

However, the current study has some limitation: the cross-sectional design, the lack of biomarker assessment, the relatively young age, the setting (ie, Navy) and the prevalence of the male gender. Moreover, it has been recently reported that the gender has no relevant impact on asthma, apart from the anxiety and the perception of asthma control.¹⁰ Thus, the present outcomes could be cautiously generalized to the AR patients. Anyway, further studies should be conducted to answer the unmet questions, including validation by prospective studies, long-term follow-up and more general population.

In conclusion, the clustering analysis could be a useful tool in the management of AR patients as it could allow to identify specific clusters of patients potentially at risk of evolving towards asthma.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ORCID

Giorgio Ciprandi  <http://orcid.org/0000-0001-7016-8421>

Giorgio Ciprandi¹ 

Michela Silvestri²

Angela Pistorio³

Maria Angela Tosca⁴

Ignazio Cirillo⁵

¹Ospedale Policlinico San Martino, Genoa, Italy

²Paediatric Pulmonology, IRCCS Istituto Giannina Gaslini, Genoa, Italy

³Epidemiology and Biostatistics Service, IRCCS Istituto Giannina Gaslini, Genoa, Italy

⁴Paediatric Allergology, IRCCS Istituto Giannina Gaslini, Genoa, Italy

⁵Navy Medical Service, La Spezia, Italy

Correspondence

Giorgio Ciprandi, Ospedale Policlinico San Martino, Genoa, Italy.

Email gio.cip@libero.it

REFERENCES

1. Brożek JL, Bousquet J, Agache I, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines-2016 revision. *J Allergy Clin Immunol.* 2017;140:950-958.
2. Ciprandi G, Cirillo I. The lower airway pathology of rhinitis. *J Allergy Clin Immunol.* 2006;118:1105-1109.
3. Ciprandi G, Klersy C, Cirillo I, Marseglia GL. Quality of life in allergic rhinitis: relationship with clinical, immunological, and functional aspects. *Clin Exp Allergy.* 2007;37:1528-1535.
4. Global Initiative for Asthma. GINA guidelines. Global strategy for Asthma Management and Prevention. <http://www.ginasthma.org/>. Accessed August 4, 2018.
5. Hsiao HW, Wu CC, Lin MC, et al. Comprehensive risk factors, asthma control, and life quality pathways in adults with asthma: a structural equation modeling analysis. *Allergy Asthma Proc.* 2016;37:31-40.
6. Desai M, Oppenheimer J. Elucidating asthma phenotypes and endotypes: progress towards personalized medicine. *Ann Allergy Asthma Immunol.* 2016;116:394-401.
7. Haldar P, Pavord ID, Shaw DE. Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med.* 2008;178:218-224.
8. Agache I, Akdis CA. Endotypes of allergic diseases and asthma: an important step in building blocks for the future of precision medicine. *Allergol Int.* 2016;65:243-252.
9. Ciprandi G, Tosca MA, Silvestri M, Ricciardolo FLM. Inflammatory biomarkers for asthma endotyping and personalized therapy. *Exp Rev Clin Immunol.* 2017;13:715-721.
10. Ciprandi G, Gallo F. The impact of gender on asthma in daily clinical practice. *Postgrad Med.* 2018;130:271-273.

DOI: 10.1111/all.13648

Exposure to 3-methyl-4-nitrophenol facilitates development of intestinal allergy

To the Editor,

It is recognized that the environmental pollution is associated with the pathogenesis of a number of diseases.¹ The sources of environmental pollution include multiple aspects, such as the automobile exhaust particles.² 3-methyl-4-nitrophenol (MNP) is one of the components of diesel-exhaust particles.³ Food contaminated by organophosphorus insecticide fenitrothion can be another source of MNP.⁴ It is reported that exposure to MNP induces renal tubule epithelial cell apoptosis and spermatogenic cell apoptosis.^{5,6} Based on the above information, we hypothesize that MNP facilitates the development of intestinal allergy. In this study, mice were exposed to MNP or/and OVA. The exposure to MNP induced intestinal

epithelial cell apoptosis, induced epithelial barrier dysfunction and facilitated the development of intestinal allergy in mice.

1 | MNP FACILITATES THE DEVELOPMENT OF INTESTINAL ALLERGY

Grouped mice were gavage-fed with MNP or/and OVA daily for 3 weeks. The mice were sacrificed 1 day after the last gavage. Samples were collected from the small intestine. The status of intestinal allergy was analyzed. The results showed that exposure of mice to both MNP- and OVA-induced intestinal allergy, including drop of the

core temperature and diarrhea in response to antigen challenge; increases in producing specific IgE, mouse mast cell protease-1, IL-4, decreases in IFN- γ , by immune cells isolated from the small intestine; and the antigen-specific CD4⁺ T cells were detected in the intestine, which did not occur in those mice treated with either MNP alone or OVA alone (Figure 1). The data suggest that exposure to MNP facilitates the development of food allergy in mice.

2 | MNP COMPROMISES INTESTINAL EPITHELIAL BARRIER INTEGRITY

To assess the effects of MNP on alternating the intestinal epithelial barrier functions, grouped mice were gavage-fed with MNP at gradient doses daily for 1, or 2, or 3 weeks, respectively. The intestinal epithelial barrier function was tested 1 day after the last gavage with MNP by assessing the absorption of FITC-dextran. The results

showed that exposure to MNP increased the absorption of FITC-dextran in an MNP dose- and time-dependent manner. Feeding mice with ethanol (the vehicle) alone did not alter the intestinal epithelial barrier function (Figure S1).

3 | MNP INDUCES INTESTINAL EPITHELIAL CELL APOPTOSIS

Published data show that exposure to MNP can induce cell apoptosis.⁶ Thus, segments of the small intestine were collected from mice after treating with MNP or saline for 3 weeks and examined by TUNEL. The results showed spot apoptotic epithelial cells in the small intestine after exposure to MNP for 3 weeks, some epithelial cells had exfoliated, which were rarely seen in mice treated with saline (Figure S2A-B). The results suggest that MNP can induce intestinal epithelial cell apoptosis. To corroborate the results, T84 cells and HT-29 cells were

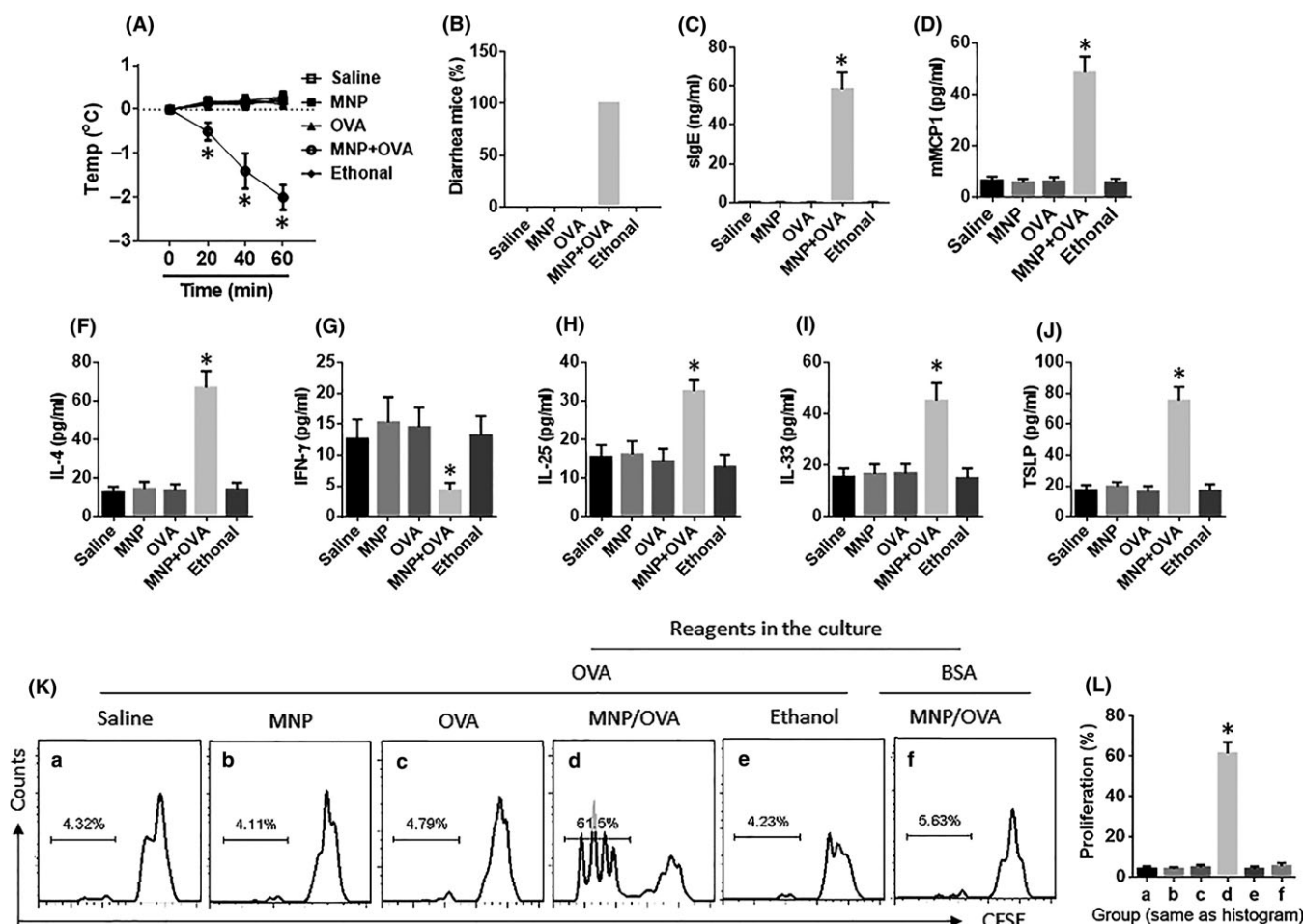


FIGURE 1 3-methyl-4-nitrophenol (MNP) facilitates the development of intestinal allergy. Grouped mice ($n = 6$ of each group) were exposed to MNP or/and OVA via gavage-feeding for 3 wk. A-B, the bars indicate the drop of core temperature (A; recorded 30 min after the last gavage) and the number of mice having diarrhea (B; recorded 0-4 h after the last gavage). The mice were sacrificed on the next day to the last gavage. C-J, the bars indicate the serum levels of OVA-specific IgE (sIgE; C, mouse mast cell protease-1 (mMCP1; D, IL-4 (E), IFN- γ (F), IL-25 (G), IL-33 (H), and TSLP (I). K, the gated histograms show T cell proliferation (by CFSE-dilution assay) after the indicated treatment above each panel and the presence of dendritic cells (T cell:dendritic cell = 5:1). L, the bars indicate the summarized data of the gated cell population in panel K. Data of bars are presented as mean $\pm$ SD. * $P < 0.01$, compared with the saline group

cultured in the presence or absence of MNP for 3 days. The cells were stained with annexin V reagents and propidium iodide (PI) and analyzed by flow cytometry. The results showed that exposure to MNP in the culture markedly induced T84 cell and HT-29 cell apoptosis in an MNP concentration-dependent manner (Figure S2C-D).

4 | EXPOSURE TO MNP INCREASES FAS/FASL EXPRESSION IN INTESTINAL EPITHELIAL CELLS

The data reported above suggest that exposure to MNP abnormally triggers the initiation of apoptosis in the intestinal epithelial cells. To test this, after gavage-feeding with MNP daily for 3 weeks, epithelial samples were collected from the small intestine of each mouse by scraping. The samples were analyzed by enzymatic assay. The results showed that exposure to MNP markedly activated caspase (casp) 8 and casp3 in the epithelium of the small intestine (Figure S3A-B). On the other hand, T84 cells were exposed to MNP in the culture at gradient concentrations for 3 days. The cell extracts were prepared and analyzed by enzymatic assay. The results showed that the exposure to MNP increased the activities of casp8 and casp3 in T84 cells in an MNP concentration-dependent manner (Figure S3C-D). We

next assessed the expression of Fas and FasL in intestinal epithelial cells after exposure to MNP. The results showed that MNP significantly increased the expression of Fas and FasL in the mouse intestinal epithelium (Figure S4) and T84 cells after exposure to MNP (Figure S5). Since casp8 and casp3 are the components of the upstream in the signal transduction pathway in expression of Fas and FasL, in separate experiments, T84 cells were exposed to MNP in the culture with or without the presence of an inhibitor of caspase (Z-VAD-FMK) for 3 days. Indeed, the presence of caspase inhibitor abolished the MNP-enhanced expression of Fas and FasL in mouse intestinal epithelial cells (Figure S4) and T84 cells (Figure S5).

5 | BLOCKING CASPASE ACTIVITIES INHIBITS MNP-FACILITATED INTESTINAL ALLERGY

Finally, we tested whether blocking caspase activities could prevent the MNP-induced intestinal epithelial barrier dysfunction and intestinal allergy. Mice were treated with MNP and OVA daily with or without Z-VAD-FMK for 3 weeks. The results showed that administration of a caspase inhibitor abolished the MNP-induced intestinal epithelial barrier dysfunction and intestinal allergy (Figure 2).

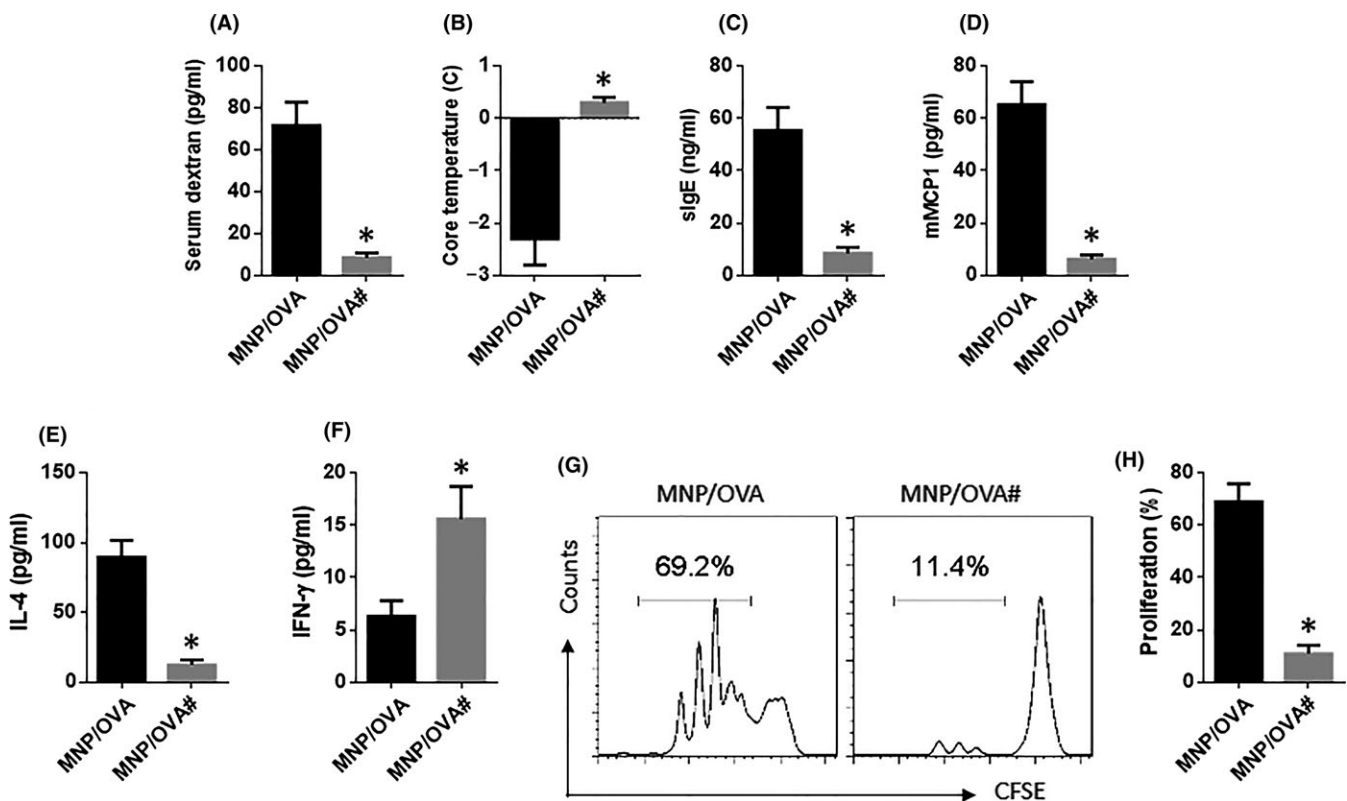


FIGURE 2 Caspase inhibitors block 3-methyl-4-nitrophenol (MNP)-induced intestinal epithelial barrier dysfunction and intestinal allergy. Mice (6 mice per group) were treated with MNP and OVA with or without Z-VAD-FMK (#) daily for 3 wk. A, the bars indicate the serum levels of dextran (the indicator of intestinal epithelial barrier permeability, tested on day 22). B-F, the bars show the indicated cytokine levels in the culture supernatant of LPMC after activated by specific antigen OVA. G, the gated histograms show the proliferation of Teff cells isolated from LPMC. H, the bars show the summarized data of panel G. The data of bars are presented as mean $\pm$ SD. * $P < 0.01$, compared with the MNP/OVA group

Treatment with Z-VAD-FMK alone did not alter the parameters (data not shown).

In summary, the present study showed exposure to MNP induced intestinal epithelial barrier dysfunction and facilitated the development of intestinal allergy, which was abolished by administration of caspase inhibitors. The results suggest that the environmental pollution may contribute to the development of allergic diseases, which may be prevented by administration with caspase inhibitors.

ACKNOWLEDGMENT

This study was supported by grants from the National Nature and Science Foundation of China (81870706, 31570932, 81700888, 81701589), the Shenzhen science, technology and innovation committee (CXZZ20140902151802864, JCYJ20170307162827158) and Guangdong Nature Science Foundation (2015A030313786).

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ORCID

Zhi-Qiang Liu  <http://orcid.org/0000-0002-7353-8827>

Zhi-Gang Liu  <http://orcid.org/0000-0002-7097-5001>

Ping-Chang Yang  <http://orcid.org/0000-0002-6806-9464>

Jiang-Qi Liu^{1,2,3}
Shan-Shan Li¹
Li-Hua Mo⁴
Dian Yu¹
Xiao-Rui Geng^{1,2,3}
Jing-Yi Hong¹
Hai-Qiong Yu⁵
Li-Teng Yang⁵
Rui-Di Xie¹
Zhi-Qiang Liu^{1,2,3} 

Zhi-Gang Liu¹ 

Ping-Chang Yang¹ 

¹Research Center of Allergy & Immunology, Shenzhen University School of Medicine, Shenzhen, China

²Longgang ENT Hospital & Shenzhen ENT Institute, Shenzhen, China

³Brain-Body Institute, McMaster University, Hamilton, Ontario, Canada

⁴Department of Pediatric Otolaryngology, Southern Medical University Shenzhen Hospital, Shenzhen, China

⁵Department of Allergy, Third Affiliated Hospital of Shenzhen University, Shenzhen, China

Correspondence

Ping-Chang Yang and Zhi-Gang Liu, Shenzhen University School of Medicine, Shenzhen, China.

Emails: pcy2356@szu.edu.cn (PY); lzg@szu.edu.cn (ZL)

Jiang-Qi Liu, Shan-Shan Li and Li-Hua Mo equally contributed to this work.

REFERENCES

1. Mannucci PM, Harari S, Martinelli I, Franchini M. Effects on health of air pollution: a narrative review. *Intern Emerg Med*. 2015;10:657-662.
2. Zhu Y, Zhang Q. Characterizing ultrafine particles and other air pollutants in and around school buses. *Res Rep Health Eff Inst*. 2014;180:3-37.
3. Taneda S, Mori Y, Kamata K, et al. Estrogenic and anti-androgenic activity of nitrophenols in diesel exhaust particles (DEP). *Biol Pharm Bull*. 2004;27:835-837.
4. Meaklim J, Yang J, Drummer OH, et al. Fenitrothion: toxicokinetics and toxicologic evaluation in human volunteers. *Environ Health Perspect*. 2003;111:305-308.
5. Yue Z, She RP, Bao HH, et al. Necrosis and apoptosis of renal tubular epithelial cells in rats exposed to 3-methyl-4-nitrophenol. *Environ Toxicol*. 2012;27:653-661.
6. Yue Z, She R, Bao H, et al. Exposure to 3-methyl-4-nitrophenol affects testicular morphology and induces spermatogenic cell apoptosis in immature male rats. *Res Vet Sci*. 2011;91:261-268.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

DOI: 10.1111/all.13651

EASI p-EASI: Predicting disease severity in atopic dermatitis patients treated with cyclosporin A

To the Editor,

Serological biomarkers offer objective and reliable outcome measures for Atopic Dermatitis (AD). A recent meta-analysis identified serum

thymus and activation-regulated chemokine (TARC/CCL17) as the best biomarker currently available for assessing disease severity in AD, showing correlation coefficient of around 0.60 with disease severity.¹

Supplemental materials

Exposure to 3-methyl-4-nitrophenol (MNP) facilitates development of intestinal allergy

Running title: MNP facilitates intestinal allergy

Jiang-Qi Liu^{1,2,3*}, Shan-Shan Li^{1*}, Li-Hua Mo^{4*}, Dian Yu¹, Xiao-Rui Geng^{1,2,3}, Jing-Yi Hong¹, Hai-Qiong Yu⁵, Li-Teng Yang⁵, Rui-Di Xie¹, Zhi-Qiang Liu^{1,2,3}, Zhi-Gang Liu¹, Ping-Chang Yang¹

1. Research Center of Allergy & Immunology, Shenzhen University School of Medicine, Shenzhen, China. 2. Longgang ENT Hospital & Shenzhen ENT Institute, Shenzhen, China. 3, Brain-Body Institute, McMaster University, Hamilton, ON, Canada. 4, Department of Pediatric Otolaryngology, Southern Medical University Shenzhen Hospital, Shenzhen, China. 5, Department of Allergy, Third Affiliated Hospital of Shenzhen University, Shenzhen, China.

*Equally contributed to this work

Corresponding authors: Dr. Ping-Chang Yang and Dr. Zhi-Gang Liu. Room A7-509 of Xili Campus. Shenzhen University School of Medicine. 1066 Xueyuan Blvd, Shenzhen 518055, China. Email: pcy2356@szu.edu.cn & lzg@szu.edu.cn. Tel: 8675586172722. Fax: 8675586671906.

Materials and methods

Reagents

The antibodies of Fas and FasL were purchased from Santa Cruz Biotech (Santa Cruz, CA). The CHIP kit, ELISA kits of OVA-specific IgE, mMCP1, IL-4 and IFN- γ were purchased from the Biomart (Beijing, China). The FITC-dextran, annexin V kit, casp8 and casp3 activation kits and Z-VAD-FMK were purchased from Sigma Aldrich (St. Louis., MO). The reagents and materials for RT-qPCR and Western blotting were purchased from Invitrogen (Carlsbad, CA). The TUNEL kit was purchased from the VAZYME (Nanjing, China). The endotoxin levels in all reagents were detected using the Limulus assay (Limulus amoebocyte lysate QCL 1000, Bio Whittaker, Walkersville, MD, USA). The reagents used in this study contained $<0.2\text{U}$ endotoxin/ $10\text{ }\mu\text{g}$ reagents.

Mice

Sex-matched (male and female) BALB/c mice (6-8 week old) were purchased from the Guangdong Experimental Animal Center (Guangzhou, China). The mice were maintained in a specific pathogen-free facility with accessing water and food freely. The using mouse in the present study was approved by the Animal Ethics Committee at Shenzhen University.

Cell culture

Immune cells were cultured in RPMI1640 medium supplemented with 10% fetal serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin and 2 mM glutamine. In the case of activating immune

cells, PMA (50 ng/ml) and ionomycin (100 ng/ml) were added to the culture. The cell viability was greater than 99% as assessed by Trypan blue exclusion assay.

Assessment of the effects of MNP on development of intestinal allergy

Sensitization: MNP was dissolved in 100% ethanol to make stock solution (10 mg MNP/ml). The stock solution was diluted 100 times with saline to make working solution (100 µg MNP/ml). Grouped mice were gavage-fed with MNP (30 µg/mouse) or/and OVA (0.1 mg/mouse) in a volume of 0.3 ml daily for 3 weeks. The ethanol control mice were fed with 3 µl ethanol (100%) in 0.3 ml saline per day.

Assessment of systemic allergic response: One day after the last gavage, mice were challenged by feeding with 5 mg OVA. The core temperature in the rectum of each mouse was recorded with a rectal thermometer 30 min after the challenge. Diarrhea was observed 0-4 h after the challenge.

Assessment of the allergic status in the intestine: The small intestine was excised from each mouse. The lamina propria mononuclear cells (LPMC) were isolated from the intestinal tissue following our established procedures {He, 2011 #27}. The LPMCs were cultured in the presence of OVA (the specific antigen; 5 µg/ml) or bovine serum albumin (BSA; used as a non-specific antigen; 5 µg/ml) for 48 h. The levels of OVA-specific IgE, mouse mast cell protease-1 (mMCP1), IL-4 and IFN-γ in the culture supernatant was determined by ELISA used as the indicators of allergic response in the intestine.

Enzyme-linked immunosorbent assay (ELISA)

Serum levels of cytokines, mMCP1 and OVA-specific IgE were determined by ELISA with commercial reagent kits following the manufacturer's instructions. The procedures of determining OVA-specific IgE include: (1) microplate wells were coated with OVA solution (10 µg/ml) and incubated at 4 °C overnight; (2) the plates were washed with TBST (Tris-buffered saline containing 0.1% Tween 20) for 3 times; (3) serum (diluted 10 times) was added to the wells (100 µl/well); the plates were incubated at 4 °C overnight; (4) the plates were washed with TBST for 3 times; (5) the plates were blocked by incubating with 1% BSA for 30 min at room temperature; (6) biotinylated anti-mouse IgE antibody was added (100 µl/well) and incubated at room temperature for 2 h; (7) washed with TBST for 3 times; (8) streptavidin (labeled with peroxidase) was added (100 µl/well) and incubated at room temperature for 2 h; (9) TMB was added to each well; the reaction was terminated by adding 2M H₂SO₄; (10) the plates were read with a microplate reader at 450 nm. The standard curve: Mouse IgE (provided by the reagent kit) was double diluted from 256 ng/ml to 2 ng/ml and coated the plate wells overnight at 4 °C; the plates were then processed through the procedures (4) to (10). Negative controls: The procedures were the same as those for samples except the anti-mouse IgE antibody in procedure 6 was replaced with isotype IgG. All samples were analyzed in triplicate.

Assessment of antigen-specific CD4⁺ T cells in the intestine (CFSE-dilution assay)

CD3⁺ CD4⁺ CD25⁻ T cells (Teff) and CD11c⁺ dendritic cells (DC) were isolated from LPMCs by magnetic cell sorting (MACS) with reagent kits following the manufacturer's instructions. The Teffs (labeled with CFSE) and DCs were cultured at a ratio of 5:1 in the presence of OVA (5 µg/ml) or BSA (5 µg/ml) for 72 h. The cells were harvested and analyzed by flow cytometry. The proliferating Teffs were regarded as the OVA-specific CD4⁺ T cells.

Assessment of the effects of MNP on intestinal epithelial barrier permeability

Grouped mice were daily gavage-fed with MNP at 10 µg/mouse, or 20 µg/mouse, or 30 µg/mouse, respectively, for one week, or two weeks, or three weeks. One day after the last gavage, mice were gavage-fed with FITC-labeled dextran at 1 mg/mouse in 0.3 ml saline. The mice were sacrificed 4 h after the gavage. The levels of dextran in the serum were determined with a fluorometer.

T84 and Caco-2 cell culture

T84 cells (passages 41-43) and HT-29 cells (passage 31-43) were purchased from ATCC (Manassas, VA) and cultured with DMEM supplemented with 10% fetal bovine serum, 0.1 mg/ml streptomycin, 100 U/ml penicillin and 2 mM glutamine. The medium was changed every day. The cells were split at 70% confluence. The cell viability was greater than 99% as checked by Trypan blue exclusion assay.

Real-time quantitative RT-PCR (RT-qPCR)

The total RNA was extracted from cells collected from relevant experiments with the TRIzol reagents. The RNA was converted to cDNA with a reverse transcription kit following the manufacturer instructions. The samples were amplified by qPCR with the SYBR Green Master Mix in the presence of relevant primers including Fas (human: acctccagtcgtgaaacat and ctcagctgtgtcttgatgc. Mouse: atgctgtggatctgggctg and ttgacagcaaatgggcctc) and FasL (Human: atagccaaccccagtacacc and gctggttgttgcaagactga. Mouse: atagccaaccccagtacacc and gctggttgttgcaagactga). The results are presented as fold change against the housekeeping gene beta actin.

Cell protein extraction

Cells were collected from relevant experiments and incubated with a lysing buffer for 30 min. The lysates were centrifuged for 10 min at 13,000 rpm. The supernatant was collected and used as the cytosolic extracts. All the procedures were performed at 4 °C.

Western blotting

The proteins were fractioned by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto a PVDF membrane. The membrane was blocked by incubating with 5% skim milk for 30 min, incubating with the antibodies of interest overnight at 4 °C and followed by incubating with the second antibodies (labeled by peroxidase) for 2 h at room temperature. The membrane was washed with Tris-buffered saline Tween 20 for 3 times after each time of incubation. The immunoblots on the membrane were developed with the enhanced chemiluminescence and photographed with an imaging device.

Assessment of apoptotic cells

The small intestinal segments were collected from mice. Cryosections were prepared and stained with a reagent kit of terminal deoxynucleotidyl transferase (TUNEL, labeled with FITC) following the manufacturer's instructions. The samples were observed under a confocal microscope. The apoptotic and non-apoptotic epithelial cells were counted in 20 randomly selected microscopical windows ($\times 400$) per sample. The counts were presented as percentage. On the other hand, cells were collected from relevant experiments and stained with propidium iodide and an annexin V kit following the manufacturer's instructions. The cells were analyzed with a flow cytometer.

Assessment of activity of casp8 and casp3.

Casp3 and casp8 activity was measured in T84 cells (1×10^6 /ml) and intestinal epithelial scraping samples. The assay was performed with a reagent kit by monitoring cleavage of the casp3- or casp8-specific fluorogenic substrate according to the manufacturer's instructions.

Statistics

The data are presented as mean $\pm$ SD. The difference between two groups was determined by Student *t* test or ANOVA followed by SNK test for multiple comparisons. $P < 0.05$ was set as a significant criterion.

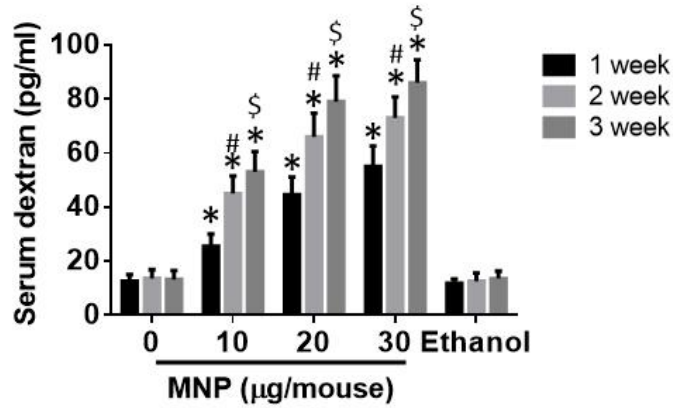


Fig. S1. MNP compromises intestinal epithelial barrier functions. Grouped mice (6 mice per group) were gavage-fed with MNP at the doses as denoted on the X axis for 1 week, or 2 weeks, or 3 weeks. The intestinal epithelial barrier permeability was tested by assessing the absorption of FITC-dextran. The bars indicate the serum levels of dextran. * $p < 0.01$, compared with the dose “0” group. # $p < 0.01$, compared with the “1 week” group. \$ $p < 0.01$, compared with the “2 week” group. Ethanol: Mice were fed with 3 μ l 100% ethanol in 0.3 ml saline per day.

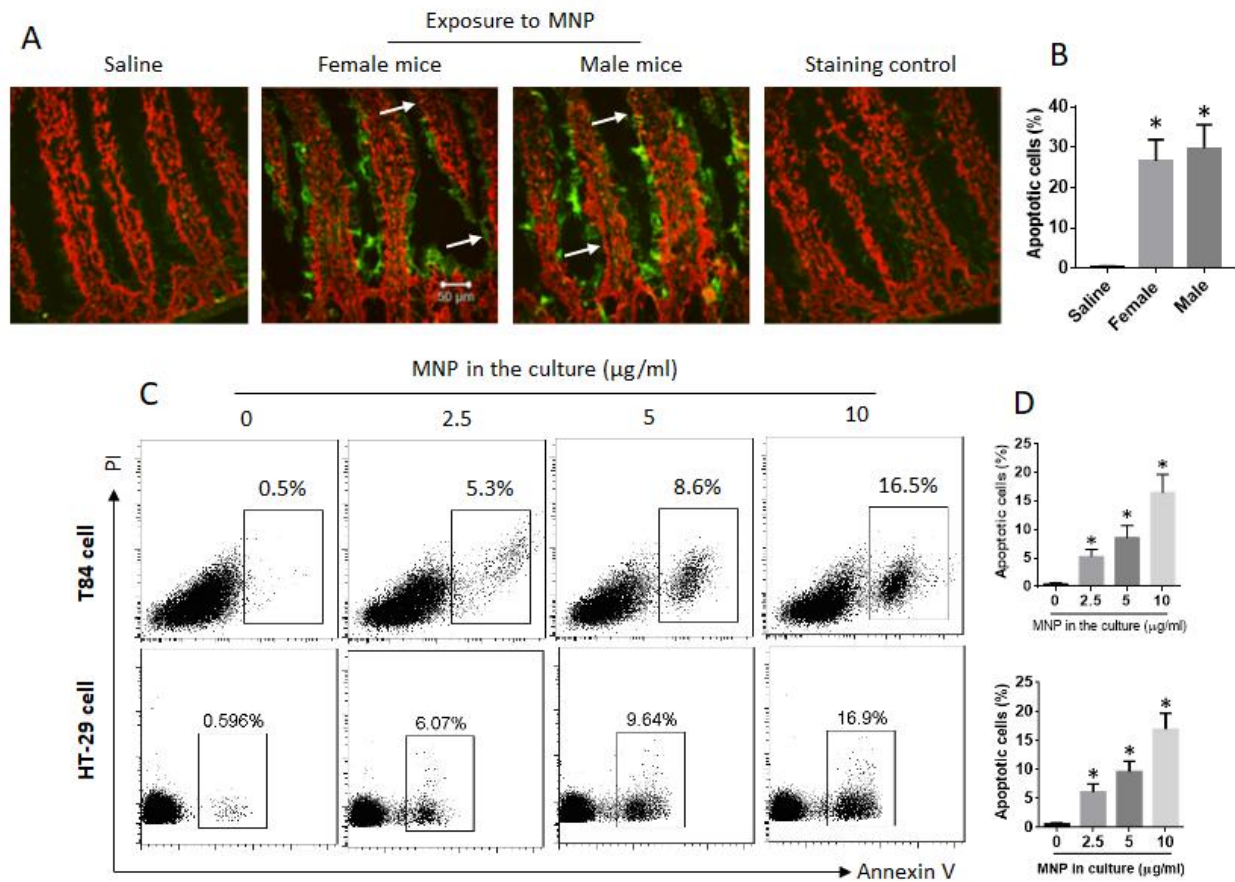


Fig. S2. MNP induces intestinal epithelial cell apoptosis. A, the representative confocal images ($\times 630$) show the apoptotic intestinal epithelial cells (in green; by TUNEL) of mice treated with saline or MNP for 3 weeks. The arrows point at the epithelial cell exfoliation. B, bars indicate the summarized apoptotic cells in panel A. C, the gated cell population in the representative flow cytometry dot plots indicates the apoptotic T84 cells. D, the bars indicate the summarized data of the gated dot plots of panel B. The data of bars are presented as mean $\pm$ SD. * $p < 0.01$, compared with the saline group (B) or the dose "0" group (D). Each group consists of 6 mice.

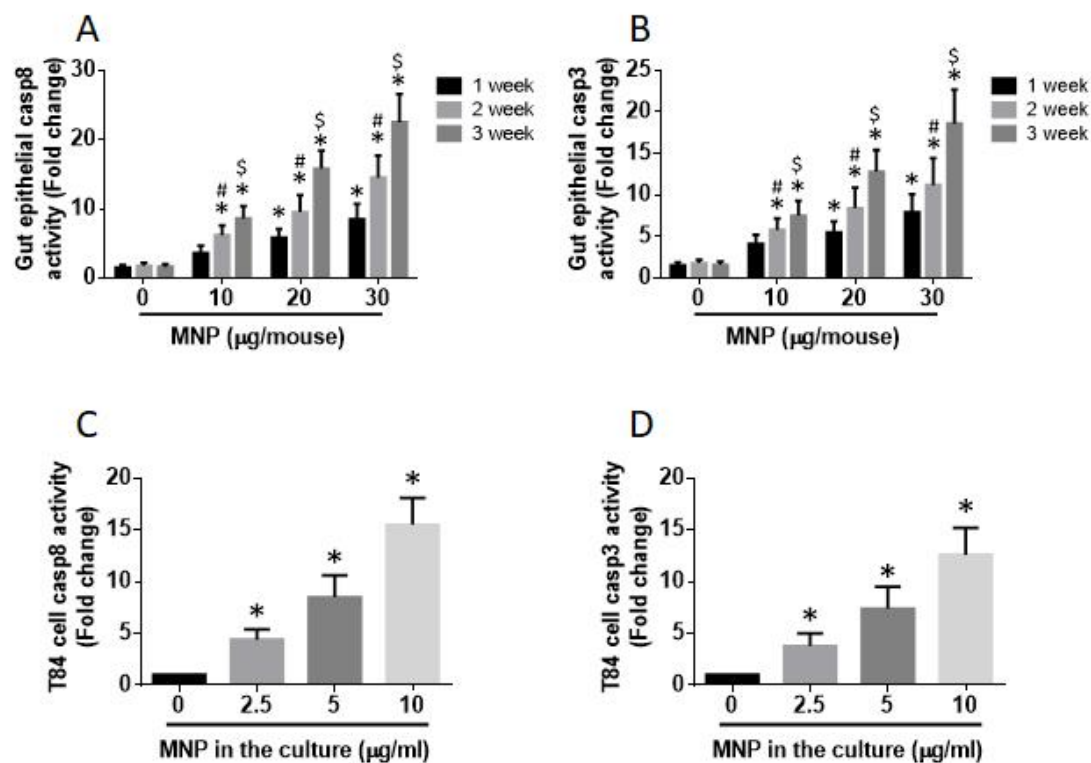


Fig. S3. MNP increases the activation of casp8 and casp3 in intestinal epithelial cells. A-B, mice (6 per group) were treated with MNP via gavage-feeding for 1-3 weeks at gradient doses. The intestinal epithelial samples were collected by scrapping. The bars indicate the activation of casp8 and casp3. C-D, the bars indicate the activation of casp8 and casp3 in T84 cells after exposure to MNP in the culture for 3 days at the indicated concentrations. The data of bars are presented as mean $\pm$ SD. * $p < 0.01$, compared with the "0" group. The data of C and D represent 3 independent experiments.

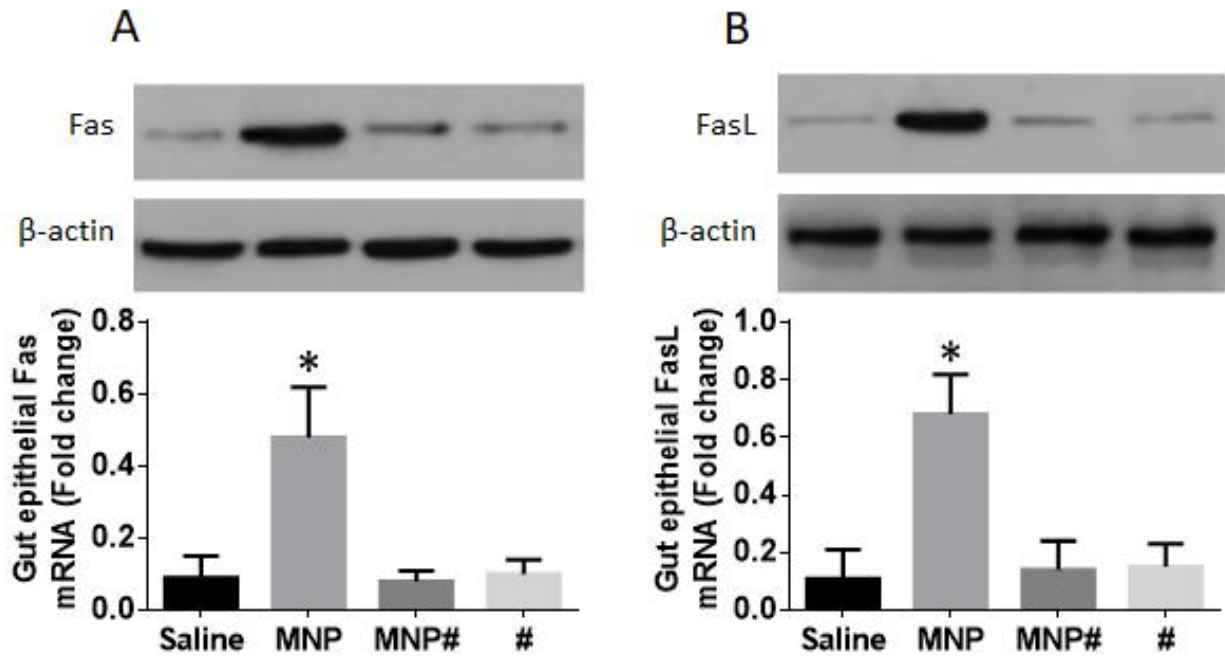


Fig. S4. Exposure to MNP up regulates Fas and FasL expression in the intestinal epithelial cells.

A-B, the levels of Fas (A) and FasL (B) in the small intestinal epithelium of mice (6 mice per group) after the treatment denoted on the X axis for 3 weeks. #: Inhibitors of caspase (Z-VAD-FMK; 1.5 mg/kg daily for mice). Data of bars are presented as mean $\pm$ SD. * $p < 0.01$, compared with the saline group.

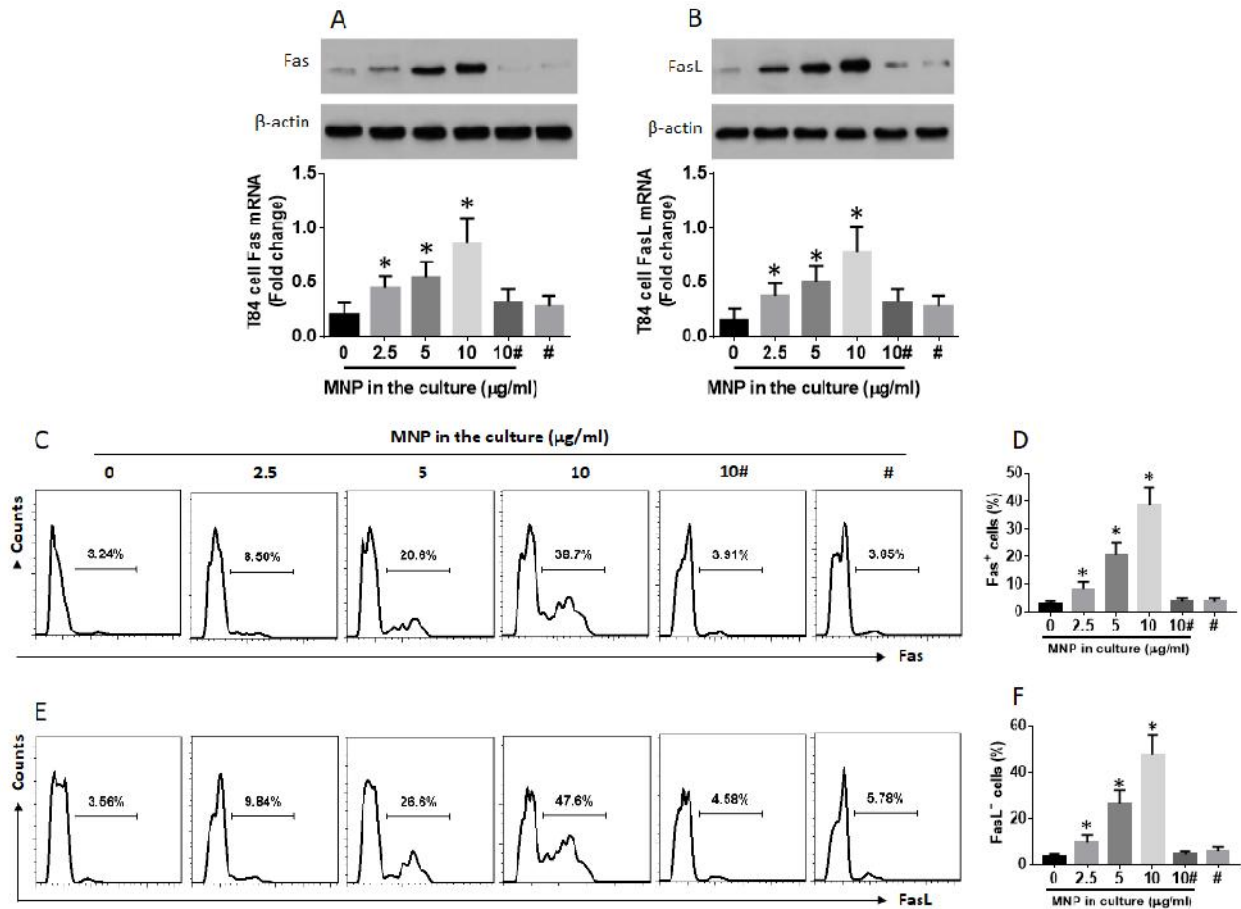


Figure S5. Exposure to MNP induces Fas/FasL expression in T84 cells. A-B, the levels of Fas (A) and FasL (B) in T84 cells after exposure to MNP at the indicated concentrations (denoted on the X axis) for 3 days. #: Inhibitors of caspase (Z-VAD-FMK; 50 μM). C-F, the T84 cells were also analyzed by flow cytometry. The gated histograms show frequency of Fas (C) and FasL (E) positive T84 cells. Bars of D and F show summarized data of C and E. Data of bars are presented as mean ± SD. *p<0.01, compared with the “0” group. The data represent 3 independent experiments.