Involvement of the Mannose Receptor and p38 Mitogen-Activated Protein Kinase Signaling Pathway of the Microdomain of the Integral Membrane Protein after Enteropathogenic *Escherichia coli* Infection

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The microdomain of the integral membrane protein (MIMP) has been shown to adhere to mucin and to antagonize the adhesion of enteropathogenic *Escherichia coli* (EPEC) to epithelial cells; however, the mechanism has not been fully elucidated. In this study, we further identified the receptor of MIMP on NCM460 cells and investigated the mechanism (the p38 mitogen-activated protein kinase [MAPK] pathway) following the interaction of MIMP and its corresponding receptor, mannose receptor. We first identified the target receptor of MIMP on the surfaces of NCM460 cells using immunoprecipitation-mass spectrometry technology. We also verified the mannose receptor and examined the degradation and activation of the p38 MAPK signaling pathway. The results indicated that MIMP adhered to NCM460 cells by binding to the mannose receptor and inhibited the phosphorylation of p38 MAPK stimulated after EPEC infection via inhibition of the Toll-like receptor 5 pathway. These findings indicated that MIMPs relieve the injury of NCM460 cells after enteropathogenic *E. coli* infection through the mannose receptor and inhibition of the p38 MAPK signaling pathway, both of which may therefore be potential therapeutic targets for intestinal diseases, such as inflammatory bowel disease.

There is a widespread assumption that bacterial species that closely adhere to the mucosa are more likely to play a contributory role in major intestinal infection and inflammatory bowel diseases (IBD) (40, 43). During such diseases, the homeostasis of the gut mucosa is altered, leading to gut barrier dysfunction (2, 47). IBD is characterized by increased numbers of *Bacteroides* spp., adherent/invasive *Escherichia coli*, enterococci, and *Clostridium perfringens* and reduced numbers of *Bifidobacterium* and *Lactobacillus* species in the gut (16, 22, 53). Maintaining normal epithelial barrier function is important in resisting the invasion of pathogens. Over the past few decades, some studies have reported that treatment with probiotics, including lactic acid bacteria, has been proven to be effective in patients with colonic inflammation (26, 35, 38, 42). Bacteria belonging to the genus *Lactobacillus* that reside in the mammalian gut play an important role in maintaining homeostasis of the gut flora by adhering to and colonizing the intestinal mucosa, where they compete with pathogenic bacteria (8, 34, 36). However, the underlying molecular mechanisms of probiotics have not been fully elucidated (13).

Surface layer proteins (SLPs), including numerous identical protein/glycoprotein subunits (3), are ubiquitous cell envelope structures of lactobacilli that can spontaneously form regular layers either in a solution or on a solid support under certain conditions (46, 51). It is reported that SLPs have been investigated in regard to the interaction of lactobacilli and the intestinal epithelium (6, 10, 15). SLPs play an important part in the binding process of lactobacilli to the intestinal epithelial cells (IECs) (5, 10, 57). However, only a few studies (10, 57) have investigated the binding process of lactobacilli SLPs, which may be because lactobacilli SLPs are difficult to isolate, purify, and synthesize (59). Therefore, the roles of SLPs in the process of adhesion to the intestine remain poorly understood (24).

Our previous studies have demonstrated that *Lactobacillus plantarum* was able to prevent enteroinvasive *E. coli* (EIEC) induced damage and then confer therapeutic effects on colorectal intestinal epithelial (Caco-2) cells (41) by competitive inhibitory effects of EIEC, immunologic regulation of the reaction of dendritic cells and T cell differentiation (32), and protection of tight junction (TJ) protein structure and function both in vitro and in vivo (31, 33). Furthermore, a small functional protein domain, the microdomain of the integral membrane protein (MIMP), was identified, and its adhesion activity was verified in a normal human colon mucosal epithelial cell line, NCM460. Our recent study (28) also described the SLP functional domain, indicating that the integral membrane protein (IMP), which was responsible for adhesion to human IECs, could mediate the adhesion of *L. plantarum* strain CGMCC 1258 to IECs. Moreover, MIMP (IMP515 to -575), the small active-domain adhesive protein within IMP, was further purified and successfully characterized using bioinformatics and molecular techniques. Competitive-inhibition assays were performed in this study to further confirm the ability of MIMP to interfere with enteropathogenic *E. coli* (EPEC) adherence to NCM460 (27). Our previous data (27) also confirmed that MIMP reduced intestinal permeability and restored the expression and distribution of TJ proteins in both NCM460 cell monolayers and interleukin-10 (IL-10) (IL-10) mice. Moreover, MIMP stimulated the expression of anti-inflammatory cytokines in colonic mucosa.
and attenuated colitis in IL-10−/− mice (27). Therefore, our above-mentioned findings indicated that MIMP was the main L. plantarum component that conferred protective effects on IECs, establishing a foundation from which the anti-infective role of MIMP could be further defined. To further analyze the protective effects of MIMP on IECs, we established transiently MIMP-expressing NCM460 cells (NCM460/MIMP) that possessed EPEC anti-infective properties related to the activation of protein kinase C-γ and occludin phosphorylation (25).

The aim of our present study was to identify the receptor of MIMP on NCM460 cells and to further investigate the mechanism (the p38 mitogen-activated protein kinase [MAPK] pathway) following the interaction of MIMP and its corresponding receptor in order to explain the molecular mechanism of MIMP in protecting against barrier dysfunction of NCM460 cells.

**MATERIALS AND METHODS**

MIMP, antibodies, and major reagents. Recombinant MIMP was expressed in *E. coli* as described in our previous studies (27, 28). Anti-MIMP antibodies were prepared by immunizing an adult male New Zealand White rabbit with MIMP. Briefly, 0.3 ml (2 g/liter) of the purified protein emulsified in complete Freund's adjuvant was injected intraperitoneally at multiple points on the back and groin (200 μg at each point) for the first immunization, and half the dose in incomplete Freund's adjuvant was used for the booster immunization after 2 weeks and repeated two more times. Fourteen days after the last booster, serum was collected and purified by affinity chromatography on a 1-ml MIMP-Sepharose 4B column and stored at −20°C until use. Primary antibodies, including those against mannose receptor (MR), occludin, phosphorylated p38 MAPK, p38 MAPK, and actin, were purchased from Santa Cruz Biotechnology (California). Secondary antibodies, including horseradish peroxidase (HRP)-conjugated anti-rabbit IgG and HRP-conjugated anti-human IgG antibodies, were purchased from Sigma (Missouri). Dulbecco's modified Eagle's medium (DMEM), Iscove's modified Dulbecco's medium, fetal bovine serum (FBS), and TRIzol reagent were obtained from Gibco (California). EDTA solution, ♂-mannose, brefeldin A, and saponin were from Sigma-Aldrich (Steinheim, Germany). Anisomycin from Streptomyces griseolus (176880-MG) and p38 MAPK activator were purchased from Merck (KGaA, Darmstadt, Germany).

**Bacterial strains, culture conditions, and the infection model.** The intestinal epithelial monolayers were treated with EPEC for 24 h in the presence or absence of MIMP, as described previously (27, 28). The EPEC strain ATCC 43887 (O111:NM; Shanghai Municipal Center for Disease Control and Prevention, Shanghai, China) was grown in static DMEM at 37°C for 24 h to allow intimate adherence and pedestal formation. Quantification of bacterial density was measured at 600 nm (Beckman DU-50 spectrophotometer), along with the CFU. Furthermore, mannose was also added to prevent type 1 fimbria-mediated binding in a concentration-dependent manner (>80% inhibition in the presence of 3% mannose). NCM460 cells were purchased from Incell Corporation (San Antonio, TX) and cultured in M3 medium supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a 95% humidified atmosphere with 5% CO2. Cells were passaged at preconfluent densities with 0.05% trypsin and 0.5 mm EDTA (Invitrogen, Carlsbad, CA) (29). NCM460 cells were passaged for 24 h before further treatment.

**Quantitative real-time PCR.** Ocludin gene expression was determined by quantitative real-time PCR. Total RNA was isolated from NCM460 cells in each group using TRIzol reagent, as previously described (21), followed by DNase I treatment. The quantity and quality of RNA was verified by the ratios of absorbance values at 260 and 280 nm and by visualization of the bands on agarose gels. For each sample, 600 ng of mRNA was used in the reverse transcription reaction (iScript kit; Bio-Rad Laboratories, Hercules, CA) according to the manufacturer’s specifications. Further analysis of the mRNA levels of each group was performed by real-time PCR with a light-cycling system (LightCycler; Roche Diagnostics GmbH, Mannheim, Germany). All values were expressed as a fold increase or decrease relative to the expression of actin. The sequences of the primers were as follows: occludin, F, 5′-GACGCTCGACTGCTACAGC-3′, and R, 5′-ATGGGACTGTCAACTCTTTC-3′; β-actin, F, 5′-CTCCA TCTTGCGCTGCTGT-3′, and R, 5′-GCTGTACCTTCACGGTTTC-3′. All values are presented as the mean and standard deviation (SD).

**Western blotting.** For Western blotting, proteins were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Massachusetts) using a semidy electroblotter (Bio-Rad) for 120 min at 100 V. The membrane was then incubated with the appropriate primary antibody for 2 h at room temperature, washed three times (20 min each time) with Tris-buffered saline (TBS) containing 0.1% Tween 20 (TBS-T buffer), and then incubated for 1 h with the appropriate HRP-conjugated secondary antibody in TBS-T buffer for 4 h at 4°C. The membrane was washed three times (60 min each time) with TBS-T buffer and developed by the enhanced chemiluminescence method (ECL kit; Pierce, Illinois) according to the manufacturer’s instructions.

**Fluorescent staining.** For the fluorescent staining of cells, NCM460 cell monolayers were cultured, treated with EPEC or MIMP according to the different groups, and then fixed in acetone-methanol (1:1) at 0°C for 5 min. Fluorescent staining of occludin protein was performed using a previously described method (41). Briefly, samples prepared as described above were permeabilized in 0.2% Triton X-100 and incubated with a primary antibody and corresponding fluorescein isothiocyanate (FITC)-conjugated specific secondary antibody in 3% nonfat milk. The fluorescence was then visualized by confocal laser scanning microscopy (CLSM) (MRC 1024; Bio-Rad).

**Immunoprecipitation.** Ni-nitrotriacyclic acid (NTA) agarose beads were resuspended by alternately inverting and gently tapping the polystyrene column (Invitrogen). The 6×His-tagged recombinant MIMP protein was incubated with gentle agitation at room temperature in the prepared purification column for 1 h. The resin was then precipitated by gravity or low-speed centrifugation (800 × g), and the supernatant was carefully aspirated. The extracted membrane protein from the NCM460 cells was mixed with the resin-MIMP protein complex and incubated at 4°C overnight, followed by three washes with wash buffer (8 M urea, 20 mM sodium phosphate, pH 6.0, 500 mM NaCl). The supernatant was stored at 4°C for SDS-PAGE analysis. A negative control was included that lacked MIMP protein.

**SDS-PAGE and MS.** Immunoprecipitation samples were mixed with loading buffer containing SDS and beta-mercaptoethanol, boiled for 3 min, centrifuged, and loaded onto 4 to 20% precast Novex Tris-glycine gels in a MiniProtein II apparatus (Bio-Rad). The gels were minimally stained with Cooamassie brilliant blue R-250. The molecular weight of the protein was determined by comparing its electrophoretic mobility with those of marker proteins. By comparison with the IgG bands, the unique band in the samples was excised from the SDS-PAGE gel and analyzed by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). The MS data were initially analyzed with SEQUEST software (Thermo Fisher Scientific, San Jose, CA) and then with Trans-Proteomics Pipeline (TPP) software (Center for Systems Biology, Institute for Systems Biology, Seattle, WA) and FASTA software to further identify the protein.

**Statistical analysis.** Statistical analyses were performed using the SPSS 10.0 system (SPSS Inc., Illinois). The SD between multiple groups was assumed to satisfy a normal distribution. Data were analyzed by one-way analysis of variance (ANOVA), assuming homogeneity of variance. The Dunnett test was used for multiple comparisons between experimental and control groups. The Student-Newman-Keuls (SNK) test was employed to examine differences between two sets of data or variables between two groups. A P value of <0.05 was considered to be statistically significant.
RESULTS

MIMP relieved the decreased occludin protein levels in EPEC-infected NCM460 cells. In comparison with the uninfected control cells, a significant decrease in occludin gene expression in NCM460 cells infected with EPEC was observed. Treatment with MIMP relieved the decrease in the occludin gene expression level induced by EPEC, similarly to uninfected control cells (Fig. 1A). The results of Western blot analysis showed that the occludin protein levels were decreased in NCM460 cells infected with EPEC (Fig. 1B) compared with the control cells. However, in NCM460 cells treated with MIMP, the occludin protein levels reached a higher level than in EPEC-infected cells. Relatively low levels of fluorescence of the occludin protein were evident in the NCM460 cells infected with EPEC (Fig. 1C). In NCM460 cell monolayers treated with MIMP before EPEC infection, the intensity and distribution of fluorescence appeared similar to those in the control cells. Both the mRNA and protein expression data confirmed that MIMP prevented the decrease in occludin protein levels induced by EPEC in NCM460 cells.

Identification of the extracted SLP responsible for adhesion by MIMP. The membrane proteins were isolated from the NCM460 cells incubated with MIMP, and protein fractions were isolated using the MIMP antibody and beads. The proteins in each group were separated and detected by SDS-PAGE. There were several bands of interest with different immunoprecipitates (IPs) pulled down by the MIMP antibody compared with the proteins pulled down by IgG (Fig. 2A). A band with a molecular mass of 160 to 190 kDa, the most strongly reacting single band, was excised from the gel and subjected to LC–MS-MS, followed by analysis using the SEQUEST, Trans-Proteomics Pipeline, and FASTA software, as described in Materials and Methods. According to the ProteinProphet algorithm (29), the putative protein was identified as mannose receptor (see Figure S1 in the supplemental material). Further analysis of this band by Western blotting identified the putative target protein using a mannose receptor antibody (Fig. 2B).

Transduction signal pathway following MIMP adhesion. As shown by the Western blot results for the EPEC-infected cells compared with those for the control cells, EPEC decreased the occludin protein levels (Fig. 3A). The occludin protein level was similar to that of control cells when the EPEC-infected cells were treated with MIMP (Fig. 3A). However, when NCM460 cells were treated with EPEC, MIMP, and anti-MR, the occludin protein level was similar to that in cells treated with EPEC alone (Fig. 3A). The anti-MR antibody abolished the protective effect of MIMP on the TJ structure in NCM460 cells. The expression of p38 MAPK and phosphorylated p38 MAPK was assessed to determine whether the signal pathway was activated. As shown in Fig. 3B, infection with EPEC led to a slight decrease in p38 MAPK, and the addition of MIMP could reverse this effect (Fig. 3B). The anti-MR antibody abolished the effect of MIMP (Fig. 3B). The phosphorylation of p38 MAPK increased significantly after infection with EPEC (Fig. 3C), and the addition of MIMP abolished this effect (Fig. 3C). However, after the anti-MR was added, the effect of
MIMP was also abolished (Fig. 3C). In Fig. 3D, we investigated the ratio of phosphorylated p38 to total p38 (pp/p38) for each group and found that pp/p38 was enhanced following the addition of EPEC, which could be relieved by MIMP. The effect of MIMP could also be abolished by anti-MR ($P < 0.001$) (Fig. 3D). Figure 3A to D indicate that MIMP abrogates EPEC-induced occludin degradation and p38 activation through a mechanism that requires the MR.

MIMP blocks the TLR5 pathway through the mannose receptor. The expression levels of Toll-like receptor 5 (TLR5) and p38 were determined by Western blotting. The results showed that EPEC enhanced the expression level of phosphorylated p38, while addition of MIMP prevented the change of phosphorylated p38 and the expression of TLR5 was inhibited. However, after anti-MR was added, the effects of MIMP were abolished (Fig. 4).

**The effect of blocking the transduction signal pathways, as detected by fluorescence staining.** Anisomycin, a bacterial antibiotic isolated from *S. griseolus*, is an extremely potent activator of p38 MAPK. In our study, we also used anisomycin to activate the p38 MAPK pathway to investigate the protective effects of MIMP. The occludin protein was distributed throughout the cytoskeleton in the NCM460 cell monolayers not infected with EPEC (Fig. 5, column 1 from the left). As shown in Fig. 4B, the intensity of fluorescence of the NCM460 cells treated with EPEC was lower than that of the control cells. MIMP increased the intensity and distribution of fluorescence to a level similar to that in the control cells (Fig. 5, column 3). The anti-MR antibody inhibited the abil-
ity of MIMP to maintain the intensity and distribution of fluorescence at normal levels (Fig. 5, column 4). The intensity of fluorescence in cells treated with the p38 MAPK activator was lower than that in the control cells, indicating that anisomycin from S. griseolus could partially abolish the protective effect of MIMP by the activation of p38 MAPK (Fig. 5, column 5).

**DISCUSSION**

It has been established that probiotics have important protective effects on the intestinal barrier function (17, 56, 58). However, the SLP potency of clinical probiotics is relatively low, and the use of large doses of probiotics may increase the risk of translocation of lactic acid bacteria, especially for clinical patients with poor gastrointestinal function (14, 23, 50). Furthermore, when antibiotics are used at the same time, the antibiotics also cause damage to the probiotics, which can reduce their therapeutic effects. In recent years, studies on the adhesion mechanism of lactobacilli have shown that SLP plays an important role in their adhesion to human IECs through their protective biological function (30). It has been reported that SLP not only can mediate the adhesion of bacteria to target cells, but also can stimulate intracellular signaling transduction pathways and block bacterial adhesion by competitive inhibition of receptor activity with a similar structure (30). However, current research on SLP has focused mainly on the high-molecular-weight proteins directly, instead of the highly effective microdomain protein of SLP. As a result of larger protein fragments, an unclear domain, and an undefined mechanism, the disease resistance of SLP is still weak, and the clinical application of SLP is still limited. Our recent study showed that the SLP functional domain MIMP, which was responsible for adhesion to human IECs, could mediate the adherence of L. plantarum to IECs (28). Moreover, we further purified and successfully characterized the biological function of MIMP (27).

**FIG 4** MIMP blocks the TLR5 pathway through the mannose receptor. (A) EPEC enhanced the expression level of phosphorylated p38, while addition of MIMP prevented the change in phosphorylated p38 and the expression of TLR5 was inhibited. However, after anti-MR was added, the effects of MIMP were abolished. (B) Quantitative analysis of the different groups. The data at each time point represent the mean value and SD obtained from 3 individual NCM460 monolayers. ***, P < 0.001 compared with the control group.

**FIG 5** Protective mechanism of MIMP in NCM460 cells infected with EPEC as detected by fluorescent staining. Columns from left to right: normal NCM460 cells, normal NCM460 cells plus EPEC, normal NCM460 cells plus EPEC plus MIMP, normal NCM460 cells plus EPEC plus MIMP plus MR antibody (MRR), and normal NCM460 cells plus EPEC plus MIMP plus anisomycin from S. griseolus (ASG). The images were collected in 1-μm increments beginning at the apical aspect of the monolayers and optically sectioning to the basolateral membrane. Original magnification, ×630.
Occludin (but not ZO-1, claudin-1, or cell adhesion proteins) has been observed to be downregulated even in non-actively inflamed tissue in ulcerative colitis (1, 19, 52). Trinitrobenzene sulfonic acid (TNBS)/ethanol-induced colitis in rats caused disruption of normal immunofluorescent staining patterns of intestinal epithelial occludin (but not ZO-1 or cingulin), even though freeze fracture strand patterns seen in electron micrographs were normal (11). Another experimental colitis model, however, the IL-2 knockout mouse, showed increased occludin protein levels and increased barrier function in the colon (4). Strategies to prevent and/or reverse occludin downregulation may be an important therapeutic target. *L. plantarum* was able to antagonize the adhesion of EPEC, as it adheres to NCM460 colonic cells. In previous studies, we determined the region of MIMP responsible for maintaining intestinal epithelial barrier function and altered TJ structure, for inhibiting intestinal permeability, and for decreasing the production of proinflammatory cytokines (27). In our study, we used quantitative real-time PCR, Western blotting, and fluorescent staining to verify the protective effects of MIMP on NCM460 cells after infection with EPEC. In comparison with the uninfected control cells, a significant decrease in occludin gene expression in NCM460 cells infected with EPEC was observed (Fig. 1A, EPEC). Treatment with MIMP prevented the decrease in the gene expression level induced by EPEC, similarly to uninfected control cells (Fig. 1A, EPEC + MIMP compared with the EPEC group; *P* < 0.001). The results of Western blot analysis showed that the occludin protein levels had decreased in NCM460 cells infected with EPEC (Fig. 1B, EPEC) compared with the control cells. However, in NCM460 cells treated with MIMP, the occludin protein levels reached a higher level than in EPEC-infected cells. Relatively low levels of fluorescence of the occludin protein were evident in the NCM460 cell infected with EPEC (Fig. 1C, EPEC). In NCM460 cell monolayers treated with MIMP before EPEC infection, the intensity and distribution of fluorescence appeared similar to those in the control cells. Both the mRNA and protein expression data confirmed that MIMP prevented the decrease in occludin protein levels induced by EPEC in NCM460 cells.

To identify the MIMP receptor on the surfaces of the NCM460 cells infected with EPEC, we employed immunoprecipitation followed by mass spectrometry and used the data to analyze protein complex components and cellular protein networks. We purified a novel component of the MIMP complex, mannose receptor, and analyzed the composition of this protein to demonstrate core complex modules and several novel subcomplex interactions. Mannose receptor is a C-type lectin carbohydrate-binding protein. The structure of mannose receptor allows it to bind to high-mannose structures on the surfaces of potentially pathogenic viruses and bacteria so that they can be neutralized by phagocytic engulfment or be engulfed by the cell (12, 45). The function of this receptor is to recognize complex carbohydrates located on glycoproteins that are a part of many different biological processes, such as cell-cell recognition, serum glycoprotein turnover, and the neutralization of pathogens, mediating the endocytosis of glycoproteins by macrophages. Antigens are targeted to dendritic cells through dendritic-cell-specific receptors, and mannose receptor is one of these targets (49). By identifying the receptor for MIMP and the subsequent transduction signal pathways in this study, we found that MIMP abrogates EPEC-induced occludin degradation and p38 activation through a mechanism that requires the MR. Because there is strong evidence that p38 is upregulated in response to EPEC flagellin, we further investigated the effects of MIMP on TLR5 signal. The results showed that MIMP is capable of blocking the TLR5 pathway through the mannose receptor and then decreasing the expression level of phosphorylated p38.

p38 MAPK is a Ser/Thr kinase belonging to the family of MAPKs. The signaling pathways induced by MAPKs are responsive to stress stimuli, such as cytokines, UV irradiation, heat shock, and cell apoptosis. Activation of protein kinase C (PKC) by exposure of cultured human corneal epithelial cells to phorbol myristate acetate (PMA) (a PKC activator) has been shown to result in an increase in paracellular permeability (9). However, when the cells were treated with PMA in the presence of a specific inhibitor of MAPK kinase, all barrier characteristics were maintained, which indicated that PKC modulates TJ function via the activation of MAPK. This was further supported by the finding that occludin distribution was altered in these cells (54). Therefore, p38 MAPK is closely related to TJ proteins. The p38 MAPK pathway is important in macrophages and polymorphonuclear neutrophils (37, 44), and inflammatory stimuli, such as lipopolysaccharide and N-formyl-methionyl-leucyl-phenylalanine, also activate p38 MAPK (7, 39, 48, 60). p38 MAPK has been demonstrated to be involved in the upregulation of expression of several inflammatory genes, such as those encoding inducible nitric oxide synthase (iNOS), IL-8, and IL-6 (20, 55). As for the mechanism of MIMP stimulation of the p38 MAPK pathway, it has been reported that p38 is upregulated in response to EPEC flagellin (18). In our study, we further verified the p38 MAPK signal pathway following the interaction of MIMP and MR. We treated NCM460 cells with anisomycin from *S. griseolus*, the p38 MAPK activator, and found that it could partially abolish the protective effect of MIMP, which further verified that MIMP might exert its protective effects via the p38 MAPK signal pathway.

Our study indicated MIMP could interact with MR and then exert its protective effects on NCM460 cells. However, it has been reported that MR mainly interacts with mannose residues, such as the structures on the surfaces of potentially pathogenic viruses, bacteria, and fungi. Therefore, one limitation of our study is that the structures of MIMP and MR may not conjugate, and the mechanism of binding between MIMP and MR also needs to be further investigated. Taken together, our findings enhance our understanding of the therapeutic effects of MIMP on the cellular and molecular mechanisms involved in intestinal barrier dysfunction and intestinal inflammation during some intestinal diseases, such as IBD.

**Conclusion.** MIMP relieves the injury to NCM460 cells after EPEC infection through the mannose receptor and the p38 MAPK signaling pathway, which may be potential therapeutic targets for intestinal barrier dysfunction.

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