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Epithelial cells modulate genes associated with NF kappa B activation in co-cultured human macrophages

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Abstract

Macrophages located in airways and the alveolar space are continually exposed to different signals from the respiratory mucosa. In this respect, epithelial cells represent an important source of cytokines and mediators modulating the state of activation and/or differentiation of mononuclear phagocytes. Many of the proinflammatory genes induced in macrophages during immune and immunopathological reactions are regulated by transcription factor NF kappa B. The aim of our study was to characterize changes in the expression of genes associated with NF kappa B activation and signalling in THP-1 human macrophages co-cultured with A549 respiratory epithelial cells. At least 4-fold upregulation of mRNA level was found in 29 of 84 tested genes including genes for multiple cytokines and chemokines, membrane antigens and receptors, and molecules associated with NF kappa B signalling. The mRNA induction was confirmed at the level of protein expression by evaluating the release of IL-6 and IL-8 and by ICAM-1 expression. Blocking of one NFκB subunit by p65 siRNA inhibited the production of IL-6 in both cell types while IL-8 release from THP-1 cells did not seem to be affected.

We conclude from our data that unstimulated respiratory epithelial cells regulate genes associated with NF kappa B dependent immune responses in human macrophages and that these interactions may play a key role in immediate responses in the respiratory mucosa.

Keywords

Co-cultures; Cytokines; Epithelial cells; Macrophages; NF kappa B

Introduction

Mononuclear phagocytes represent highly efficient effector cells of innate immunity mechanisms and in the case of dendritic cells are responsible for the initiation of adaptive immune responses. Traditionally, peripheral blood monocytes are recruited during their development (more extensively in inflammation) into tissue compartments and consequently mature into macrophages or dendritic cells. There are several lines of evidence showing that tissue macrophages are not a homogeneous population of cells and different subpopulations can be defined with respect to phenotype or cell function (Gordon and Martinez, 2010 and Mantovani et al., 2007). The level of maturation is probably a key factor in macrophage heterogeneity. For example, human alveolar macrophages sampled by bronchoalveolar

lavage can reflect either a phenotype of mature tissue macrophages or less mature monocyte-like cells (Striz et al. 1993). Moreover, the phenotypic pattern of mononuclear phagocytes undergoes prominent changes in the course of immune a immunopathological reactions.

Macrophages located in the airways and the alveolar space are continually exposed to different signals from the respiratory mucosa which is known to regulate a number of immune responses (Bulek et al. 2010). In this respect, epithelial cells represent an important source of cytokines and mediators modulating the state of activation and/or differentiation of mononuclear phagocytes. We have shown previously that human monocytes co-cultured in a direct cell–cell contact with primary bronchial epithelial cells change their phenotype into a pattern resembling mature alveolar macrophages. Some of the changes, such as the induction of ICAM-1 (CD54) also were inducible by soluble factors. Moreover, similar results were obtained when using cell lines (Striz et al. 2001).

Many of the proinflammatory genes induced in macrophages during their activation are regulated by transcription factor NF kappa B, which functions as a homo or heterodimer of five constituent proteins: c-REL, RelA (p65), RelB, p50, and p52 (Wang et al. 2007). This transcription factor regulates genes involved in multiple stages of immune responses including activation of both cells participating in innate immunity cells and acquired immunity mediated by lymphocytes and dendritic cells (Wan and Lenardo 2010).

The aim of our study was to characterize changes in the expression of genes associated with NF kappa B activation and signalling in human macrophages co-cultured with respiratory epithelial cells. Furthermore, the potential role of transcription factor NFκB in cell–cell interactions was evaluated using p65-siRNA.

Methods

Culture conditions

The A549 alveolar type II-like cell line (from ATCC) was cultured in Iscove's modified Dulbecco's medium (IMDM) with 10% fetal calf serum (FCS) and l-glutamine, penicillin, streptomycin (Sigma–Aldrich) until confluency in 6-well tissue culture plates. The medium was removed and the cells were washed twice with Earle's Balanced Salt Solution (EBS). THP-1 cells (monocyte/macrophage cell line) were cultured in the same medium as A549 cells and in co-culture experiments, the cells were physically separated with 0,4 μm pore-filters. At different times (0, 4, 8, 12 and 24 h) we harvested the cells separately to evaluate gene and protein expression.

Quantitative RT-PCR (qRT-PCR)

The qRT-PCR analysis was performed using RT^2 Profiler PCR Arrays (Common Cytokines Array Kit) from SABiosciences (USA) on the instrument ABI 7900HT. This kit contains 84 different specific primers for NFκB related genes, plus controls for genomic contamination and reverse transcription and positive PCR controls (Table 1). Data were evaluated with a web-based program provided by the manufacturer.

Flow cytometry

The expression of CD54 (ICAM-1) was measured by flow cytometry (Cytomics FC 500, Beckman Coulter) using PE labeled monoclonal antibody (Beckman Coulter). Isotype specific IgG1 mouse immunoglobulin (Beckman Coulter) was used as a control. The viability of the cells was detected by 7-amino-actinomycin D (7-AAD Viability Dye, Beckman Coulter). Non-viable cells can be characterized as they are stained by 7-AAD, while living cells, retaining their membrane integrity, are impermeable to 7-AAD, are unstained and 7-AAD negative.

Cytokine levels

Simultaneous determination of the concentrations of IL-6 and IL-8 released from co-cultured cells was assessed by the immunoluminescent x-MAP technology, Luminex^{100} System (Luminex Corporation, Austin, Texas, USA), based on the laser analysis of analyte-specific antibodies that are coated onto microparticles. In the first step, 50 μl of the samples/standards was incubated with 50 μl of microparticles for 3 h at RT on a horizontal orbital microplate shaker. After washing the beads to remove unbound substances, we added 50 μl of the secondary antibodies conjugated with biotin to each well and incubated the samples/standards for another 1 h. After the incubation, we washed the beads to remove unbound secondary antibodies and added 50 μl of the Streptavidin-PE. After 30 min incubation and another washing, the microparticles were resuspended in 100 μl of wash buffer and read using a Luminex analyzer.

RNA interference by p65-siRNA suppression of NFκB

Targeting p65 siRNA and non-targeting control siRNA (Dharmcon, Inc, Lafayette, CO) were introduced into the cells using a method previously described with modification (Liu et al. 2008). Briefly, A549 cells were cultured in 1% FCS–DMEM and THP-1 cells were cultured in 10% FCS–RPMI 1640 without antibiotics and after washing with PBS, the cells were treated with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) containing p65-siRNA or non-targeting control siRNA (final concentration 100 nM in Opti-MEM) for 6 h. Then, the cells were re-fed with 0.5% FCS–RPMI, and THP-1 cells plated on top of confluent monolayer of A549 cells. Control cultures with isolated cells were prepared. The levels of IL-6 and IL-8 released into culture media under different culture conditions were evaluated by ELISA.

IL-6 ELISA

IL-6 levels in culture supernatants were quantified using a sandwich ELISA. Briefly, 96 well flat bottomed microtiter plates (Immulon, Chantilly, VA) were coated with 200 μl/well of purified goat anti-hIL-6 antibody (R&D, Minneapolis, MN) diluted 1:1000 in Voller's buffer for 24 h at 4 °C. After 3× washing in PBS, 0.25% Tween-20 (washing solution), undiluted culture supernatants and serial dilutions of standard rIL-6 (Sigma, St. Louis) were incubated at room temperature for 90 min. Plates were rinsed 3 times with PBS–Tween followed by the addition of rabbit anti-human IL-6 antibody (Calbiochem, LaJolla, CA) diluted 1:1000 in washing solution. After 1 h incubation and washing 3 times, human serum absorbed peroxidase conjugated goat anti-rabbit antibody (ICN Biomedicals, Costa Mesa, CA) was

added at a 1:2000 dilution for a 1 h incubation. The plates were then washed again three times with PBS–Tween and the substrate solution orthophenyldiamine (Sigma, St. Louis) at 10 ng/ml in a solution of 0.01% H_2O_2 was added. The reaction was terminated with 27.5 μl of 3 M sulfuric acid and plates were read at 490 nm in an automated ELISA reader.

IL-8 ELISA

IL-8 levels in culture supernatants were quantified using a sandwich ELISA. The 96 well flat bottomed microtiter plates (Dynatech, Chantilly, VA) were coated with 200 μl/well of goat anti-human IL-8 antibody (R&D, Minneapolis, MN) diluted 1:2000 in Voller‘s buffer for 24 h at 4 °C. After 3× washing in PBS–Tween, undiluted culture supernatants and serial dilutions of standard human recombinant rIL-8 (Sigma, St. Louis, MO) were incubated at room temperature for 90 min. Plates were rinsed 3 times with PBS–Tween followed by the addition of rabbit anti-human IL-8 antibody (Upstate Biotechnology Inc., Lake Placid, NY) diluted 1:2000 in washing solution. After a 1 h incubation, dishes were washed 3 times, and peroxidase conjugated goat anti-rabbit antibody (Sigma, St. Louis, MO) was added at a 1:2000 dilution for a 1 h incubation. The plates were then washed again and orthophenyldiamine (Sigma, St. Louis, MO) dissolved in methanol (1 mg/ml) and diluted (10 ng/ml) in distilled water containing 0.01% H_2O_2 was added. The subsequent reaction was terminated with 27.5 l of 3 M sulfuric acid and plates were read at 490 nm at an ELISA reader.

Statistical analysis

Statistical analysis was performed using the Mann–Whitney *U* test and Student's *t*-test, for qRT-PCR data, a web-based program provided by the manufacturer (SABiosciences) was used.

Results

Genes induced in THP-1 monocytes/macrophages after a co-culture with A549 epithelial cells

At least 4-fold upregulation of mRNA level was found in 29 out of 84 tested genes. In the early phases of co-culture (4 h), upregulation of proinflammatory cytokine genes IL-1 beta and TNF alpha (more than 40-fold increase in mRNA level compared to basal expression) was detected together with increased expression of IL-8/CXCL8 chemokine (the highest mRNA expression), EGR1, HMOX1, CCL2, JUN, LTA, IRAK2, FOS, IFN beta 1, and ICAM-1 (Table 2). Macrophages isolated after 8 h of co-culture upregulated gene expression of IL-1R-type I 1 and CD40. After 12 h of co-culture with epithelial monolayers, other genes associated with NF kappa B activation are upregulated including REL, transcription co-activator BCL3, MALT gene, NFκB1 subunit, TNFRSF member 10b, IFN alpha 1, IL-6 and IL-10. After 24 h, mRNA for CD27, Fas ligand (CD178), TLR3, CSF2, CSF3, IL-1 alpha and IFN gamma are induced.

In most of the genes evaluated, the increase in mRNA expression was followed by their sequential down-regulation, on the other hand, IL-6 and IL-10 gradually increased the expression in 24 h period.

During co-culture, only a limited number of genes showed transient down-regulation compared to basal levels; these included Gap junction protein alpha 1, MAP3 kinase 1, and coagulation factor II (thrombin) receptor.

CD54 expression and cytokine release induced by co-culture of THP-1 monocytes/ macrophages with A549 epithelial cells

Co-culture with a monolayer of A549 cells induced the expression of ICAM-1 (CD54) on the surface of THP-1 cells (Fig. 1). The changes were more pronounced in a direct co-culture without separation of cells by filter insert. When looking at the cytokine levels in culture media, IL-8 was released earlier than IL-6 (Fig. 2 and Fig. 3).

Effect of p65 suppression on IL-6 release by A549/THP-1 co-culture

Suppression of the NFκB subunit by p65 demonstrated that this pathway is involved in the regulation of IL-6 in both cell types (Fig. 4). The release of IL-6 was inhibited in a co-culture of p65 siRNA transfected THP-1 cells with control A549 cells ($p < 0.0001$) and similarly in a co-culture of control THP-1 cells with siRNA p65 transfected A549 cells ($p < 0.0001$) as compared with control A549/THP-1 co-culture.

Effect of p65 suppression on IL-8 release by A549/THP-1 co-culture

The release of IL-8 was inhibited only in a co-culture of p65 transfected A549 cells with control THP-1 cells ($p < 0.0003$) or p65 transfected THP-1 cells ($p < 0.0053$). Isolated suppression of p65 only in THP-1 did not affect IL-8 production (Fig. 5).

Discussion

Coordinated efforts of different cell populations present in the lung are important for rapid and effective responses to stimuli inhaled from the environment. Epithelial cells in both the airways and alveoli are able to release multiple chemokines that regulate the influx of different types of immune cells (Mercer et al., 2009, Sachse et al., 2006 and Vroling et al., 2007), proinflammatory cytokines that amplify immune responses (Ge et al., 2010 and Hashimoto et al., 2000), and regulate functions of T lymphocytes (Regamey et al. 2007), as well as growth factors (Leigh et al. 2008) and mediators of repair (Howat et al. 2002). In the lung, macrophages are the predominant population of “professional” immune cells and are thus continuously affected by mediators released from the respiratory epithelium. It is likely that both soluble factors and direct cell–cell contacts can play a role in this communication. We have shown, using an *in vitro* model, that THP-1 macrophages co-cultured with filter separated A549 epithelial cells upregulate the transcription of numerous proinflammatory genes of cytokines, chemokines and signalling molecules within hours. The highest mRNA expression at 4 h of co-culture was found for IL-8/CXCL8, a major chemokine regulating the influx of neutrophils. This was followed by early growth response protein 1 which induces activation and differentiation of lymphocytes (Gomez-Martin et al. 2010). The induction of macrophage-related proinflammatory cytokines TNF alpha and IL-1 beta was also very early and robust. On the other hand, slightly delayed induction of the anti-inflammatory cytokine IL-10 mRNA was detected, which increased gradually during the 24 h period. Delayed release of IL-10 could function as a control mechanism to dampen and/or

terminate inflammation induced by earlier expression of pro-inflammatory genes. Suppression of inflammation by delayed induction of some NF kappa B related genes is also consistent with recent findings that this transcription factor is associated not only with inflammatory responses but also mediates pathways leading to resolution of inflammation (Han et al., 2009 and Lawrence and Fong, 2010). This is also supported by the observation that A549 cells reduce NO production by alveolar macrophages (Rubovitch et al. 2007).

Findings at the protein level, consistent data with the mRNA induction, were found in the release of IL-6 and IL-8 and ICAM-1 expression. These proteins were selected as we have previously studied their regulation in primary cultures of human bronchial epithelial cells and human blood monocytes and know that similar regulatory mechanisms are transferable to studies of cell lines. Both macrophages and epithelial cells might contribute as a source of IL-6 and IL-8. It has been found in previous study, that a co-culture of A549 cells with mononuclear phagocytes modulates the release of cytokines and chemokines in response to endotoxin and staphylococcal exotoxins (Krakauer 2002), hyperoxia (Hjort et al. 2003), and microparticles (Alfaro-Moreno et al. 2008).

Membrane expression of ICAM-1 on THP-1 macrophages was induced to a higher degree when the cells were in a direct contact with epithelial cells as compared to filter-separated conditions. This observation is consistent with our data using primary cells and the interaction probably involves multiple ligand signalling since our preliminary attempts to block it with a large panel of monoclonal antibodies against major contact and co-stimulatory molecules have not shown any specific ligand dependence (data not shown).

NF kappa B regulates multiple genes involved in different stages of immune responses and controls also many functions of macrophages and epithelial cells. This transcription factor plays a key role in the regulation of proinflammatory cytokine genes (TNF alpha, IL-1beta, IL-6), Toll-like receptors, IL-1 and TNF receptors. NF kappa B triggers activation of MAPK and STAT proteins and downstream leads to induction of interferons (Biswas and Lewis 2010). RNA interference targeting the p65 (RelA) subunit of NF kappa B has been shown to modulate functions of primary epithelial cells in our previous study (Liu et al. 2008) and we thus decided to use this approach in the current co-culture model. We have found that THP-1 cells lacking p65 are defective in IL-6 release after co-culture with epithelial cells. In contrast, IL-8 concentrations were not significantly inhibited by blocking THP-1 p65 using siRNA in the same set of experiments, suggesting regulation through different pathways. A role for NF kappa B is not excluded, however, as atypical NF kappa B pathways, involving p105, p100, and RelB (Sun and Ley 2008) are responsible for regulation of IL-8 gene transcription. Negative results have been obtained in experiments targeting p65 to modulate VEGF release from THP-1 cells (data not shown).

We conclude that unstimulated respiratory epithelial cells regulate the expression of multiple genes in human macrophages, that this is, at least partially due to NF kappa B dependent signaling and that these interactions might play a key role in immediate responses in the respiratory epithelium.

Acknowledgements

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Abbreviations

NF	nuclear factor
mRNA	messenger ribonucleic acid
ICAM	intercellular adhesion molecule
IL	interleukin
hIL	human interleukin
rIL	recombinant interleukin
siRNA	small interfering ribonucleic acid
qRT-PCR	quantitative reverse transcription real-time polymerase chain reaction
Ig	immunoglobulin
ELISA	enzyme-linked immunosorbent assay

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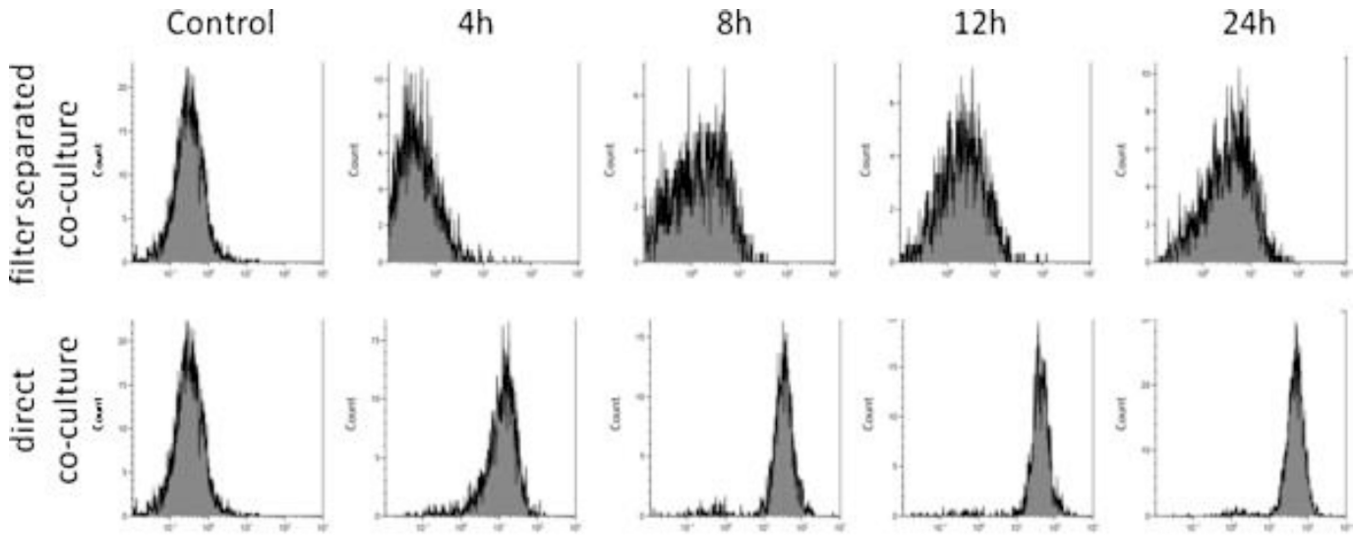

Fig. 1.

ICAM-1 (CD54) expression on THP-1 cells co-cultured with A549 epithelial monolayers. THP-1 cells were either directly co-cultured with confluent A549 cells in the same well or separated by a filter insert. At different time points (4, 8, 12, 24 h) THP-…

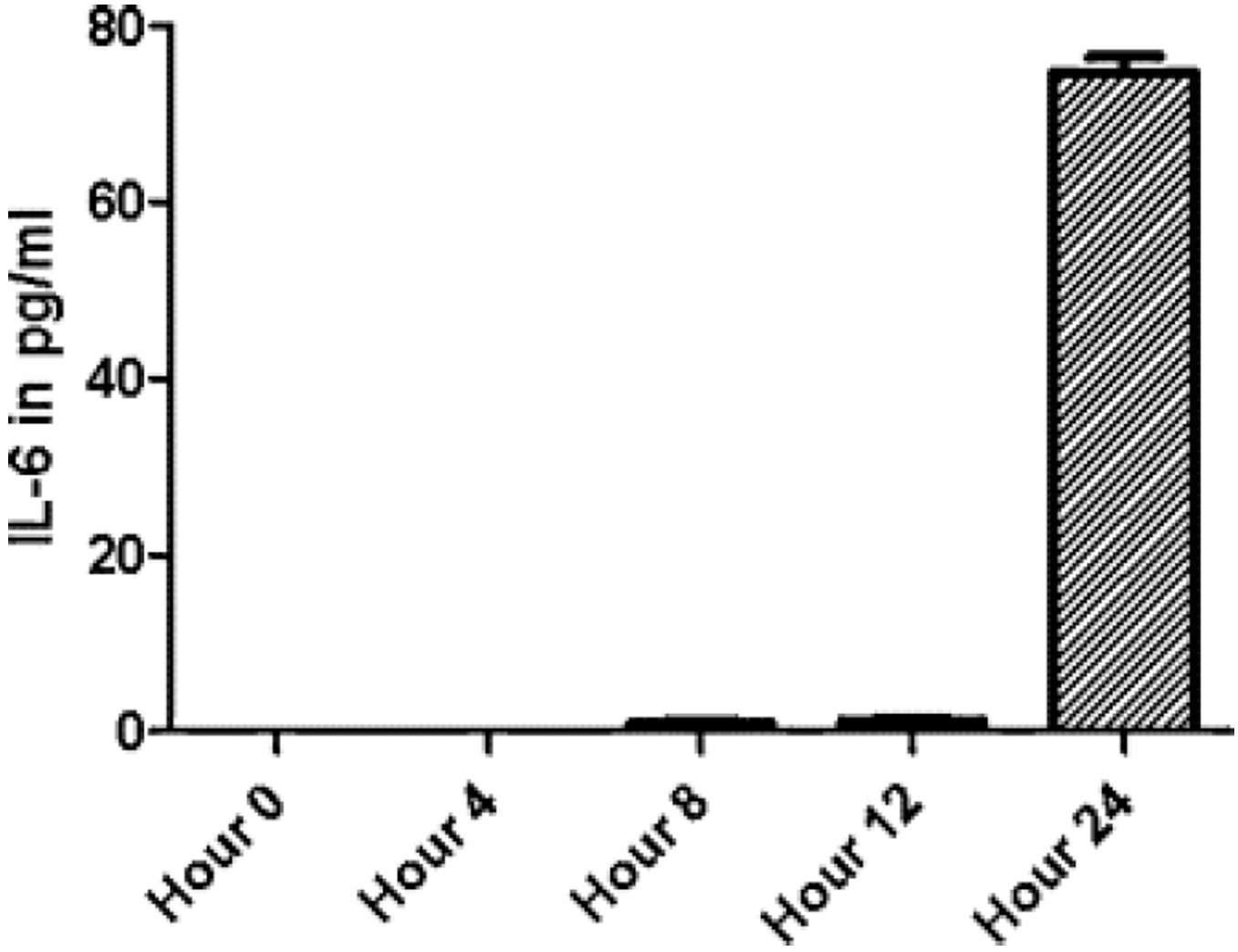

Fig. 2.

IL-6 induction by a co-culture of THP-1 monocytes/macrophages with A549 epithelial cell line. Both cell lines were co-cultured for 24 h and at different time points (4, 8, 12, 24 h) the supernatants with floating THP-1 cells were aspirated, centrifuged…

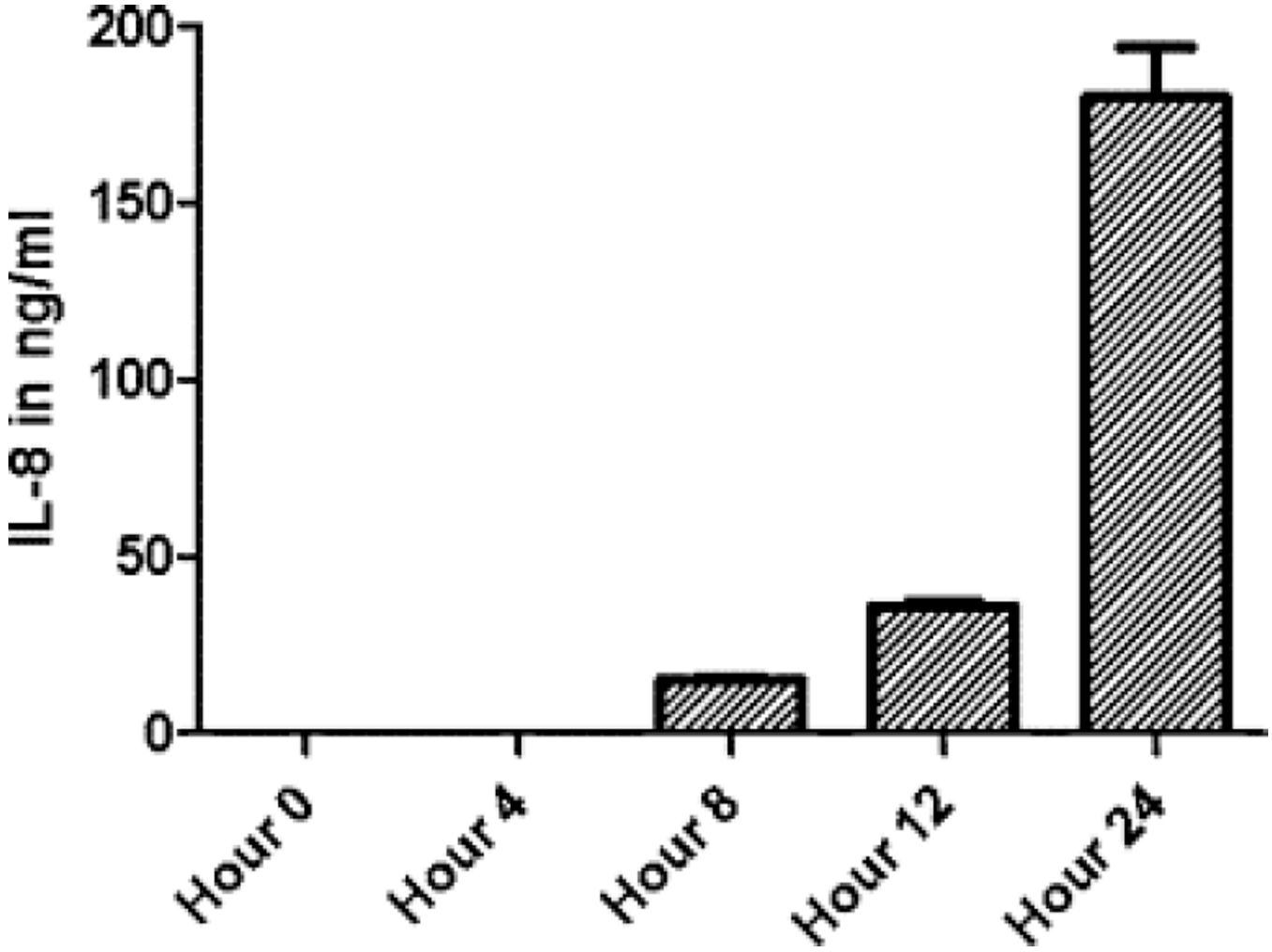

Fig. 3.

IL-8 induction by a co-culture of THP-1 monocytes/macrophages with A549 epithelial cell line. Both cell lines were co-cultured for 24 h and at different time points (4, 8, 12, 24 h) the supernatants with floating THP-1 cells were aspirated, centrifuged…

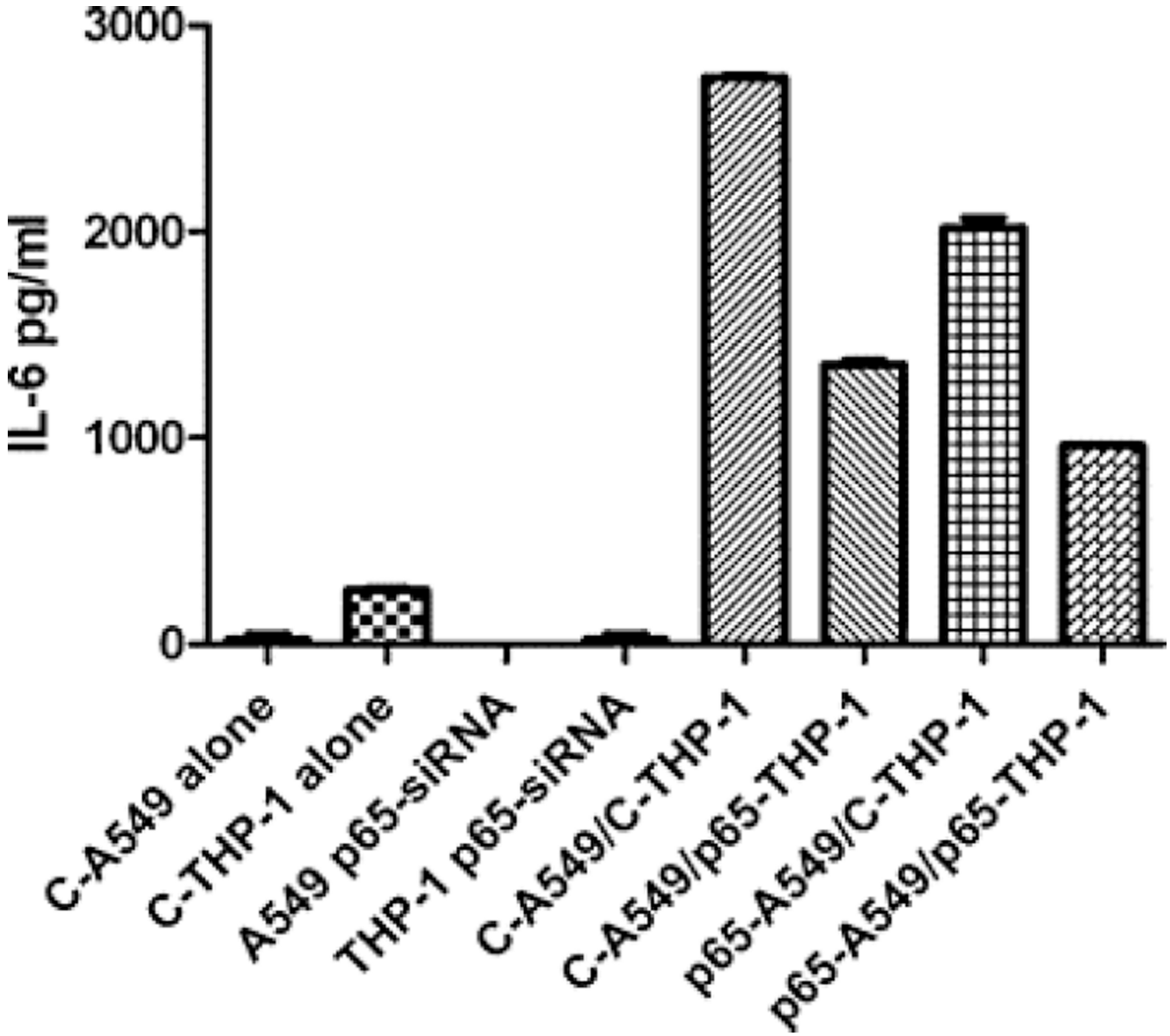

Fig. 4.

P65-siRNA effect on IL-6 release by A549/THP-1 co-culture. A549 and THP-1 cells were transfected with p65-siRNA or non-targeting control siRNA. Then, the THP-1 cells were plated on top of confluent monolayer of A549 cells with relevant control culture…

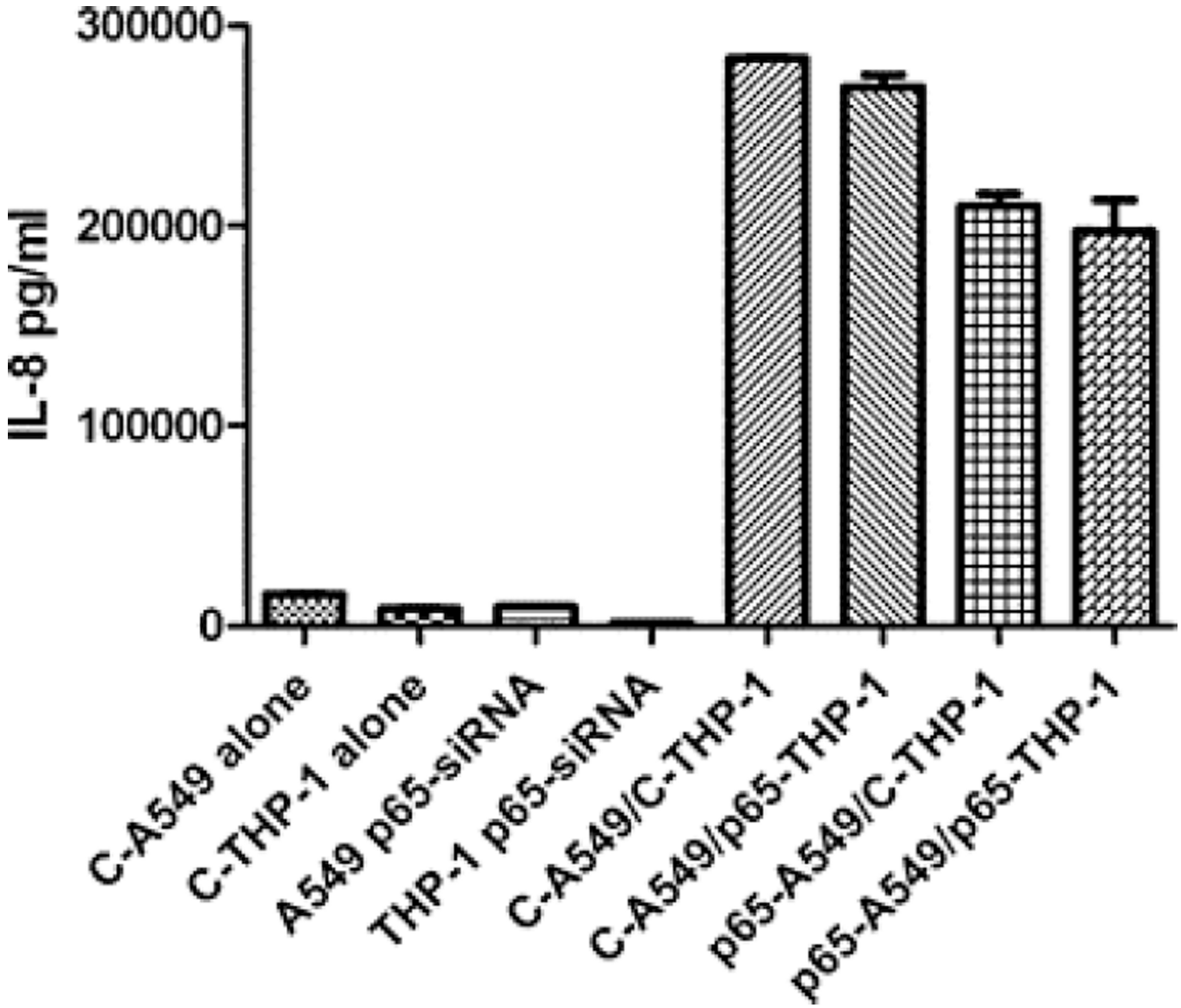

Fig. 5.

P65-siRNA effect on IL-8 release by A549/THP-1 co-culture. After the transfection of A549 and THP-1 cells with p65-siRNA or non-targeting control siRNA, the cells were co-cultured for 24 h with relevant control culture conditions of isolated cells. The…

Table 1

Abbreviations of genes evaluated.

Symbol	Description
AGT	Angiotensinogen (serpin peptidase inhibitor, clade A, member 8)
AKT1	V-akt murine thymoma viral oncogene homolog 1
ATF1	Activating transcription factor 1
BCL10	B-cell CLL/lymphoma 10
BCL3	B-cell CLL/lymphoma 3
CFB	Complement factor B
BIRC2	Baculoviral IAP repeat-containing 2
NOD1	Nucleotide-binding oligomerization domain containing 1
CASP1	Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)
CASP8	Caspase 8, apoptosis-related cysteine peptidase
CCL2	Chemokine (C-C motif) ligand 2
CD40	CD40 molecule, TNF receptor superfamily member 5
CFLAR	CASP8 and FADD-like apoptosis regulator
CHUK	Conserved helix-loop-helix ubiquitous kinase
CSF2	Colony stimulating factor 2 (granulocyte-macrophage)
CSF3	Colony stimulating factor 3 (granulocyte)
SLC44A2	Solute carrier family 44, member 2
EDARADD	EDAR-associated death domain
LPAR1	Lysophosphatidic acid receptor 1
EGR1	Early growth response 1
ELK1	ELK1, member of ETS oncogene family
F2R	Coagulation factor II (thrombin) receptor
FADD	Fas (TNFRSF6)-associated via death domain
FASLG	Fas ligand (TNF superfamily, member 6)
FOS	V-fos FBJ murine osteosarcoma viral oncogene homolog
GJA1	Gap junction protein, alpha 1, 43kDa
HMOX1	Heme oxygenase (decycling) 1
HTR2B	5-hydroxytryptamine (serotonin) receptor 2B
ICAM1	Intercellular adhesion molecule 1
IFNA1	Interferon, alpha 1
IFNB1	Interferon, beta 1, fibroblast
IFNG	Interferon, gamma
IKBKB	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta
IKBKE	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon
IKBKG	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma
IL10	Interleukin 10
IL1A	Interleukin 1, alpha
IL1B	Interleukin 1, beta
IL1R1	Interleukin 1 receptor, type I

Symbol	Description
IL6	Interleukin 6 (interferon, beta 2)
IL8	Interleukin 8
IRAK1	Interleukin-1 receptor-associated kinase 1
IRAK2	Interleukin-1 receptor-associated kinase 2
JUN	Jun oncogene
LTA	Lymphotoxin alpha (TNF superfamily, member 1)
LTBR	Lymphotoxin beta receptor (TNFR superfamily, member 3)
MALT1	Mucosa associated lymphoid tissue lymphoma translocation gene 1
MAP3K1	Mitogen-activated protein kinase kinase kinase 1
MYD88	Myeloid differentiation primary response gene (88)
NLRP12	NLR family, pyrin domain containing 12
NFκB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
NFκB2	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)
NFκBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
PPM1A	Protein phosphatase 1A (formerly 2C), magnesium-dependent, alpha isoform
RAF1	V-raf-1 murine leukemia viral oncogene homolog 1
REL	V-rel reticuloendotheliosis viral oncogene homolog (avian)
RELA	V-rel reticuloendotheliosis viral oncogene homolog A (avian)
RELB	V-rel reticuloendotheliosis viral oncogene homolog B
TRIM13	Tripartite motif-containing 13
RHOA	Ras homolog gene family, member A
RIPK1	Receptor (TNFRSF)-interacting serine-threonine kinase 1
SLC20A1	Solute carrier family 20 (phosphate transporter), member 1
STAT1	Signal transducer and activator of transcription 1, 91kDa
TBK1	TANK-binding kinase 1
TICAM2	Toll-like receptor adaptor molecule 2
TLR1	Toll-like receptor 1
TLR2	Toll-like receptor 2
TLR3	Toll-like receptor 3
TLR4	Toll-like receptor 4
TLR6	Toll-like receptor 6
TLR7	Toll-like receptor 7
TLR8	Toll-like receptor 8
TLR9	Toll-like receptor 9
TMED4	Transmembrane emp24 protein transport domain containing 4
TNF	Tumor necrosis factor (TNF superfamily, member 2)
TNFAIP3	Tumor necrosis factor, alpha-induced protein 3
TNFRSF10A	Tumor necrosis factor receptor superfamily, member 10a
TNFRSF10B	Tumor necrosis factor receptor superfamily, member 10b
TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A
CD27	CD27 molecule
TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10

Symbol	Description
TNFSF14	Tumor necrosis factor (ligand) superfamily, member 14
TRADD	TNFRSF1A-associated via death domain
TICAM1	Toll-like receptor adaptor molecule 1

Table 2

mRNA expression in THP-1 cells after a co-culture with A549 epithelial monolayers. THP-1 cells were co-cultured with a monolayer of A549 cells separated by a filter insert. After 4 h, 8 h, 12 h, and 24 h, the THP-1 cells were aspirated, mRNA extracted and the expression of mRNA evaluated by qRT-PCR. The experiment has been repeated three times with consistent results. Data were correlated with mRNA expression of unstimulated THP-1 cells and statistically evaluated with a web-based program provided by the manufacturer. Bold are the values of at least 4-fold change of mRNA expression.

Upregulated												
Symbol	95% CI	Fold reg.	p-Value	95% CI	Fold reg.	p-Value	95% CI	Fold reg.	p-Value	95% CI	Fold reg.	p-Value
IL8	(147.30, 296.04)	221.67	0	(174.85, 197.32)	186.08	0	(207.99, 245.93)	226.96	0	(27.32, 30.74)	29.03	0
EGR1	(82.59, 111.12)	96.85	0	(115.54, 124.96)	120.25	0	(45.21, 65.48)	55.34	0	(7.73, 18.74)	13.23	0.01
TNF	(58.66, 69.62)	64.14	0	(41.25, 48.81)	45.03	0	(18.15, 22.35)	20.25	0	(22.34, 29.40)	25.87	0
IL1B	(29.80, 55.01)	42.41	0	(32.09, 59.42)	45.75	0	(286.84, 483.44)	385.14	0	(1.43, 3.28)	2.35	0.02
HMOX1	(31.26, 39.29)	35.28	0	(26.57, 30.66)	28.61	0	(25.31, 29.56)	27.43	0	(3.32, 8.68)	6	0.02
CCL2	(6.93, 11.50)	9.21	0	(9.83, 10.96)	10.4	0	(31.39, 33.10)	32.24	0	(4.05, 5.20)	4.62	0
JUN	(6.24, 12.00)	9.12	0	(5.80, 7.05)	6.43	0	(7.27, 13.11)	10.19	0	(2.48, 4.01)	3.25	0
LTA	(6.94, 8.66)	7.8	0	(7.91, 9.49)	8.7	0	(5.49, 6.90)	6.2	0	(5.44, 10.86)	8.15	0.01
IRAK2	(3.50, 7.43)	5.46	0.01	(4.30, 6.67)	5.49	0	(17.13, 23.24)	20.19	0	(1.38, 2.61)	1.99	0.03
FOS	(4.30, 6.28)	5.29	0	(5.10, 7.15)	6.13	0	(9.26, 11.60)	10.43	0	(5.49, 8.71)	7.1	0
IFNB1	(2.11, 8.17)	5.14	0	(1.81, 6.41)	4.11	0	(0.00001, 9.74)	4.77	0.14	(2.12, 8.58)	5.35	0
ICAM1	(3.38, 5.53)	4.45	0	(5.01, 7.21)	6.11	0	(11.25, 18.77)	15.01	0	(0.86, 1.88)	1.37	0.18
IL1R1	(3.05, 3.91)	3.48	0	(3.74, 4.41)	4.08	0	(5.51, 7.17)	6.34	0	(1.73, 2.57)	2.15	0
CD40	(2.08, 3.54)	2.81	0	(4.63, 7.59)	6.11	0	(3.54, 7.86)	5.7	0.01	(1.65, 2.45)	2.05	0
BCL3	(2.42, 3.07)	2.75	0	(2.24, 3.24)	2.74	0	(11.64, 14.65)	13.15	0	(0.47, 0.79)	1.59	0.02
CD27	(1.79, 3.54)	2.66	0	(1.10, 3.01)	2.06	0.05	(1.21, 2.68)	1.94	0.02	(2.98, 8.06)	5.52	0.01
NFκB1	(2.02, 2.52)	2.27	0	(1.63, 2.04)	1.84	0	(4.10, 5.20)	4.65	0	(0.77, 1.24)	1.01	0.92
IL6	(1.42, 2.98)	2.2	0.04	(2.95, 3.61)	3.28	0	(2.79, 7.21)	5	0.03	(6.43, 10.26)	8.34	0
MALT1	(1.04, 2.70)	1.87	0.09	(2.11, 2.34)	2.23	0	(9.36, 10.57)	9.96	0	(1.02, 1.54)	1.28	0.1
TNFRSF10B	(1.61, 1.95)	1.78	0	(2.23, 2.69)	2.46	0	(4.46, 7.40)	5.93	0	(1.07, 1.38)	1.22	0.04
IL10	(1.41, 1.65)	1.53	0	(1.45, 2.53)	1.99	0.02	(3.31, 7.95)	5.63	0.01	(6.43, 10.26)	8.34	0
IFNA1	(1.41, 1.65)	1.53	0	(0.94, 0.96)	1.05	0	(1.40, 1.77)	1.58	0	(6.43, 10.26)	8.34	0
CSF2	(1.41, 1.65)	1.53	0	(0.39, 2.57)	1.48	0.36	(1.13, 1.41)	1.27	0.02	(6.43, 10.26)	8.34	0
CSF3	(1.41, 1.65)	1.53	0	(0.94, 0.96)	1.05	0	(1.13, 1.41)	1.27	0.02	(6.43, 10.26)	8.34	0

FASLG	(1.41, 1.65)	1.53	0	(0.94, 0.96)	1.05	0	(1.13, 1.41)	1.27	0.02	(6.43, 10.26)	8.34	0
IFNG	(1.41, 1.65)	1.53	0	(0.94, 0.96)	1.05	0	(1.13, 1.41)	1.27	0.02	(6.43, 10.26)	8.34	0
TLR3	(1.41, 1.65)	1.53	0	(1.07, 1.15)	1.11	0	(1.13, 1.41)	1.27	0.02	(6.43, 10.26)	8.34	0
IL1A	(1.19, 1.29)	1.24	0	(1.45, 2.97)	2.21	0.03	(4.49, 8.15)	6.32	0.01	(0.54, 0.77)	1.53	0.01
REL	(1.11, 1.19)	1.15	0	(0.93, 1.08)	1.01	0.83	(4.25, 5.27)	4.76	0	(0.52, 0.67)	1.68	0
Downregulated												
F2R	(0.13, 0.37)	3.99	0.02	(0.14, 0.35)	4.14	0.02	(0.09, 0.31)	4.99	0.02	(0.19, 0.63)	2.45	0.06
MAP3K1	(0.25, 0.52)	2.58	0	(0.62, 0.68)	1.54	0	(0.43, 0.52)	2.11	0	(0.22, 0.27)	4.08	0
GJA1	(0.40, 0.70)	1.81	0.02	(0.78, 1.34)	1.06	0.75	(3.35, 6.17)	4.76	0	(0.39, 1.01)	1.42	0.21