Research Article

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EXPRESSION OF COMPLEMENT RECEPTOR IMMUNOGLOBULIN IN HUMAN MONOCYTE-DERIVED MACROPHAGES

Nateelak Kooltheat [1], Prapakorn Wisitpongpun [1], Pachuen Potup [1], Yordhathai Thongsri [1], Sutatip Pongcharoen [2], Bernard Malissen[3] and Kanchana Usuwanthim [1]*

[1]Cellular and Molecular Immunology Research Unit, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok 65000, Thailand

[2]Faculty of Medicine, Naresuan University, Phitsanulok 65000, Thailand

[3]Centre d'Immunologie de Marseille-Luminy and Centre d'Immunophenomique, Aix-Marseille Universite, INSERM U1104 and US012, CNRS UMR7280 and UMS3367, 13288 Marseille Cedex 09, France

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***Corresponding author**
E-mail: kanchanau@nu.ac.th

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ABSTRACT

The complement receptor of the immunoglobulin (CRIg) is exclusively express by macrophages. This complement receptor play critical role not only in innate immunity but also adaptive immune response. Previous study has suggested that PKCα involved in the regulation of CRIg expression in human macrophages. In this study, we have examined the role of glucocorticoid receptor in CRIg expression in human monocyte-derived macrophages (MDMs). Human macrophages nucleofected with plasmid containing short hairpin RNA against glucocorticoid receptor showed reduced expression of glucocorticoid receptor mRNA and protein by RT-qPCR and Western blot analysis, respectively. Glucocorticoid receptor-deficient MDMs showed decreased expression of CRIg mRNA and protein (long and short form). In contrast, cell deficient in glucocorticoid receptor showed increased expression of CRIg in response to dexamethasone. The finding indicates that glucocorticoid receptor involves in the regulation of CRIg expression and dexamethasone promotes the up-regulation of CRIg expression via glucocorticoid receptor.

Keywords: Complement receptor immunoglobulin; Glucocorticoid receptor; Monocyte-derived macrophages

INTRODUCTION

Macrophages can effectively defence infected pathogen using receptors binding to ligands on pathogen surface mediated endocytosis. Phagocytosis of opsonized particle requires the recognition of complement receptors. Complement receptor immunoglobulin (CRIg), a recently discovered complement receptor, enhances complement-mediated phagocytosis of macrophages and can negatively regulate T cell activation.[1] CRIg is transmembrane protein type 1 immunoglobulin superfamily which encoded by *VISIG* gene located in X chromosome.[2] CRIg play critical role in both innate and adaptive immune system. Previous studies showed that CRIg is the receptor for the complement fragment C3b and mediated in phagocytosis of C3b-opsonised pathogen.[3,4] Binding of CRIg to opsonised pathogen also inhibits the alternative pathway of complement activation. Inhibition of C3 and C5 convertase by CRIg prevent the formation of membrane attack complex and lysis of target pathogen, which in turn inhibit the inflammatory response of the macrophage. Regulation of CRIg expression was revealed to be mediated by the stimulation of glucocorticoid drug dexamethasone, which has anti-inflammatory and immunosuppressive activities.[5] Previous study demonstrated that the glucocorticoid receptor (GR) agonist, dexamethasone, caused a marked up-regulation of CRIg expression. Evidence suggested that dexamethasone regulate CRIg expression via protein kinase Cα and also inhibit the activation of arachidonate.[6] It is interesting that glucocorticoid receptor agonist not only has ability to decrease inflammation but also can up-regulate CRIg expression. However, the signaling pathway of GR regulate CRIg expression remain unclear.

Thus, a better understanding of the GR works may provide insights into possible anti-inflammatory therapy. These evidence lead to determine the pathway of GR regulate CRIg expression in human monocyte-derived macrophages (MDMs). The present study is an attempt to focus on the GR knockdown using short hairpin RNA (shRNA) nucleofection in human MDMs that associate the expression and signaling pathway of CRIg regulation.

MATERIALS AND METHODS

Isolation and differentiation of human peripheral blood monocytes

Human monocytes were isolated from leukocytes-rich blood component obtained with permission from Naresuan University Hospital, Phitsanulok, Thailand (IRB no. 364/58). Blood component were equally diluted with Hank's balanced salt solution (HBSS), layered above 1.077 g/ml Lymphoprep density gradient solution (Axis-Shield PoC AS, Oslo, Norway) and then centrifuged at 2500 rpm, 30 min, 25°C for the isolation of peripheral blood mononuclear cell (PBMCs). Cells were washed once with HBSS and centrifuged at 1800 rpm for 5 min. PBMCs were diluted with RPMI-1640 medium (Thermo Fisher Scientific, Inc., New York, NY, USA), layered above 1.064 g/ml hyper-osmotic Percoll solution (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), and centrifuge at 2100 rpm, 15 min, 25°C for the isolation of human monocytes. For the separation of platelet, cells suspension was layered on 1.068/ml iso-osmotic Percoll solution followed by centrifugation at 1500 rpm, 15 min, 25°C. Pellet of human monocytes was suspended with RPMI-1640 and washed with HBSS followed by centrifugation at 1800, 1500, 1200 and 900 rpm, respectively.

Isolated monocytes were cultured in RPMI-1640 supplemented with 10% FBS (Thermo Fisher Scientific, Inc., New York, NY, USA). Cells were cultured at 37°C in humidified incubator with 5% CO_2 for 14 days until fully differentiated to human monocyte-derived macrophages (MDMs).[7,8]

Fluorescence-activated cell sorting analysis

Human monocytes and macrophages were tested for the expression of membrane surface marker by using FACS analysis. Monocytes and macrophages were analysed by immunostaining with PE-conjugated anti-CD14 (BioLegend, Inc., San Diego, CA, USA) and FITC-conjugated anti-CD16 (BD Biosciences, San Jose, CA, USA), respectively.
Isolated cells were suspended with staining buffer solution in 12 × 75 round-bottom test tube to a concentration of 10^6 cells/ml and then centrifuged at 300 × g, 8 min, 4°C. Cell supernatant was discarded, fluorescence-conjugated antibody was added to the final concentration of 2 µg/10^6 cells in 100 µl, and the reaction was incubated at 4°C for 1 h in the dark. Cells were washed twice with2 ml of staining buffer and centrifuged at 300 × g, 6 min, 4°C. Stained cell pellet was resuspended with 400 µl of staining buffer and analysed by FACS using FC 500 Flow Cytometer (Beckman Coulter, Inc., Indianapolis, IN, USA).

Stimulation of CRIg expression

Expression of CRIg was stimulated by dexamethasone (United States Biological, Salem, MA, USA). Macrophages were plated at the density of 2×10^5 cells/well in 24-well plate and stimulated for 24 h by 12.50 ng/ml dexamethasone. Cells were collected for the quantitation of genes expression by RT-qPCR, and for the investigation of CRIg expression by western blot.

Bacterial culture and DNA plasmid isolation

Culturing of bacteria containing lentiviral shRNA plasmid in this study was approved by the Institutional Biosafety Committee of Naresuan University (NUIBC GM 59-04-16). Stbl3*E-coli* containing lentiviral shRNA plasmid were streaked onto brain-heart infusion (BHI) agar plate containing 100 µg/ml ampicillin antibiotic and incubated at 30°C for 18 h. The isolated colony were picked and cultured in BHI broth containing 100 µg/ml ampicillin at 30°C for 5 h to obtain a growth phase culture.

Lentiviral shRNA plasmids were extracted from *E.coli* by using QIAGEN Plasmid Plus Midi Kit (QIAGEN, Hilden, Germany). Growth-phase culture of Stbl3*E-coli* containing lentiviral shRNA plasmid was obtained for 35 ml and centrifuged at 6000 × g for 15 min at 4°C to collect bacterial pellet. The pellet was completely resuspended with 4 ml of Buffer P1, mixed by inverting, and incubated at room temperature for 3 min. The cell lysate was mixed with 4 ml of Buffer S3 by inverting, transferred to QIAfilter Cartridge, and incubated at room temperature for 10 min. The cell lysate was filtered through the QIAfilter Cartridge and mixed with 2 ml of Buffer BB. The lysate was transferred to QIAGEN Plasmid Plus spin column on QIAvac 24 Plus and filtered through the column. DNA plasmid on the filter column was sequentially washed by 700 µl of Buffer ETR and 700 µl of Buffer PE with vacuum. Residual wash buffer was removed from filter column by centrifuging at 10000 × g for 1 min. Plasmid DNA was eluted from filter column to 1.5 ml microcentrifuge tube by 200 µl of Buffer EB with centrifugation of 5000 × g for 1 min.

Knockdown of GR

Knockdown of GR were performed using *GR/NR3C1*-shRNA specific plasmid. In order to deliver shRNA plasmid into human macrophages, Ingenio Electroporation Solution (Mirus Bio LLC., Madison, WI, USA) and Lonza-Amaxa Nucleofector 2b Device (Lonza Group AG, Basel, Switzerland) were used. MDMs were harvested using detach buffer containing 50 mM EDTA and 0.04% lidocaine hydrochloride in HBSS solution. Cells were transferred to 50 ml centrifuge tube and washed twice with HBSS with centrifugation at 500 × g for 5 min. Cell supernatant was discarded, cell pellet was resuspended with HBSS. Cells were counted, 10^7 cells of macrophages were transferred to microcentrufuge tube and centrifuged at 500 × g for 5 min. Cell supernatant was completely discarded, cell pellet was resuspended with 200 µl of Ingenio Electroporation Solution containing 20 µg/ml of *GR/NR3C1* -shRNA or nontargeting control shRNA. Cell suspension was transferred to 0.2 mm transfection cuvette, put into Lonza-Amaxa Nucleofector 2b device, and electroporated with optimal pulse for human primary macrophages (Protocol Y-010). Viable electroporated cells were counted, transferred to 24-well plate at the density of 2×10^5 cells/well, and allowed to rest for 48 h before additional experimentation.

RNA isolation

Total RNA from transfected macrophages was isolated by TRIzol reagent (Thermo Fisher Scientific, Inc., New York, NY, USA) containing phenol and guanidine isothiocyanate. Cultured macrophages were directly lysed inside the well plate by 200 µl of TRIzol reagent. Homogenized sample was transferred to polypropylene microcentrifuge tube and 100 µl of chloroform were added to perform phase separation. Reaction was vigorously mixed, incubated for 3 min at room temperature, and centrifuged at 12000 × g, 15 min, 4°C. The upper aqueous phase containing RNA was transferred to new tube, 100 µl of isopropanol was added for the precipitation of RNA stand for 10 min at room temperature and centrifuged at 12000 × g, 15 min, 4°C. Reaction supernatant was discarded, RNA pellet was washed once with 500 µl of 75% ethanol in RNase-free water followed by the centrifugation at 7500 × g, 5 min, 4°C. RNA pellet was solved by 50 µl of RNase-free water followed by an incubated for 10 min at 55°C.

Gene expression analysis

Isolated RNA from samples was converted to cDNA using Tetro cDNA Synthesis Kit (Bioline Reagents Limited, London, UK). Reaction contains 5 µl of 1 µg/ml RNA template, 1 µl of $Oligo(dT)_{18}$ Primer, 1 µl of 10mM dNTP mix, 5 µl of 5x RT Buffer, 1 µl of RiboSafe RNase Inhibitor, 1 µl of 200 U/ µl Tetro Reverse Transcriptase, and 7 µl of RNase-free water. Reverse transcription was performed in thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) setting at 45°C for 30 min, and 85°C for 5 min.

Gene expression was evaluated by qPCR using SensiFAST SYBR No-ROX Kit (Bioline Reagents Limited, London, UK). Reaction was prepared by mixing the following reagents; 10 µl of 2x SensiFAST SYBR No-ROX Mix, 0.8 µl of 10 µM forward primer, 0.8 µl of 10 µM reverse primer, 5 µl of 1 ng/ml cDNA template, and 3.4 µl of RNase-free water. Oligonucleotide primers specific to genes used in this study are showed in Table 1 (Bio Basic Inc., Markham, ON, Canada).[5,9] The qPCR cycling was done in CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA), set as 3 min

of initial denaturation at 95°C, 45 cycles of 10 sec denaturation at 95°C followed by 30 sec of annealing and elongation at 60°C. RT-qPCR data was analysed by normalized gene expression using $2^{-\Delta\Delta C_T}$ method.[10]

Table 1: Oligonucleotide primer used in this study

Gene	Sequence (5' → 3')		Tm (°C)	Amplicon Size (Base Pair)
ACTB:	Actin beta [*Homo sapiens*], Reference Gene			
	F:	CATGTACGTTGCTATCCAGGC	60.8	250
	R:	CTCCTTAATGTCACGCACGAT	60.2	
VSIG4:	V-set and immunoglobulin domain containing 4 [Homo sapiens], CRIg			
	F:	TCCTGGAAGTGCCAGAGAGT	60.2	112
NR3C1:	Nuclear receptor subfamily 3 group C member 1 [Homo sapiens], GR			
	F:	ATAGCTCTGTTCCAGACTCAACT	60.5	111
	R:	TCCTGAAACCTGGTATTGCCT	60.8	

Protein extraction

Total protein from macrophages was extracted using cell lysis buffer (150 mM NaCl, 50 mM Tris, 1.0% NP-40) with DTT solution, and Halt Protease/Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific, Inc., New York, NY, USA). Cell culture supernatant from experimental 24-well plate was discarded and cells were washed twice with cold HBSS. Cold RIPA buffer was added to the cells at 100 µl/well, and then keep on ice for 5 min. Cells were scrapped and cell lysate was transferred to 1.5 ml microcentrifuge tube. The lysate was centrifuged at 14000 × g for 15 min to discrete cell debris, then supernatant containing protein extract was collected for further analysis.

Quantification of total protein concentration was performed by Bradford coomassie-binding, colorimetric method. Using 96-well plate, 5 µl of diluted sample or series of protein standard was added. Then 250 µl of coomassie reagent was added to each well and mixed with plate shaker at RT for 30 sec. The reaction was further incubated at RT for 10 min, then measured for the absorbance at 595 nm by microplate reader (PerkinElmer Inc., Waltham, MA, USA).

SDS-PAGE and Western blot analysis

In order to prepare proteins from experimental samples for separation by polyacrylamide gel electrophoresis, 25 µl of 1000 µg/ml protein extract was mixed with the equal volume of 2X Leammli loading buffer, and heated to denature at 95°C for 5 min. Samples were then mixed and centrifuged at 16000 × g for 1 min to separate cell debris and precipitated proteins. For the separation of proteins by SDS-PAGE, 40 µl of each protein sample was loaded to the well of mini 12% SDS-PAGE gel, along with Spectra Multicolor Broad Range Protein Ladder (Thermo Fisher Scientific, Inc., New York, NY, USA). Gels were placed in Mini-PROTEAN Tetra cell (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and ran in Tris/Glycine/SDS buffer for 1 h at constant 100 V. SDS-PAGE gel and PVDF membrane were placed in Mini Trans-Blot Module (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and transfer in Towbin buffer with SDS for 1 h at constant 100 V.

For western blot analysis, PVDF blotting membrane was blocked with 2.5% polyvinyl alcohol in TBST (0.05% Tween-20 in tris-buffered saline solution) at RT for 1 h with shaking. The membrane was then incubated overnight in primary antibody at 4°C with shaking. Monoclonal primary antibodies used in this study were mouse anti-β-actin (C4), mouse anti-human Z39Ig/CRIg (6H8), mouse anti-human GR (G-5) (Santa Cruz Biotechnology, Inc., Dallas, TX, USA). Membrane was washed 4 times with TBST at RT, each for 5 min with shaking. The membrane was then incubated in HRP-conjugated goat anti-mouse IgG (H+L) secondary antibody (Thermo Fisher Scientific, Inc., New York, NY, USA) at RT for 1 h with shaking.

For the detection of protein bands by enhanced chemiluminescence (ECL), the PVDF blotting membrane was washed 4 times with TBST at RT, each for 5 min with shaking. The membrane was soaked in SuperSignal West Pico ECL substrate (Thermo Fisher Scientific, Inc., New York, NY, USA) for 5 min, and placed in ChemiDoc XRS+ Imaging System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Chemiluminescence signal of blotted membrane was acquired, bands of proteins were detected and normalized by Image Lab software (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Experimental design and statistical analysis

Three-independent experiments were performed in this study. One-way ANOVA and Bonferroni multiple comparisons test were used to analyze data using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA). Confidential interval of 99% ($p = 0.01$) was used in all statistical analysis.

RESULTS

Cell isolation

Monocytes were isolated from buffy coat and cultured to differentiate into MDMs. MDMs were fully differentiated on day 14 of culture in completed RPMI medium. Monocytes and macrophages were stained with anti-CD14-PE and anti-CD16-FITC. The expressions of surface marker were 92.87% $CD14^+$ and 84.91% $CD16^+$ (Figure 1).

Generation of GR-deficient MDMs

MDMs were nucleofected with plasmid containing shRNA against *GR/NR3C1*. Nucleofected cells were cultured for 3 days and then RNA and protein were extracted for mRNA and protein expression by RT-qPCR and western blot. The results showed that *GR* mRNA levels were significantly reduced (~72%) in GR-shRNA transfected cell compare to control (Figure 2).

The expression of GR protein was decrease by Western blot analysis. Quantitative results were obtained by normalizing the band intensities of GR-shRNA to β-actin. These results showed that GR protein significantly decreased in GR-shRNA transfected cell compare to control groups (Figure 3).

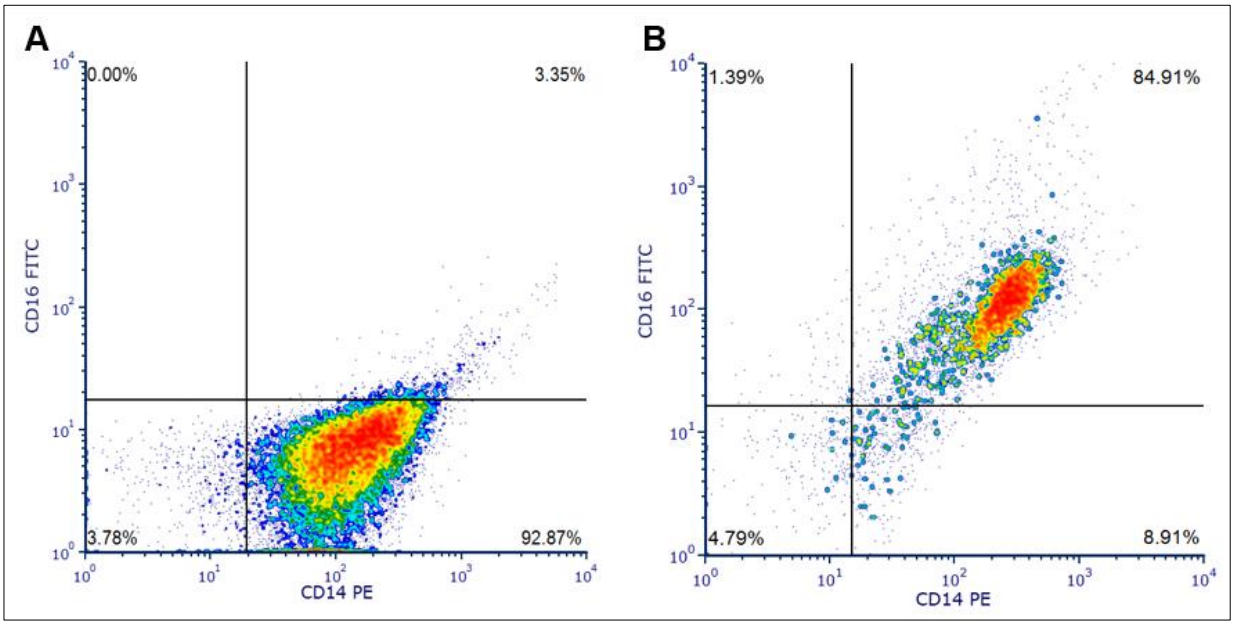

Figure 1: Flow-cytometry analysis of percent purity of monocytes and macrophages with anti-CD14-PE/CD16-FITC.
(A) Numbers in the lower right quadrant represent 92.87% of monocytes ($CD14^+CD16^-$).
(B) Numbers in the upper right quadrant represent 84.91% of macrophages ($CD14^+CD16^+$).

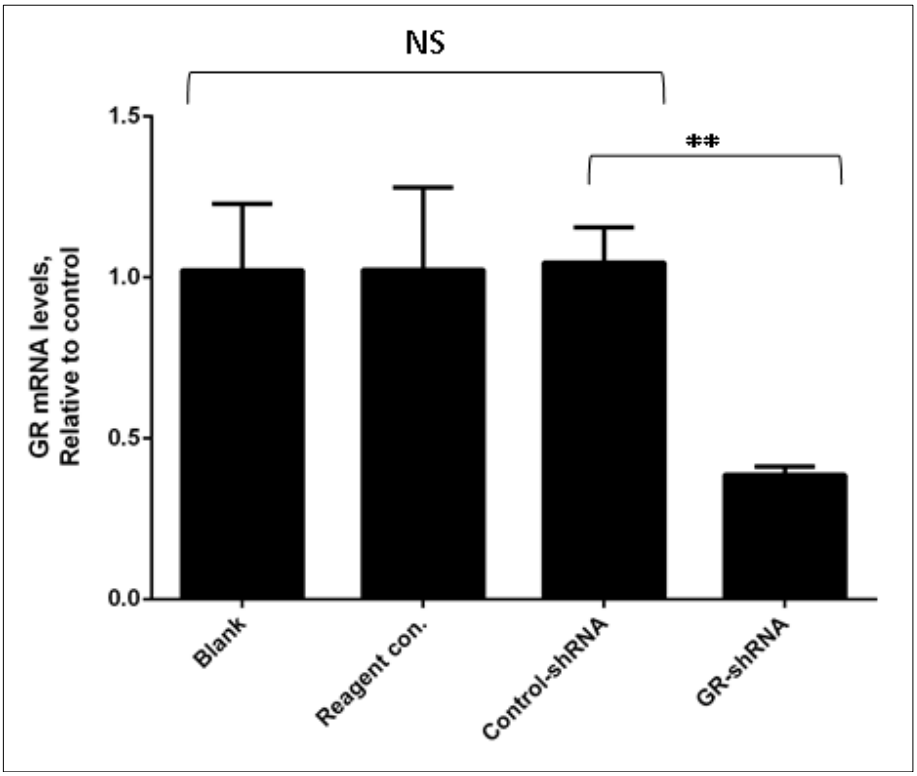

Figure 2: Expression of GR mRNA in human MDMs by Quantitative RT-qPCR.
Bar graph showing the mean ± SEM of non-nucleofection MDMs (Blank), MDMs with nucleofection solution (Reagent control), MDMs with negative-sense (Control-shRNA) and MDM with shRNA plasmid against *GR/NR3C1*(GR-shRNA). The mRNA levels were calculated by Delta–delta Ct method normalize to β-*actin* housekeeping gene (**p<0.005).

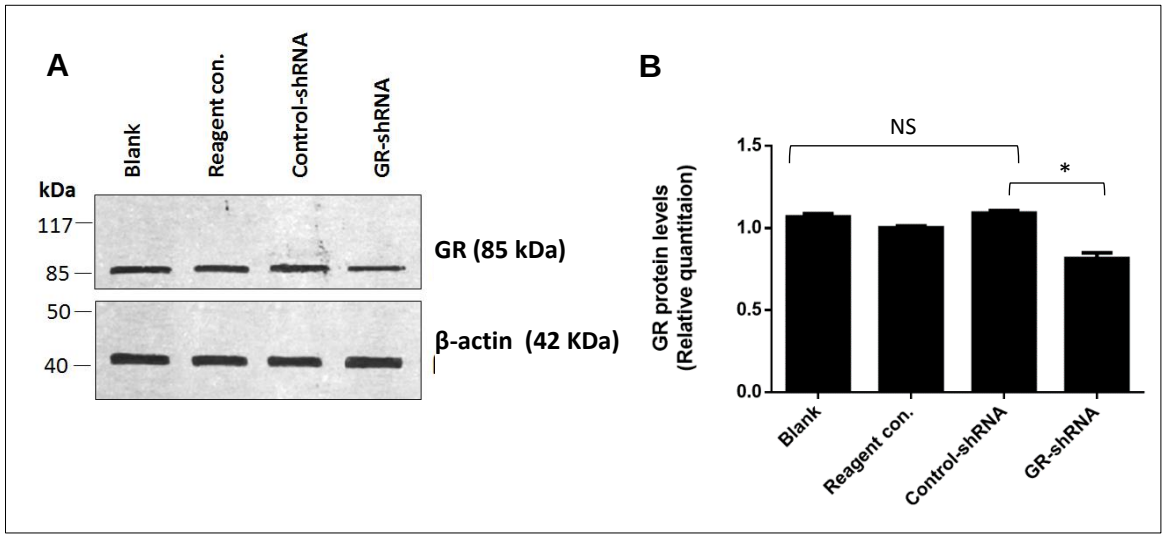

Figure 3: Expression of GR protein in human MDMs by Western blot analysis.
(A)Four band of GR expression by western blot. (B) Quantitative results of GR protein by normalizing the band intensities. Data are presented as means ± SEM of four conditions (*p<0.05).

CRIg expression in GR/NR3C1-deficient MDMs

Transfected macrophages were normalized expression analysis of *GR/NR3C1* and *CRIg/VSIG4* genes in absence and presence of dexamethasone. Results showed that dexamethasone significantly up-regulated expression of *GR/NR3C1* and *CRIG/VSIG4* genes in macrophages (p < 0.01). GR/NR3C1-shRNA transfected cells significantly down-regulated expression of both *GR/NR3C1* and *CRIg/VSIG4* genes to 0.39 and 0.50 fold of untreated control (p < 0.01). Stimulation of GR/NR3C1 knocked-down macrophages with dexamethasone can significantly up-regulated *GR/NR3C1* and *CRIG/VSIG4* genes from 0.39 and 0.50 to 1.01 fold of untreated control macrophages (p < 0.01) (Figure 4).

Transfected macrophages were normalized expression analysis of GR/NR3C1 and CRIg/VSIG4 proteins in absence and presence of dexamethasone. Results showed that dexamethasone significantly increased expression of GR/NR3C1 and CRIg/VSIG4 proteins in macrophages (p < 0.01). GR/NR3C1-deficient MDMs significantly decreased expression of both GR/NR3C1 and CRIg/VSIG4 proteins to 0.54 and 0.50 fold of untreated control macrophages (p < 0.01). Stimulation of GR/NR3C1 knocked-down macrophages with dexamethasone can significantly increased GR/NR3C1 and CRIg/VSIG4 proteins from 0.54 and 0.50 to 1.09 and 1.01 fold of untreated control (p < 0.01) (Figure 5 and 6).

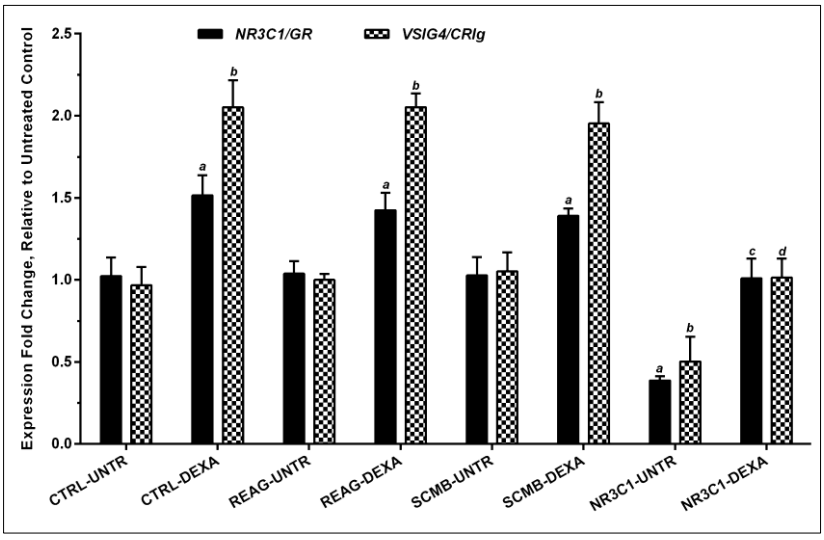

Figure 4: Effect of *GR/NR3C1* knocked-down on the expression of *GR/NR3C1* and *CRIg/VSIG4* genes.
Human macrophages were transfected with GR/NR3C1 shRNA
for the silencing of GR/NR3C1 gene. GR/NR3C1 knocked-down macrophages were stimulated with dexamethasone for 24 h. Expression of GR/NR3C1 and CRIg/VSIG4 genes were analysed by RT-qPCR. Data was normalized and presented as mean with SD of 3-independent experiments. CTRL, Control Macrophages; REAG, Reagent Control; SCMB, Scramble shRNA Transfected; NR3C1, NR3C1 shRNA Transfected; UNTR, Untreated; DEXA, Dexamethasone
a,GR/NR3C1 gene expression, compared to untreated control macrophages; p < 0.01
b,CRIg/VSIG4 gene expression, compared to untreated control macrophages; p < 0.01
c, GR/NR3C1 protein expression, compared to NR3C1- UNTR; p < 0.01
d, CRIg/VSIG4 protein expression, compared to NR3C1- UNTR; p < 0.01

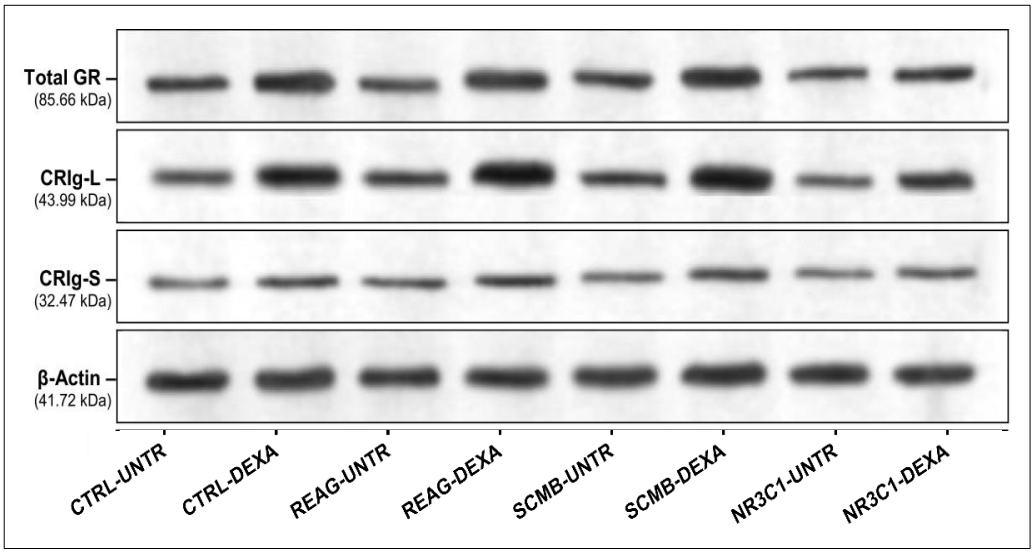

Figure 5: Effect of GR/NR3C1 knocked-downon the expression of GR/NR3C1 and CRIg/VSIG4 proteins by Western blot

Human macrophages were transfected with GR/NR3C1 shRNA for the silencing of GR/NR3C1 gene. GR/NR3C1 knocked-down macrophages were stimulated with dexamethasone for 24 h. Expression of GR/NR3C1 and CRIg/VSIG4 protein were analysed by Western blot. CTRL, Control Macrophages; REAG, Reagent Control;SCMB, Scramble shRNA Transfected; NR3C1, NR3C1 shRNA Transfected; UNTR, Untreated; DEXA, Dexamethasone

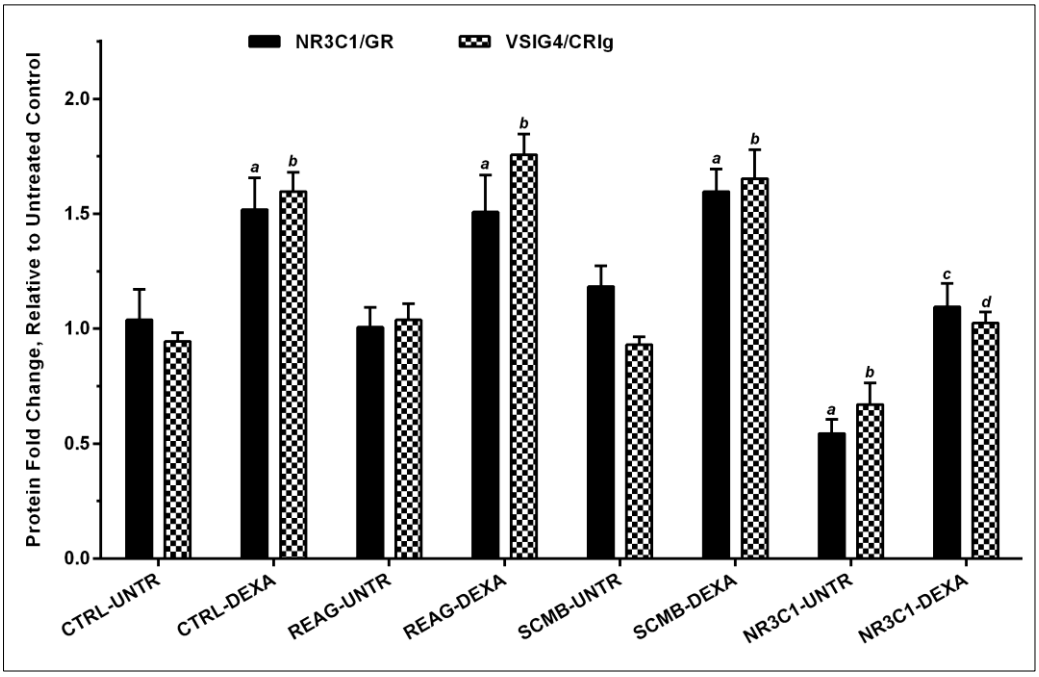

Figure 6: Western blot analysis on the effect of GR/NR3C1 knocked-down

on the expression of GR/NR3C1 and CRIg/VSIG4 proteins Human macrophages were transfected with GR/NR3C1 shRNA for the silencing of GR/NR3C1 gene. GR/NR3C1 knocked-down macrophages were stimulated with dexamethasone for 24 h. Expression of GR/NR3C1 and CRIg/VSIG4 protein were analysed by western blot. Data was normalized and presented as mean with SD of 3-independent experiments CTRL, Control Macrophages; REAG, Reagent Control; SCMB, Scramble shRNA Transfected; NR3C1, NR3C1 shRNA Transfected; UNTR, Untreated; DEXA, Dexamethasone

a,GR/NR3C1 protein expression, compared to untreated control macrophages; $p < 0.01$

b,CRIg/VSIG4 protein expression, compared to untreated control macrophages; $p < 0.01$

c, GR/NR3C1 protein expression, compared to NR3C1- UNTR; $p < 0.01$

d, CRIg/VSIG4 protein expression, compared to NR3C1- UNTR; $p < 0.01$

DISCUSSION

Our data show that GR/NR3C1-deficient MDMs can be successfully performed by nucleofection with GR/NR3C1-targeted shRNA. The result showed that GR/NR3C1 was specifically reduced in these cells (Figure 2 and 3). Because the GR/NR3C1-deficient MDMs showed decrease expression of CRIg mRNA and protein levels (Figure 4-6), the result suggest that GR can regulate CRIg expression. Our studies have now revealed that the up-regulation of CRIg expression in GR/NR3C1-deficient MDMs by dexamethasone.

Gorgani et al reported that mediators of inflammation may control CRIg expression. Arachidonate is down-regulation of CRIg expression in human macrophages.[5] In addition TNF-α, IL-1β/ IL-6 have different potency of the ability to decrease CRIg expression. Furthermore, consistent with Guo et al reported that IFN-γ down-regulated the expression of CRIg on human macrophages.[11] Meanwhile IL-10 and TGF-1β, although considered to be immunosuppressive cytokines, they have opposite effect, IL-10 increased but TGF-1β decreased CRIg expression. The data reveal that cytokines can operate work to control CRIg expression. The steroid anti-inflammation agent, dexamethasone can also up-regulate of CRIg expression.[5] Ma et al showed that the down regulation of CRIg expression in macrophages by LPS could be reduced by making the cells PKCa deficient. They suggest that agents regulate the expression via PKC α.[6] Dexamethasone is a synthetic glucocorticoid compound with potent anti-inflammatory properties.[12] Dexamethasone is used to treat inflammatory and autoimmune conditions such as rheumatoid arthritis, allergic reactions, and certain skin disorders. Dexamethasone can cross cell membranes and binds with high affinity to cytoplasmic glucocorticoid receptors.[13] Complex of glucocorticoid and GR can bind the glucocorticoid response elements, which results in a modification of transcription and protein synthesis. The anti-inflammatory actions of dexamethasone are involve phospholipase A_2 inhibitory proteins, lipocortins, which control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes.[14,15] Ma et al suggest that dexamethasone could direct action via the glucocorticoid receptor.[6]

In order to investigate the significance of GR/NR3C1 on *CRIg/VSIG4* expression, the expression of *CRIg/VSIG4* under *GR/NR3C1*-silenced condition using shRNA transfection method. *GR/NR3C1*-deficient macrophages show significantly decreased expression of mRNA and protein levels, compared to untreated control, reagent control, and scramble shRNA-transfected ($p < 0.01$). Our results show silencing of *GR/NR3C1* significantly down-regulated *CRIg/VSIG4* gene and protein expression, compared to control ($p < 0.01$) (Figure 4 and 6). Results suggested that GR/NR3C1 positively regulated transcription of *CRIg/VSIG4* gene. Our findings imply that dexamethasone activate CRIg expression via glucocorticoid receptor. It is therefore to tempting that GR and CRIg could be novel targets for developing anti-inflammatory therapies.

Results suggested that GR/NR3C1 was the transcription factor that positively regulates expression of CRIg/VSIG4. Mechanism of glucocorticoid, translocation of GR/NR3C1, and functions of GR/NR3C1 as transcriptional regulator were described in previous studies.[16,17] GR/NR3C1 normally expresses in cytoplasmic compartment of cells. Binding of glucocorticoid to GR changes conformation of GR/NR3C1 complex. GR/NR3C1 becomes hyperphosphorylated and dissociates from its accessory heat shock protein 90 (HSP90). Glucocorticoid-bounded GR/NR3C1 then translocate to nucleus through importin nucleocytoplasmic transporter. GR/NR3C1 forms homodimer inside the nucleus and binds to glucocorticoid response elements at gene promoters. Activities of GR/NR3C1 include direct transactivation, tethered transactivation, direct transrepression, and tethered transrepression.[16,17]

CONCLUSION

The data demonstrate that glucocorticoid receptor involves in the regulation of CRIg expression and dexamethasone promotes the up-regulation of CRIg expression via glucocorticoid receptor.

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