrent, intractable inflammatory disease. Despite the use of several drugs, there remains a need for new treatments due to considerations of cost and efficacy. Peptides were externally administered to mice with BD symptoms for 10 days. Symptom changes were observed and recorded. Immune organs were analyzed using flow cytometry, real-time PCR, confocal, and electron microscopy. The peptide suppressed IL-17 and RORγt mRNA genes in a dose-dependent manner under *in vitro* IL-17 induction conditions. After *in vivo* administration to symptomatic mice, IL-17, RORγt, and TNFα mRNA were suppressed in peripheral blood leukocytes and spleen. Additionally, the frequency of CD83-positive leukocytes was downregulated. These effects resulted in the shrinking of ulcers and improvement of symptoms in mice. In splenocyte primary culture, the peptide was delivered intracellularly and reached the mitochondria and endoplasmic reticulum. When administered to mouse skin, the peptide reached the dermis. Transmission electron microscopy analysis revealed that macrophages in the peritoneum of BD mice administered the peptide were restored to a level similar to that of macrophages in normal mice. In contrast, macrophages from untreated BD mice were filled with intracellular vesicles and were significantly larger in size. The peptide remained detectable for more than 48 hours in the peritoneum of mice. A peptide was discovered that has the function of suppressing pathology-related cytokine molecules that cause the deterioration of BD symptoms. This peptide improved the symptoms of ulcers in BD mice and was found to be safe in a single toxicity test, confirming its potential to be developed as a treatment in the future.

Materials & Methods BD mice were induced by inoculation of HSV-1 into the scratched earlobe using the ICR mouse strain. HSV was applied twice at 10-day intervals. Mice were maintained in conventional animal facilities. The experiment was performed with the approval of IACUC. The peptides were synthesized and identified by HPLC.

Results

Fig 1. Peptides administration to BD mice



- The peptide was administered topically to the skin of Behcet's disease mice with skin ulcers.
- Administered once daily for 10 days

Fig 2. TEM of peritoneal macrophages isolated from BD mice administered peptides

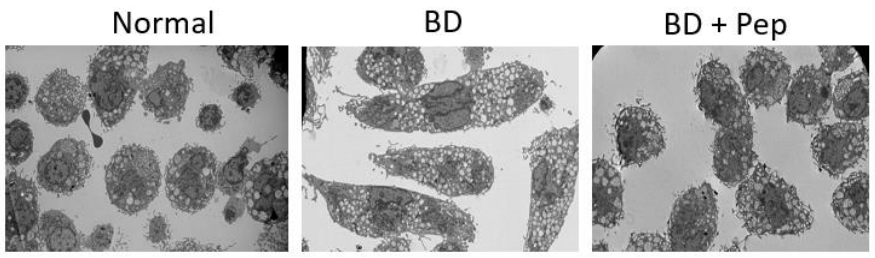
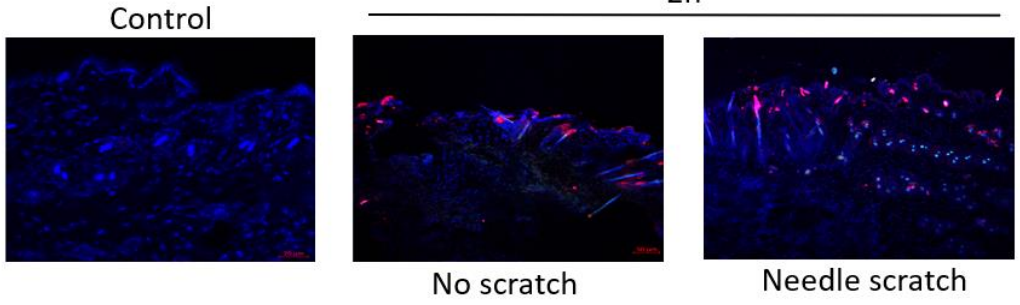
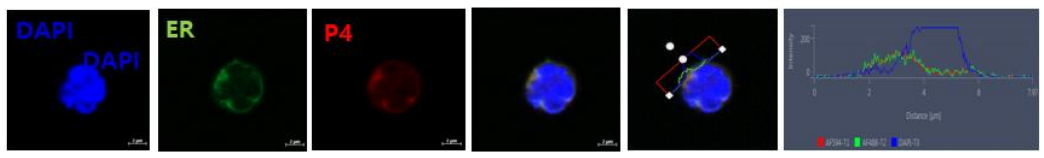


Fig 3. Topically administered peptides are delivered to the dermis



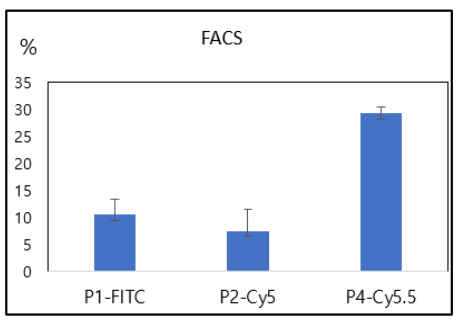
- The peptides were delivered to the dermis

Fig 4. Peptides transport into intracellular organelles after peptides treatment in primary cultures of *in vitro* spleen cells



- Peptide colocalize in ER confirmed through intensity profile

Fig 5. After 48 hours of intraperitoneal administration of peptides to mice, peritoneal cells were analyzed by FACS



- After administering the fluorescently labeled peptides to the mouse abdominal cavity, peritoneal cells were isolated and the frequencies of fluorescently labeled cells were confirmed by FACS.

Fig 6. Confirmation of peptide dose dependence in primary culture of spleen cells under Th17 induction condition by RT-PCR

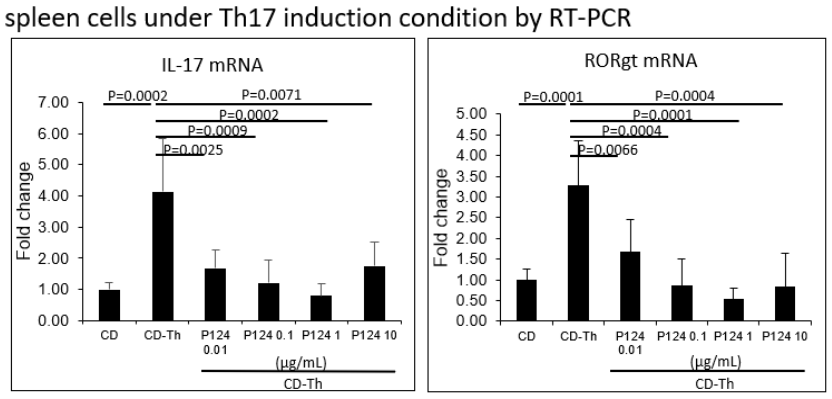


Fig 7. Changes in IL-17 and TNFα mRNA expressions after intraperitoneal injection of peptides after Th17 induction in normal mice (*in vivo*).

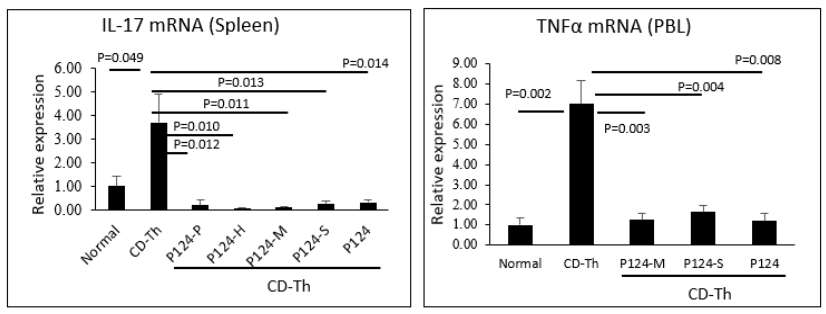
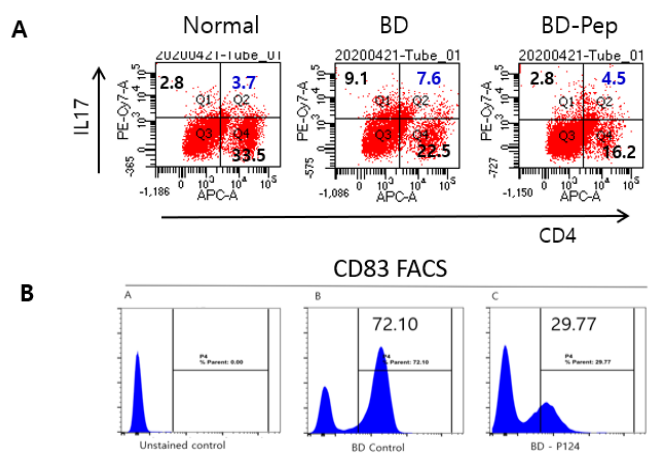


Fig 8. Changes in IL-17 and RORgT expression after peptide treatment in BD mice



- A. *(In vivo)* FACS analysis of spleen cells from BD mice administered peptides showed that the frequency of IL-17 positive cells was reduced compared to BD mice not administered peptides.
- B. *(In vivo)* After topical skin administration of peptides to BD mice, PBL were separated, cells were stained with CD83 antibody, and their frequencies were analyzed by FACS. The frequency of CD83-positive cells, a marker of dendritic cell activation, was reduced in peptide-treated BD mice.

Conclusion

- Peptides have immunomodulatory and anti-inflammatory properties
- Peptides have tissue regeneration properties
- Peptides penetrate cell membranes
- Peptides colocalize with endoplasmic reticulum within cells
- Peptides are delivered to skin tissues after application as external agents
- Peptides remain in the mouse abdominal cavity for at least 48 hours and in skin tissues for at least 2 hours