GRIM-19 Interacts with Nucleotide Oligomerization Domain 2 and Serves as Downstream Effector of Anti-bacterial Function in Intestinal Epithelial Cells*

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Nucleotide oligomerization domain 2 (NOD2) functions as a mammalian cytosolic pathogen recognition molecule, and variants have been associated with risk for Crohn disease. We recently demonstrated that NOD2 functions as an anti-bacterial factor limiting survival of intracellular invasive bacteria. To gain further insight into the mechanism of NOD2 activation and signal transduction, we performed yeast two-hybrid screening. We demonstrate that GRIM-19, a protein with homology to the NADPH dehydrogenase complex, interacts with endogenous NOD2 in HT29 cells. GRIM-19 is required for NF-κB activation following NOD2-mediated recognition of bacterial muramyl dipeptide. GRIM-19 also controls pathogen invasion of intestinal epithelial cells. GRIM-19 expression is decreased in inflamed mucosa of patients with inflammatory bowel diseases. GRIM-19 may be a key component in NOD2-mediated innate mucosal responses and serve to regulate intestinal epithelial cell responses to microbes.

Crohn disease (CD) is an inflammatory bowel disease (IBD) characterized by chronic inflammation of the intestine. The pathogenesis of CD is complex and appears to consist of three interacting elements: genetic susceptibility factors, priming by the enteric microflora, and immune-mediated tissue injury (1–3). Innate immunity relies on the expression of a restricted variety of receptors, including Toll-like receptors (a family of trans-membrane protein) and NODs (a family of intracellular bacterial sensors), able to recognize highly conserved microbial motifs (4). CARD15/NOD2 gene, located on chromosome 16q12, has been identified as the first susceptibility gene for CD (5, 6). The role of the two N-terminal CARD domains, a nucleotide-binding domain, and multiple C-terminal leucine-rich repeat regions of NOD2 remains to be established (7).

NOD2 recognizes muramyl dipeptide (MDP-LD) and subsequently activates NF-κB through an incompletely understood pathway involving RIP2/RICK and members of the Toll-like receptor-sensing cascade (8–13). Recent studies leave unclear whether NOD2 is a regulator of TLR2 responses that may be disregulated when NOD2 3020insC mutant associated with CD is present (14–16). A direct interaction between NOD2 and transforming growth factor-β-activated kinase 1 (TAK1) was recently shown, and TAK1 regulates NOD2-mediated NF-κB activation (17). In addition, NF-κB activation induced by Staphylococcus pneumoniae depends on NOD2 (18). A recent study of NOD2-deficient mice shows loss of protective immunity in response to bacterial muramyl dipeptide (19). In addition, these mice are susceptible to listeria infection via the oral route. We have previously shown that the 3020insC mutant of CARD15/NOD2 has impaired function as a defensive factor against intracellular bacteria in intestinal epithelial cells (IEC) (19). We demonstrate here that GRIM-19 is an interactor necessary for NF-κB and anti-bacterial effects of NOD2.

MATERIALS AND METHODS

Cell Culture and Transfection—SW480, HT29, Caco-2, T84, Colo205, HCT116, HEK293, COS7, THP-1, and Jurkat cells were obtained from the American Type Culture Collection (Manassas, VA). HEK293 and COS7 cells were cultured in Dulbeco’s modified Eagle’s medium (Cellgro Mediatech Inc., Herndon, VA) supplemented with 10% (v/v) heat-inactivated fetal calf serum (Atlanta Biologicals Inc., Norcross, GA). THP-1 and Jurkat cells were cultured in RPMI medium (Cellgro Mediatech Inc.) containing 10% heat-inactivated fetal calf serum. All the other IEC lines were cultured as described previously (19). Cells were transfected with a cationic lipid (Lipofectamine 2000; Invitrogen) according to the manufacturer’s protocols.

Yeast Two-hybrid Screening—Yeast two-hybrid screening was performed by MATHCMAKER GAL4 Two-System 3 according to the manufacturer’s protocol (BD Biosciences Clontech, Palo Alto, CA). Briefly, pGBKTT-NOD2 was generated by PCR methods from pCMV-FLAG-NOD2 vector (19). pGBKTT-NOD2 was transfected into the AH109 yeast strain. Expression of Myc-tagged NOD2 protein in yeast extract was confirmed by Western blot analysis using anti-Myc monoclonal antibody (Covance, Richmond, CA) and affinity-purified anti-NOD2 anti-sera (19). Screening was performed using a bone marrow pretransformed library (BD Biosciences Clontech) according to the manufacturer’s protocols. For immunostaining experiments, cells were transfected using the TransIT transfection reagents kit (Mirus Corp., Madison, WI) according to the manufacturer’s protocols.

Construction of Expression Plasmids—Expression tagging of CARD15/NOD2 (19) mammalian expression vector (pCDNA4/HisMAX-GRIM-19) was generated by PCR from cDNA from T84 cells. FLAG-tagged NOD2 mammalian expression vector (pCMVFLAG-NOD2) was previously con-
structured (19) and GFP-tagged NOD2 mammalian expression vector (pEGFP- 
C1-NOD2) was generated by restriction methods from pCMV-
FLAG-NOD2. pCI CARD4/NOD1- 
HA expression vector was kindly 
provided by Dr. John Berin (Millennium Pharmaceutica 
Inc.). (Two oli 
gonucleotides, 19 residues in length (gtgggtgatcagggta 
etagggacttagntggtc) specific to the human grin-19 cDNA were selected 
for synthesis of siRNA (20). pSUPER vector for siRNA was purchased 
from Oligoengine (Seattle, WA). The depletion of endogenous GRIM-19 
expression by siRNA was confirmed by RT-PCR.

Immunoprecipitation and Immunoblotting Experiments—Cells grown 
on 6-well plates. Medium was removed, and 300 µl of 1% Triton 
lysis buffer (1.25% sodium dodecyl sulfate, 2.5% glycerol, 62.5 mmol/ 
liter Tris/HCl, pH 6.8, 5% -mercaptoethanol) supplemented with pro 
tease inhibitor mixture (Complete Mini; Roche Applied Science) 
was added. Supernatant of cell lysate was obtained by centrifugation 
at 12,000 × g for 10 min. Protein concentration was determined using the 
DC protein assay kit (Bio-Rad). Proteins were blotted onto polyvinyl 
diene difluoride membranes and stained for FLAG-NOD2 using anti 
FLAG monononal antibody (Sigma), for Xpress-GRIM-19 using anti-
Xpress monononal antibody (Invitrogen), and for NOD1- 
HA using anti-HA monononal antibody (Roche Applied Science). Two milligrams 
of cell lysate were immunoprecipitated with 2 µg of anti-FLAG, anti-
Xpress, or anti-HA monomonal antibodies and 100 µl of Hiptrap pro 
eline A/G PLUS (Amersham Pharma Biotech, Piscataway, NJ) secondary antibodies. 

2 h with primary antibody (mouse monoclonal anti-Xpress antibody 
W11003) and for NOD1- 
HA primary antibody (mouse monoclonal anti-FLAG antibody and/or 
rabbit polyclonal anti-FLAG antibody (Sigma). Immunostaining 
was performed, respectively, with Texas-Red-conjugated anti-mouse 
IgG or with fluorescein isothiocyanate-conjugated anti-rabbit IgG (Vec 
tor Laboratories) secondary antibodies. Salmonella typhimurium was 
detected with rabbit antibody to Salmonella fluorescein conjugate 
(Biological Industries, Saco, ME). Coverslips were mounted in 
Vectashield (Vector Laboratories) and examined with a confocal laser 
scanning microscope.

Bacterial Invasion Assays—Invasion assays were performed with 
Salmonella enterica serovar typhimurium or Escherichia coli TOP10 
(Invitrogen). Monolayers were seeded in 24-well tissue culture plate 
with 1 ml of cell culture medium without antibiotic and with 
heat-inactivated fetal calf serum at a multiplicity of infection (MOI) of 
10 bacteria/epithelial cell. After a 2-h incubation period at 37 °C, 
monolayers were washed twice with PBS. Fresh cell culture medium 
containing 100 µg/ml gentamicin (Sigma) was added for 1 h to kill 
extracellular bacteria. The epithelial cells were then lysed with 
1% Triton X-100 in deionized water. Samples were diluted and plated onto 
Luria-Bertani agar plates to determine the number of colony-forming 
units corresponding to the number of intracellular bacteria.

Reverse Transcription-Polymerase Chain Reaction—Total RNA of 
IEC lines were extracted using Trizol (Invitrogen) following the manu 
facturer’s protocols. For reverse transcription, 2 µg of total RNA were 
transcribed by the FirstScript First-strand Synthesis system (Invitro 
gen). Real-time RT-PCR was performed in an ABI Prism 7000 sequence 
detector using the SYBR Green JumpStart™ detection system. Briefly, 
50 ng of the reverse transcribed cdNA were used for each PCR 
reaction with 200 nM of forward and reverse primers. Primers for PCR were 
forward 5-acccaggggtggagct-3, reverse 5-tctagacctcatctc-3 (GRIM-19, 194 bp); forward 5-ttcctctgtgctcgtgctt-3, reverse 5-gccagcctg-
gtctgtctgctgctggattatcttcgggtgctacacc-3 (MDP-LD, 440 bp). The PCR program used was 50 °C for 2 min, then 95 °C for 10 
min followed by 40 cycles of 95 °C 15 s, 60 °C 15 s, and 72 °C 15 s. The threshold cycle (CT) values were obtained for the reactions reflecting 
quantity of the template in the sample. GRIM-19 Delta CT (ΔCT) was 
calculated by subtracting the GAPDH CT value from the GRIM-19 CT 
value and thus represented the relative quantity of the target molecule 
after normalizing with the internal standard GAPDH. The GRIM-19 ΔCT values of Caco-2 cells infected with Salmonella or transfected with 
pcDNA/His/MAX-GRIM19 were expressed as percentage of GRIM-19 
ΔCT values of control Caco-2 cells.

RESULTS

GRIM-19 Is a New Binding Partner of NOD2—A yeast two-
hybrid screen was performed to identify cellular proteins that 
interact with NOD2. The N-terminal deletion construct, NOD2 
without the CARD15 domain, was used as bait (Fig. 1A). We 
screened a human bone marrow cdNA library expressing pro 
teins fused to the AD transcriptional activation domain. One 
positive clone was identified to encode the human GRIM-19 
protein, a novel cell death-related gene (21). Co-expression of 
NOD2 and GRIM-19 in yeast survival assays in S.D./– Ade 
/His/–Leu/–Trp/X-gal-selective medium confirmed a strong 
interaction between these two proteins (data not shown).

Association of NOD2 and GRIM-19—To confirm the interac 
tion of NOD2 and GRIM-19 in mammalian cells, we transfected 
COS7 or HEK293 cells with FLAG-tagged NOD2 and Xpress-
tagged GRIM-19. As shown in Fig. 1B, GRIM-19 was detected in 
anti-FLAG immunoprecipitates from NOD2 co-transf 
 
ected, but not from cells co-transfected with the control plasmid 
(Fig. 1B). A reciprocal immunoprecipitation/blotting experi 
ment using anti-Xpress monononal antibody also showed 
NOD2 co-precipitating with GRIM-19 (Fig. 1B). To study the possible physiological significance of NOD2/GRIM-19 interac 
tion, we investigated endogenous interaction between NOD2 
and GRIM-19. For this, we used the HM2559 rabbit antisem 
against NOD2 previously generated (19) and a newly produc 	body uct, anti-FLAG antibody and/or anti-HA antibody against NOD2 
against NOD2 against NOD2.

Antibacterial Activity—Co-expression of 

GRIM-19 and NOD2 Colecocalize in Caco-2 and COS7 Cells—
To determine in which cellular compartment NOD2 and 
GRIM-19 interact, we examined their subcellular localization
GRIM-19 Mediates NOD2 Anti-bacterial Activity

**Fig. 1.** Association between CARD15/NOD2 and GRIM-19 in mammalian cells. A, schematic representation of CARD15-less NOD2 construct used as bait for yeast two-hybrid screening. B, COS7 cells were transfected with FLAG-tagged NOD2 and/or Xpress-tagged GRIM-19. The cell lysates were immunoprecipitated with anti-FLAG antibody (top left panel) or with anti-Xpress antibody (top right panel). The precipitates were fractionated through 4–12% or 4–20% Tris-glycine SDS-PAGE and blotted with anti-FLAG or anti-Xpress monoclonal antibodies. Total cell lysates (TCL) were subjected to Western blot analysis with anti-Xpress antibody or anti-FLAG antibody to detect GRIM-19 or NOD2 in transfected COS7 cells. Caco-2 cells were transfected with Xpress-tagged GRIM-19 and infected with *S. typhimurium*. GRIM-19 was expressed in Caco-2 cells (2.26-fold), whereas infection with a non-pathogenic and non-invasive *E. coli* had no effect on grim-19 mRNA expression (Fig. 4).

**Fig. 2.** Colocalization between GFP-NOD2 and Xpress-GRIM-19 in mammalian cells. Caco-2 and COS7 cells were co-transfected with GFP-tagged NOD2 and Xpress-tagged GRIM-19. GRIM-19 was detected by confocal microscopy using monoclonal anti-Xpress as primary antibody, followed by Texas Red-conjugated anti-mouse IgG.

by immunofluorescence confocal microscopy. COS7 and Caco-2 cells were transfected with Xpress-GRIM-19 and GFP-NOD2 expression plasmids (Fig. 2). The NOD2 protein was observed throughout the cytoplasm and also near the plasma membrane. In COS7 and Caco-2 cells co-expressing GFP-NOD2 and Xpress-GRIM-19, GRIM-19 partially colocalized with NOD2 in intracellular vesicles but not near the membrane (Fig. 2).

**GRIM-19 Is Expressed in IBD Tissues and Intestinal Epithelial Cell Lines**—Expression of GRIM-19 was also determined in colonic biopsies from Crohn disease and ulcerative colitis patients taken from both involved and non-involved areas and compared with expression in mucosal biopsies obtained from controls without IBD. In the non-involved mucosa from IBD patients, grim-19 mRNA expression was comparable with that in control patients. In contrast, grim-19 mRNA expression was significantly decreased in involved areas from mucosa of both ulcerative colitis and CD patients (Fig. 3A). Expression of grim-19 mRNA was also assessed by RT-PCR in several human intestinal epithelial cell lines, the THP-1 macrophage cell line, and Jurkat cells. GRIM-19 was expressed in THP-1, Jurkat cell lines, and in all the IEC lines used in this study (Fig. 3B).

The effect of bacterial invasion on GRIM-19 expression was evaluated. Caco-2 cells were infected with invasive *S. typhimurium* and non-pathogenic and non-invasive *E. coli* for 2 h at an MOI 100. Invasive ability of *S. typhimurium* was verified using gentamicin protection assay (data not shown). *S. typhimurium* infection up-regulated grim-19 mRNA expression in Caco-2 cells (2.26-fold), whereas infection with a non-pathogenic and non-invasive *E. coli* had no effect on grim-19 mRNA expression (Fig. 4).

**Functional Role of GRIM-19 in Caco-2 Cells**—To determine the effect of GRIM-19 on cell death, we assessed a non-destructive bioluminescence cytotoxicity assay on Caco-2 cells. Cells were transfected with Xpress-tagged GRIM-19 or FLAG-tagged NOD2 or infected with *S. typhimurium*. Overexpression of GRIM-19 or NOD2 did not induce cell death under the conditions of this study, whereas infection by *S. typhimurium* induced cell death (Fig. 5A). To determine whether GRIM-19 protein results in an alteration of the functional outcome of bacterial survival, untransfected and transiently transfected Caco-2 cells were infected with *S. typhimurium*. After a 2-h incubation period with the monolayer, the percentage of intracellular bacteria in comparison with the inoculum was significantly decreased (72.0 ± 5.4%) in Caco-2 cells expressing GRIM-19 in comparison with non-transfected cells or cells transfected with vector alone (Fig. 5B). Consistent with these findings, *S. typhimurium* invasion increased (162.0 ± 43.8%) in Caco-2 cells harboring a plasmid encoding the grim-19 siRNA-1 (Fig. 5B), which significantly decreased grim-19 mRNA levels (data not shown), whereas the control grim-19 siRNA (sequence 2), which has no effect on grim-19 mRNA, did not modify *S. typhimurium* invasive ability. To confirm these results, immunostaining was performed with Caco-2 cells transfected with Xpress-tagged GRIM-19 and infected with *S. typhimurium*. As shown in Fig. 5C, few bacteria were found in Caco-2 cells expressing GRIM-19, whereas numerous bacteria were observed in adjacent cells that did not express GRIM-19 (Fig. 5C). Consistent with these results, the mean number of intracellular bacteria found in Caco-2 cells expressing GRIM-19 after immunostaining examination by confocal microscopy is significantly lower (7.5 bacteria/cell) (p < 0.001) than that of untransfected cells (14.8 bacteria/cells) (Fig. 5D). Thus, GRIM-19 protected host cells by reducing the intracellular survival of *S. typhimurium*.

**Retinoic Acid and IFN-β Exert Anti-bacterial Activity by Inducing GRIM-19 Expression**—To investigate whether endoge-
**GRIM-19 Mediates NOD2 Anti-bacterial Activity**

We provide evidence here that *Salmonella* infection increases *grim-19* mRNA in infected Caco-2 cells, whereas expression remains unchanged in Caco-2 cells infected with non-invasive *E. coli*. Epithelial cells of the human intestinal mucosa are the initial site of host invasion by bacterial enteric pathogens. Human colonic epithelial cells were shown to undergo the apoptotic role that promotes tumor growth, and GW112 could be involved in the regulation of cellular apoptosis under inflammatory conditions in the digestive system.

Present and previous studies indicate that GRIM-19 binds additional proteins that play a crucial role in IBD, including NOD2, Stat3, and GW112. GRIM-19 binds Stat3 in various cell types but did not bind other Stat proteins, such as Stat1 or Stat5a (25), and the interaction between GRIM-19 and Stat3 suppresses Stat3 activity. Stat3 has a critical role in the development and regulation of innate immunity, and deletion of Stat3 during hematopoiesis causes Crohn disease-like pathogenesis and lethality in mice (27). GRIM-19 has also been reported to bind GW112, a protein expressed in various human normal and malignant tissues with higher expression in organs/tumors of the digestive system. GW112 plays an anti-apoptotic role that promotes tumor growth, and GW112 could be involved in the regulation of cellular apoptosis under inflammatory conditions in the digestive system (28).

We provide evidence here that *Salmonella* infection increases *grim-19* mRNA in infected Caco-2 cells, whereas expression remains unchanged in Caco-2 cells infected with non-invasive *E. coli*. Epithelial cells of the human intestinal mucosa are the initial site of host invasion by bacterial enteric pathogens. Human colonic epithelial cells were shown to undergo apoptosis following infection with different invasive bacteria, transfecting with siRNA specific for *grim-19*. Transfection with *grim-19* siRNA-1 significantly decreased *grim-19* mRNA level and inhibited the MDP-LD-driven response to NOD2. NF-κB activation in HEK293 transfected with NOD2 and *grim-19* siRNA-1 after MDP-LD stimulation was only 50% of that observed in HEK293 transfected only with NOD2 or with pSUPER control vector (Fig. 7A). Control *grim-19* siRNA, which did not knock down *grim-19* mRNA, had no significant effect on NF-κB activation via NOD2 after MDP-LD stimulation.

The anti-bacterial activity of NOD2 was also dependent on the presence of GRIM-19. The invasive ability of *S. typhimurium* decreased in HEK293 cells overexpressing NOD2 compared with non-transfected HEK293 cells (Fig. 7B). This effect was reversed in the presence of *grim-19* siRNA-1 (Fig. 7B), suggesting that anti-bacterial activity conferred by NOD2 correlates with NF-κB activation.

**DISCUSSION**

We have previously shown that NOD2, but not the NOD2 mutant 3020insC associated with CD, protects intestinal epithelial cells against *Salmonella* infection (19). In the present study, we performed yeast two-hybrid screening and identified GRIM-19 as an interacting protein with NOD2 in mammalian cells. GRIM-19, gene associated with retinoid-IFN-induced mortality 19, is located on chromosome 19 and induces cell death in a number of tumor cell lines. GRIM-19 protein expression is induced by the combination of IFN-β and all-trans-retinoic acid RA (21, 22). The subcellular location of GRIM-19 action remains to be established. Originally GRIM-19 was observed in the nucleus (21) and more recently in both nucleus and cytoplasm (23). Its nuclear, but also cytoplasmic, distribution and punctate staining patterns observed in cells prompted speculation that GRIM-19 might interact with various protein or protein complexes to regulate cellular responses (21, 23). GRIM-19 is also a subunit of the mitochondrial NADPH:ubiquinone oxidoreductase (respiratory complex I) (24) and colocализed with mitochondria in MCF-7 and COS1 cells (25). Recently, GRIM-19 was detected in the native form in mitochondrial complex I. Homologous deletion of GRIM-19 in mice causes embryonic lethality at embryonic day 9.5 (26). In the present study, we showed a cytoplasmic colocalization of GRIM-19 and NOD2. Furthermore, interaction between GRIM-19 and NOD2 is NOD2-specific; no binding was observed with NOD1, another NOD protein family member.

Present and previous studies indicate that GRIM-19 binds additional proteins that play a crucial role in IBD, including NOD2, Stat, and GW11. GRIM-19 binds Stat3 in various cell types but did not bind other Stat proteins, such as Stat1 or Stat5a (25), and the interaction between GRIM-19 and Stat3 suppresses Stat3 activity. Stat3 has a critical role in the development and regulation of innate immunity, and deletion of Stat3 during hematopoiesis causes Crohn disease-like pathogenesis and lethality in mice (27). GRIM-19 has also been reported to bind GW112, a protein expressed in various human normal and malignant tissues with higher expression in organs/tumors of the digestive system. GW112 plays an anti-apoptotic role that promotes tumor growth, and GW112 could be involved in the regulation of cellular apoptosis under inflammatory conditions in the digestive system (28).
such as enteroinvasive E. coli or Salmonella. Apoptosis in response to bacterial infection may function to delete infected and damaged epithelial cells and restore epithelial cell growth regulation and epithelial integrity (29). It has previously been shown that after invasion of intestinal macrophages, virulence proteins secreted by Salmonella specifically induce apoptotic cell death by activating the cysteine protease caspase-1 (30).

GRIM-19 has been shown to interact with multiple proteins such as mitochondrial NADH:ubiquinone oxidoreductase and to have several biological activities, including cell growth, transcription, and cell death (21). Here, we show that under the conditions of this study, GRIM-19 did not induce cell death. Expression of GRIM-19 in Caco-2 cells decreased the invasive ability of S. typhimurium, revealing its protective role in IEC.

Given that the overall transfection efficiency in the Caco-2 cells was 30–35%, the 28% reduction in colony-forming units suggests that GRIM-19 is highly effective in controlling intracellular survival in those cells expressing the transfected protein. Down-regulation of GRIM-19 expression using siRNA increased the invasive ability of S. typhimurium. Consistent with these findings, the invasive ability of S. typhimurium also decreased in Caco-2 cells expressing increased endogenous GRIM-19 induced by the combination of IFN-β/RA. In addition, siRNA against GRIM-19 restored invasive activity of S. typhimurium in Caco-2 cells stimulated with IFN-β/RA, confirming that endogenous GRIM-19 protects cells against bacteria. We also show that expression of GRIM-19 is decreased in IBD tissues from both ulcerative colitis and CD patients in affected areas, compared with uninvolved tissue. This decrease could be due to the loss of epithelium in the involved area or to the down-regulation of GRIM-19 expression in inflamed areas in IBD patients.

The question remains how does GRIM-19 function in the innate immune mucosal response? It appears from our data...
and 10 ng of HEK293 cells were transfected with 1 ng of NOD2 expression plasmid. mRNA level was measured by RT-PCR using specific primers. B, Decreased expression of GRIM-19 using specific siRNA in 19026 determined in the absence or presence of 1 ng of MDP-LD. Importantly, NF-κB response to MDP-LD. GRIM-19 acts downstream of NOD2 and is required for GRIM-19 Mediates NOD2 Anti-bacterial Activity.

that GRIM-19 acts as a key component of the innate immune mucosal response by modulating NF-κB activation via NOD2. Decreased expression of GRIM-19 using specific siRNA in HEK293 overexpressing NOD2 decreased NF-κB activation in response to MDP-LD. Importantly, NF-κB activation correlates with a decrease in the number of viable intracellular S. typhimurium, indicating that NOD2 anti-bacterial activity is dependent on NF-κB activation. In addition to its effects on NOD2-mediated NF-κB activation, it is possible that GRIM-19 has other functions that contribute to its effects on epithelial response to invasive bacteria. GRIM-19 is a subunit of the mitochondrial NADPH-ubiquinone oxidoreductase (24–26). The NADPH enzyme complex catalyzes the transfer of electrons from NADPH to molecular oxygen, generating reactive oxygen species (ROS), in particular superoxide anion. Reactive oxygen species operate on a variety of physiological processes, including host defense against pathogens (31). The best known O2−-producing enzyme is the phagocyte-associated respiratory enzyme NADPH oxidase burst that plays a crucial role in the process of killing microorganisms (31). Helicobacter pylori lipopolysaccharide stimulated Toll-like-receptor 4 signaling and activated the NADPH oxidase 1 (32, 33). In addition, a yeast two-hybrid screen led to the observation of a direct interaction of TLR4 with NADPH oxidase 4 that mediates lipopolysaccharide-induced reactive oxygen species generation and NF-κB activation (34). It is possible that GRIM-19 increases the production of reactive oxygen species in intestinal epithelial cells after bacterial invasion, protecting the intestinal mucosa against pathogens.

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