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Original Article

PDIA3 knockdown in dendritic cells regulates IL-4 dependent mast cell degranulation

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Abstract: Background: A previous study found that expression of protein disulfide isomerase A3 (PDIA3) in dendritic cells (DCs) is a potential mediator of exaggerated intestinal mucosal immunity response in a rodent model of irritable bowel syndrome (IBS). Aim: The aim of this study was to explore the roles of PDIA3 in the interaction between DCs and mast cells (MCs) in the context of IBS. Methods: This study investigated the effects of shRNA-mediated knockdown of PDIA3 expression in a dendritic cell line and spleen lymphocyte co-cultures. Resultant co-culture supernatants were used to challenge MC. PDIA3 expression was assessed using q-PCR and Western blotting. Expression of surface markers was analyzed by flow cytometry. $CD4^+$ and $CD8^+$ T-cell proliferation were examined using Cell Trace CFSE kits. Cytokine production was determined by ELISA testing. MC viability was ascertained using CCK-8 production. MC degranulation was determined by ELISA and transmission electron microscope (TEM). Results: Knockdown of PDIA3 expression in a dendritic cell line decreased the production of interleukin-4 in subsequently performed co-cultures of a dendritic cell line with spleen lymphocytes. Challenge with such lysates provoked a decrease in the viability and functionality of MC. Conclusion: Co-culture experiments of dendritic cell lines and spleen lymphocytes revealed the roles of PDIA3 expression, as determined by IL-4 production and co-culture supernatant-provoked MC activation. These observations confirm that PDIA3 has a role in the interaction between DCs and MC activation. However, results simultaneously argue for further research concerning the usefulness of PDIA3 as a potential therapeutic target in IBS.

Keywords: Protein disulfide isomerase A3, irritable bowel syndrome, dendritic cell line, mast cell, cell activation

Introduction

Irritable bowel syndrome (IBS) can have debilitating consequences. However, its pathogenesis remains poorly understood, hampering the development of rational therapy. Activation of immune cells and cytokines may play an important role in the pathogenesis of IBS [1]. IBS is characterized by colonic mast cell (MC) infiltration and degranulation, occurring in the proximity of mucosal innervation. This characteristic of IBS has been associated with disease severity, in general, and frequency of abdominal symptoms, in particular [2, 3].

However, it is clear that, other than MCs, various other immune cells play important roles in the pathogenesis of IBS [4, 5]. In this context, dendritic cells (DCs) are interesting. Previous studies [6, 7] have shown that, in experimental IBS, DC acts as an upstream element in the activation of MCs in patients with IBS, possibly through T lymphocytes. *In vitro* DC-mediated T lymphocyte activation assays have shed light on the underlying mechanisms, showing the resulting production of cytokines, such as cytokines IL-4 and IL-9. These cytokines, produced through the interaction between DCs and T lymphocytes, can activate MCs. These cultures can help in understanding this aspect of IBS pathogenesis.

A previous work showed that protein disulfide isomerase A3 (PDIA3) expression is significantly increased in the ileocecal intestinal mucosa of stressed rats, considered a model of IBS [8].

Table 1. shRNA vectors employed in this study

NO.	DNA Synthesis (5'-STEM-Loop-STEM-3')
Pdia3-RNAi (24692-1)-a	Ccgg gcTATCTACAACGAGAAGCTA CTCGAG TAGCTTCTCGTTGTAGATAGC TTTTTg
Pdia3-RNAi (24692-1)-b	aattcaaaaa gcTATCTACAACGAGAAGCTA CTCGAG TAGCTTCTCGTTGTAGATAGC
Pdia3-RNAi (24693-1)-a	Ccgg cgCTTACTATGATGTGGACTA CTCGAG TAGTCCACATCATAGTAAGCG TTTTTg
Pdia3-RNAi (24693-1)-b	aattcaaaaa cgCTTACTATGATGTGGACTA CTCGAG TAGTCCACATCATAGTAAGCG
Pdia3-RNAi (24694-1)-a	Ccgg gcCAACACAAACACCTGTAAT CTCGAG ATTACAGGTGTTTGTGTTGGC TTTTTg
Pdia3-RNAi (24694-1)-b	aattcaaaaa gcCAACACAAACACCTGTAAT CTCGAG ATTACAGGTGTTTGTGTTGGC

Furthermore, compared to controls, patients with irritable bowel syndrome-diarrhea (IBS-D) show upregulation of colonic PDIA3 expression. This expression positively correlates to increased tryptase levels, a specific marker for MC activation and degranulation [9]. Other studies have shown that PDIA3 performs various other functions, including an important role in the quality control of newly synthesized glycoproteins [10, 11]. In addition, PDIA3 is an essential component of the peptide loading complex of MHC class I molecules. PDIA3 plays an important role in the presentation of auto-antigens [12]. These findings suggest that the effects of PDIA3 on DCs play an important role in the etiology of IBS, as it appears an upstream element in MC activation.

The current study sought to clarify the relationship of PDIA3 in DC and MC activation, especially aiming to assess the potential roles of PDIA3 in the interaction between these two cell types.

Materials and methods

Animals

Four-to-six-week-old female C57BL/6 mice were obtained from the Animal Laboratory of Zhejiang Chinese Medical University, Hangzhou, China. These mice were maintained in a pathogen-free facility at Zhejiang Chinese Medical University, according to guidelines set by the Chinese Government.

Dendritic cell line-JAWSII

Dendritic cell line JAWSII, originally derived from C57BL/6mice, was obtained from the Cell Bank of Chinese Academy of Sciences, Shanghai, China. This dendritic cell line was cultured according to supplier protocol. These cells were cultured in a complete medium, consisting of MEM-α (Gibco, NY, USA), ribonucleosides, deoxyribonucleosides, 4 mM L-glutamine, and 1 mM sodium pyruvate, in 20% fetal bovine serum (FBS) (Gibco, NY, USA) and 5 ng/mL of murine-recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) (Invitrogen, Garlsbad, CA, USA). This culture medium was renewed every other day.

Dendritic cell line phenotyping

Fluorescence-activated cell sorting (FACS) (Becton Dickinson Immunocytometry Systems, San Jose, CA) was used to characterize phenotypes of the dendritic cell line through analyzing expression of surface molecules. The maturation status of DCs was analyzed by performing dual staining with anti-CD11c (fluorescein isothiocyanate [PE]-conjugated, eBioscience, California, USA) and anti-CD86, MHC I, or MHC II (I-A) (all APC-conjugated, eBioscience, California, USA), respectively. PE- or APC-labeled antibodies were used as a negative isotype control. The samples were investigated using a BD LSR II system (BD Biosciences, Franklin Lakes, NJ, USA). Analysis was performed using Cell Quest software.

Construction of lentiviral vectors

Short hairpin RNA (shRNA) fragments were hybridized with synthesized sense and antisense oligonucleotides. A hybridized shRNA fragment of PDIA3 was cloned into the lentiviral vector pGC-LV-GFP. **Table 1** displays the shRNA vectors used in this study.

A recombinant lentivirus was produced by transfecting 293T cells. This recombinant lentivirus was designated as PDIA3 *lenti*. Titers of PDIA3lenti were determined by green fluorescent protein (GFP) expression. PDIA3 *lenti* and the negative control *lenti* were constructed through a similar process. Negative control *lenti* contained a scrambled fragment of shRNA.

PDIA3 lenti transduction

When the dendritic cell line was 50% confluent, lentiviral vectors, which had a multiplicity of infection (MOI) of 30, were added to the culture (serum-free medium). After 8 hours, this serum-free medium was changed into a complete medium. This complete medium was subsequently refreshed by replacing half of its volume with a new medium every alternate day. Four days after transfecting the dendritic cell line, the cell cultures were examined by fluorescent microscopy. Stably-transfected dendritic cell lines were selected by subjecting cultures to puromycin (5 μg/mL) selection. Gene and protein expression levels of PDIA3 were determined by qPCR, while successful knock down of PDIA3 was established by Western blotting.

Isolation of spleen lymphocyte and CFSE labeling

Following sacrifice of the animals, spleens were removed and gently disaggregated between frosted glass slides. Disaggregated spleens were then filtered to obtain a single-cell spleen suspension. Using mouse Percoll, approximately 1×10^7 lymphocytes were obtained per mouse. These lymphocytes were then diluted to a final concentration of 0.5×10^6-10×10^7/mL.

Assessing cell proliferation, 5 μM of Carboxy fluorescein diacetate succinimidyl ester (CFSE) (Molecular Probes, Eugene, OR, USA), was added to the cells. This medium was kept for five minutes at room temperature in the dark. These cells were then washed three times with a dilution solution containing 5% heat-inactivated FCS. This solution was diluted 10 times in an excess volume of PBS (20°C). Cells underwent sedimentation by carrying out centrifugation at 300 g for 5 minutes at 20°C. The supernatant was discarded and the cells were subsequently used for further experimentation.

Co-culture of a dendritic cell line and purified splenic lymphocytes

Transfected dendritic cell lines were seeded in triplicate in round-bottom 96-well plates. Dendritic cell lines acted as stimulating agents (2.5×10^5 cells/well) in the subsequent reaction. Purified splenic lymphocytes, which acted as responder cells (2×10^5/well), were added to the dendritic cell line cultures until the total volume of these cultures became 200 μl.

Responder lymphocytes were cultured in the absence of dendritic cell lines and used as control cells. This cell culture was maintained for seven days at 37°C in a humidified atmosphere containing 5% CO_2. Next, CFSE-labeled lymphocytes were harvested and stained with anti-CD4 (PE-conjugated, eBioscience, California, USA) and anti-CD8 (PE-conjugated, eBioscience, California, USA) antibodies, respectively. Proliferation of $CD4^+$/$CD8^+$ T cells was examined by flow cytometry. Interleukin- 4/9 levels in the supernatant culture were determined by ELISA testing. The remaining supernatant was used to challenge P815 cells.

P815 cells and C48/80 incubation

P815 cells, employed as an *in vitro* model for MCs, were originally derived from mast cell carcinomas in mice. In these experiments, P815 cells were purchased from the Cell Bank of the Chinese Academy of Sciences, Shanghai, China. According to supplier protocol, these cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) (Gibco, NY, USA). DMEM was supplemented with 100 units/mL of penicillin and 100 μg/mL of streptomycin. This culture medium was renewed every other day. Compound 48/80 (C48/80) is the N-methyl- of methoxy phenethylamine and formaldehyde condensation polymer. This preparation can activate P8-15. While performing activation assays, P8-15 cells were exposed to 10 μg/mL of C48/80 (Sigma, St. Louis, Missouri, USA) for 60 minutes. These cells were then washed with phosphate buffered saline (PBS).

CCK8 assay

Cells were seeded at a density of 1×10^6 cells/mL. Next, these cells were challenged in either co-culture or control supernatant under appropriate conditions. After incubation of the cell culture for 48 hours, P815 cells were harvested by centrifugation and washed twice with cold PBS. The vitality of P815 cells was examined by performing a CCK-8 assay, according to manufacturer protocol (Dojindo, Shanghai, China).

Tryptase assay and ultrastructure observation

P815 cells were seeded at a density of 1×10^6 cells/mL and incubated in the harvested super-

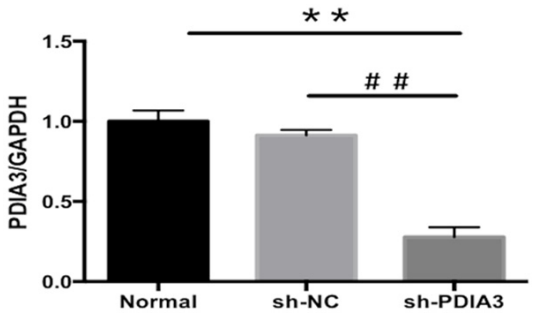

Figure 1. Gene expression of PDIA3 in JAWSII assessed by qPCR. Depicted are results from cells (labeled as "normal", cells with a scrambled sh-RNA lentivirus (labeled as sh-NC) and a sh-PDIA3 group **P < 0.01, compared with normal group; ##P < 0.01versus the sh-NC group. Data are representative of three independent experiments.

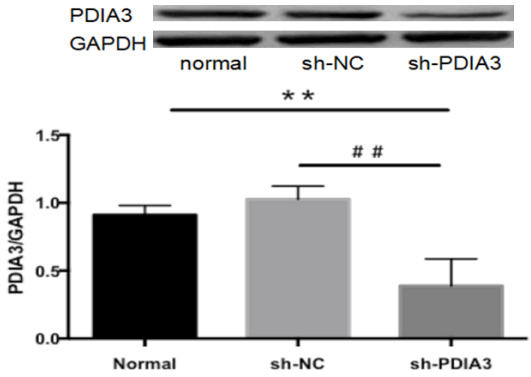

Figure 2. Protein expression of PDIA3 in dendritic cell lines, as detected by Western blot. Depicted are results from cells (labeled as "normal", cells with a scrambled sh-RNA lentivirus (labeled as sh-NC) and a sh-PDIA3 group **P < 0.01 versus normal group; ##P < 0.01 versus sh-NC group. Data are representative of three independent experiments.

natant. It was collected following co-culturing of the dendritic cell line and purified splenic lymphocytes. After incubating these cells for 72 hours, the supernatant was harvested and investigated by performing a commercial tryptase assay, according to the manufacturer protocol (Shanghai Westang Bio-tech Co. Ltd, Shanghai, China). Absorbance was recorded at 450 nm/630 nm using a microplate ELISA reader. Degranulation was observed by a transmission electron microscope (TEM), according to routine procedures.

Statistical analysis

SPSS 17.0 software was used to perform statistical analyses. Data are expressed in terms of mean ± SD. All *in vitro* experiments were performed in triplicate. One-way analysis of variance (ANOVA) was used to compare each group. P < 0.05 indicates statistical significance. Graph Pad Prism software was used to create the figures.

Results

Experimental modulation of PDIA3 expression

Evaluating the success of the current experimental approach, qPCR and Western blotting techniques were used to detect PDIA3 levels in dendritic cell lines. A clear expression of PDIA3 was observed in the dendritic cell line and expression of PDIA3 was substantially reduced in JASWII cells with a PDIA3-targeting sh-RNA (P < 0.01 for RNA, **Figure 1**; P < 0.01 for protein, **Figure 2**). Sh-NC and JASWII cells were not statistically different in this assay. It was concluded that present cultures are suitable for investigating the effects of PDIA3 expression on DC phenotypes.

Effects of PDIA3 expression on dendritic cell lines

To evaluate the effects of PDIA3 expression on dendritic cell line phenotypes, the experimental groups were analyzed with flow cytometry, before and after performing the lipopolysaccharide (LPS) challenge. Regardless of transduction or PDIA3 knockdown *per se*, dendritic cell lines remained viable and maintained their immature phenotypes (**Figure 3**). After LPS-induced differentiation, dendritic cell lines employed for this experimentation showed comparable upregulation of MHC class II and CD86 in each experimental group (**Figure 4**). Thus, PDIA3 expression did not influence properties of dendritic cell lines. Therefore, present cultures are suitable for investigating the potential effects of these cells in co-culture and MC activation experiments.

PDIA3 effect on naïve T-cell responses

CFSE analysis can be used to determine the effects of altered PDIA3 expression in dendritic cell lines on cell proliferation in co-cultures. A lower fluorescent signal indicates a greater number of cell divisions. Purified splenic lymphocytes were labeled with CFSE and co-cultured for 7 days with dendritic cell lines, in

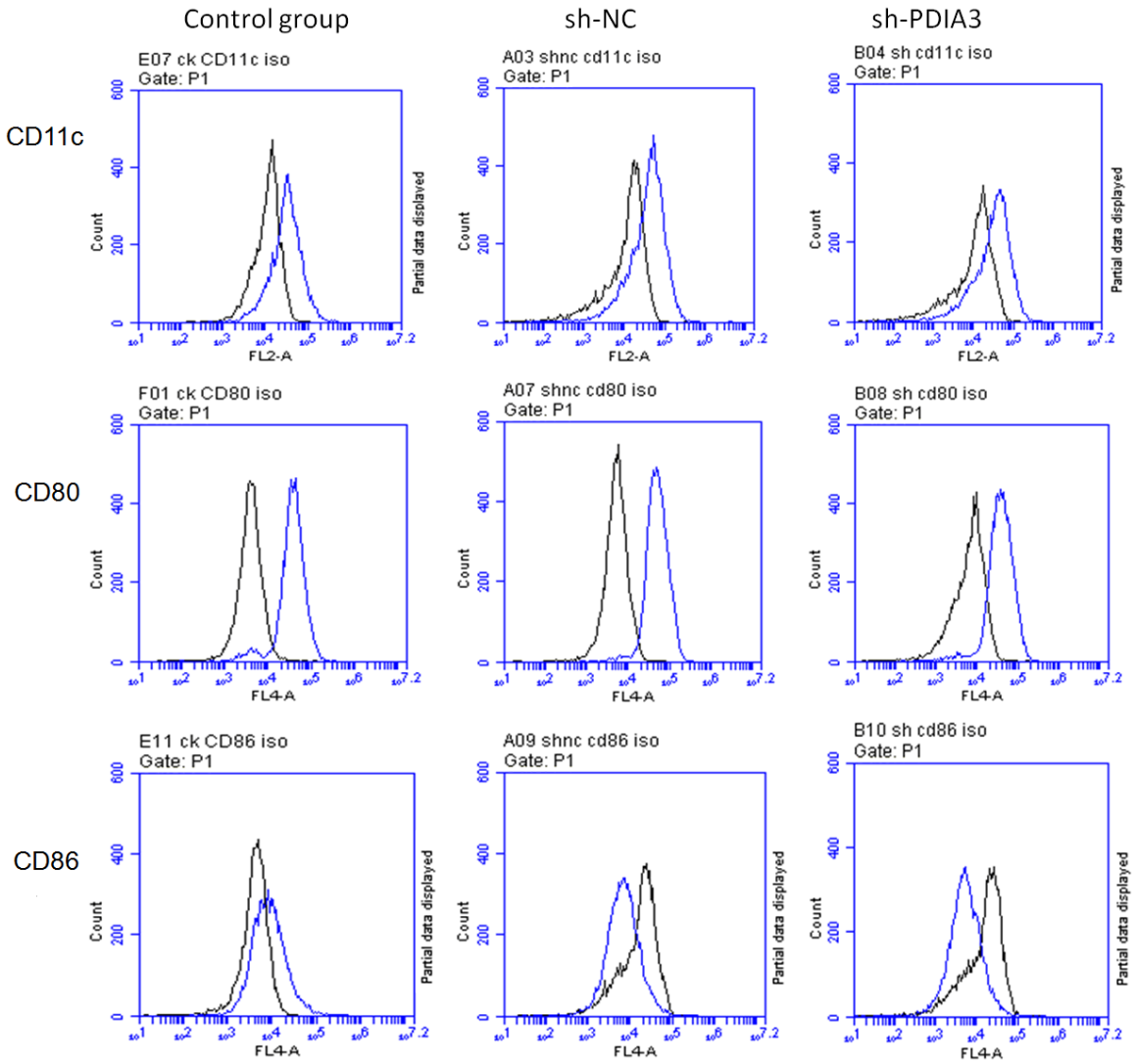

Figure 3. Phenotypes of the dendritic cell line following transduction after transduction in three groups detected by flow cytometry. Depicted are results from cells (labeled as "normal", cells with a scrambled sh-RNA lentivirus (labeled as sh-NC) and a sh-PDIA3 group. The black curve represents the isotype control for each experiment. The blue curves represent specific staining for CD11c, CD80, CD86, MHCI, and MHCII, respectively, as indicated. There is little evidence that PDIA3 expression affects dendritic cell line immunophenotype. Data are representative of three independent experiments.

which PDIA3 expression was modified. Subsequently, CFSE expression was analyzed in both $CD4^+$ or $CD8^+$ T-cell compartments using flow cytometry. Dendritic cell lines stimulated the proliferation of both the subgroups of lymphocytes. However, there was only slight proliferation of T-cells in the absence of dendritic cell lines. There were no statistically significant differences between normal dendritic cell lines, sh-NC dendritic cell lines, or sh-PDIA3 dendritic cell lines ($P > 0.05$; **Table 2**). It was concluded that experimental modification of PDAI3 expression in DCs does not interfere with lymphocyte stimulation. Subsequently, experiments were initiated to determine whether the production of MC-activating cytokines was affected by levels of PDAI3 expression in these co-cultures.

Effects of DC PDIA3 expression on production of MC-relevant cytokines

Co-culture supernatants from the different experimental groups were harvested following co-culturing for 7 days. They were probed for levels of IL-4 and IL-9. As expected, dendritic cell lines

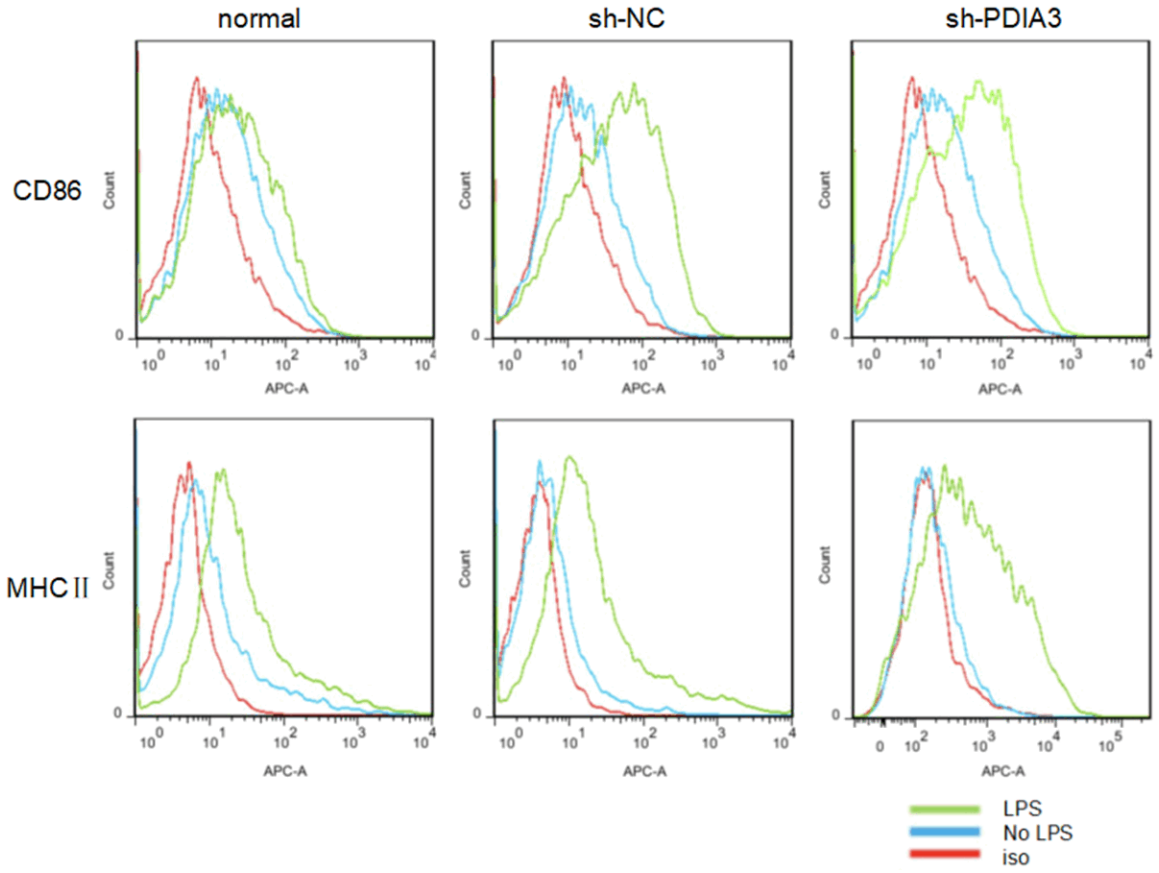

Figure 4. Phenotypes of dendritic cell line following LPS treatment. Depicted are results from cells (labeled as "normal", cells with a scrambled sh-RNA lentivirus (labeled as sh-NC) and a sh-PDIA3 group. The red curves represent isotype controls. The blue curves represent the phenotype of dendritic cell line without LPS stimulation. The yellow curves represent phenotypes of dendritic cell line after LPS treatment. Results appear independent of transduction or PDIA3 status. Data are representative of three independent experiments.

Table 2. Mean fluorescence intensity (MFI) of CFSE-labeled splenic lymphocytes ($CD4^+$ T/$CD8^+$ T) following 7 days of co-culture with differentiated dendritic cell line

Groups	$CD4^+$ T	$CD8^+$ T
Normal	26993.50 ± 582.67**	22831.67 ± 430.00##
Sh-NC	26554.67 ± 474.09**	23189.17 ± 688.95##
Sh-PDIA3	26526.33 ± 149.95**	23949.67 ± 311.51##
$CD4^+$ T	30058.50 ± 491.14	------
$CD8^+$ T	------	29378.83 ± 1007.47

**P < 0.01 versus $CD4^+$ T group; ##P < 0.01 versus $CD8^+$ T group.

potently stimulated production of both cytokines. Importantly, however, in the absence of PDAI3 expression, dendritic cell lines lost the capacity to provoke either IL-4 production (1.38 ± 0.21 pg/mL in sh-PDIA3 dendritic cell line stimulated cultures *versus* 2.59 ± 0.39 pg/mL in cultures stimulated by cells or 2.44 ± 0.77 pg/mL in cultures stimulated by sh-RNA cells, 0.73 ± 0.71 pg/mL in supernatants from unstimulated lymphocytes; $P < 0.05$). No significant differences in IL-9 production were found between the sh-PDIA3 group (5.79 ± 0.93 pg/mL), normal group (5.66 ± 0.29 pg/mL), control sh-RNA group (6.15 ± 0.91 pg/mL), and un-stimulated lymphocytes (4.59 ± 0.59 pg/m; $P > 0.05$). Thus, the loss of DC PDIA3 expression specifically affected production of the MC-relevant cytokine IL-4 in co-culture supernatants. Hence, these results prompted investigation of the potential functional consequences of this altered cytokine profile.

Effects of PDIA3 expression in DCs on co-culture-derived supernatants with respect to MC viability

P815, an MC model, was exposed to co-culture supernatants from each experimental group for 48 hours. These MC were examined for viability

using a CCK-8 assay. In this assay, higher optical density (OD) values indicate better MC viability. Strikingly, OD values of the sh-PDIA3 group (0.31 ± 0.01) were substantially lower than those of the PDIA3-proficient groups (0.36 ± 0.01 in cells, 0.37 ± 0.02 in the control sh-RNA group and 0.34 ± 0.01 in the unstimulated lymphocytes group; $P < 0.05$). This indicates that co-cultures, stimulated by the PDAI3-deficient dendritic cell line, were less proficient in supporting MC function.

Tryptase production

It appears that the PDIA3-deficient dendritic cell line is less capable of supporting T cell-stimulated MC functionality. This notion was substantiated in experiments used to determine the production of tryptase in MC. Tryptase was released strongly by PDIA3-proficient groups: cells (503.67 ± 7.51 pg/mL) and control sh-RNA group (492.33 ± 21.89 pg/mL), as they were stimulated by co-culture supernatants. Tryptase production was substantially reduced in the sh-PDIA3 group (408.46 ± 35.32 pg/mL; $P < 0.05$). The unstimulated lymphocytes group showed 347.03 ± 16.64 pg/mL of tryptase release. Thus, PDAI3 expression in dendritic cell lines is essential for downstream MC activation.

MC ultrastructure

With respect to their ultrastructure, unstimulated MCs are characterized by the presence of high electron density particles. Upon degranulation, however, these particles disappear. There is also a general cell swelling, accompanied by the disappearance of particulate matter, observed among other characteristic changes. Accordingly, these changes are observed using transmission electron microscopy following 72 hours of incubation of MC with supernatants from co-cultures stimulated by PDIA3-proficient dendritic cell lines. Coculture supernatants from cultures stimulated by dendritic cell lines deficient in PDIA3 do not provoke this effect (**Figure 5**). Depicted in this figure are the results from cells that were not transduced (labeled as "normal"), cells transduced with a scrambled sh-RNA lentivirus (labeled as sh-NC), and cells transduced with sh-PDIA3 vectors. Shown are transmission electron microscope pictures with a final magnification of 4,200 times. Ultrastructural network changes are indicated by an arrow. Thus, it seems that PDIA3 is pivotal for steering co-cultures towards a MC-stimulating cytokine profile.

Discussion

The current study worked from the assumption that visceral hypersensitivity in IBS relates to specific properties of intestinal immune cells, resulting in alternative production of cytokines [13]. Mechanisms driving alternative behavior remain only partly understood. However, present results indicate an important role for PDIA in this respect. PDIA3 [11], as a component of the protein disulfide isomerase (PDI) family, is a protein whose expression is induced by cellular stress. Alternatively named ERp57, its functionality seems to be confined to the endoplasmic reticulum (ER). There it is especially relevant in the context of the assembly of the major histocompatibility complex class 1, as well as in the quality control and proper folding of glycoproteins. The functionality of PDIA3 is manifested in liver disease [14, 15], renal fibrosis [16], airway epithelial apoptosis and fibrosis [17], and cancer [18]. In the colonic mucosa of IBS-modeling rats, high expression of PDIA3 has been observed [8]. Thus, the current study focused on the relationship between PDIA3 and IBS.

DCs, as potent antigen-presenting cells necessary to establish effective adaptive immune responses, play an important role in the pathogenesis of experimental IBS. DCs can induce T-cell differentiation through antigen presentation to this compartment. Depending on the cytokine environment, DCs provoke either Th1 (associated with IL-2, IL-12 and IFN-γ) or Th2 (associated with IL-4, IL-5 and IL-9) response [19]. With respect to experimental IBS, a previous study in mice established that, for post-infectious IBS (PI-IBS), the acute stage involves Th2 immunity, whereas later Th1 and Th17 immune responses become dominant [4]. Dendritic cell lines used in this study were derived from an immature DC line isolated from bone marrow cells of a p53-knockout C57BL/6 mouse. This cell model has proven successful for studies aimed at better understanding antitumor [20, 21] and immune/inflammatory responses [22]. It was postulated that PDIA3 is expressed on DCs, based on previous proteomic analysis of the colonic mucosa in a rat IBS model. Upon detection of PDIA3 expression on the dendritic cell line, based on immunophenotyping results, it was found that transduction does not affect the maturation status or im-

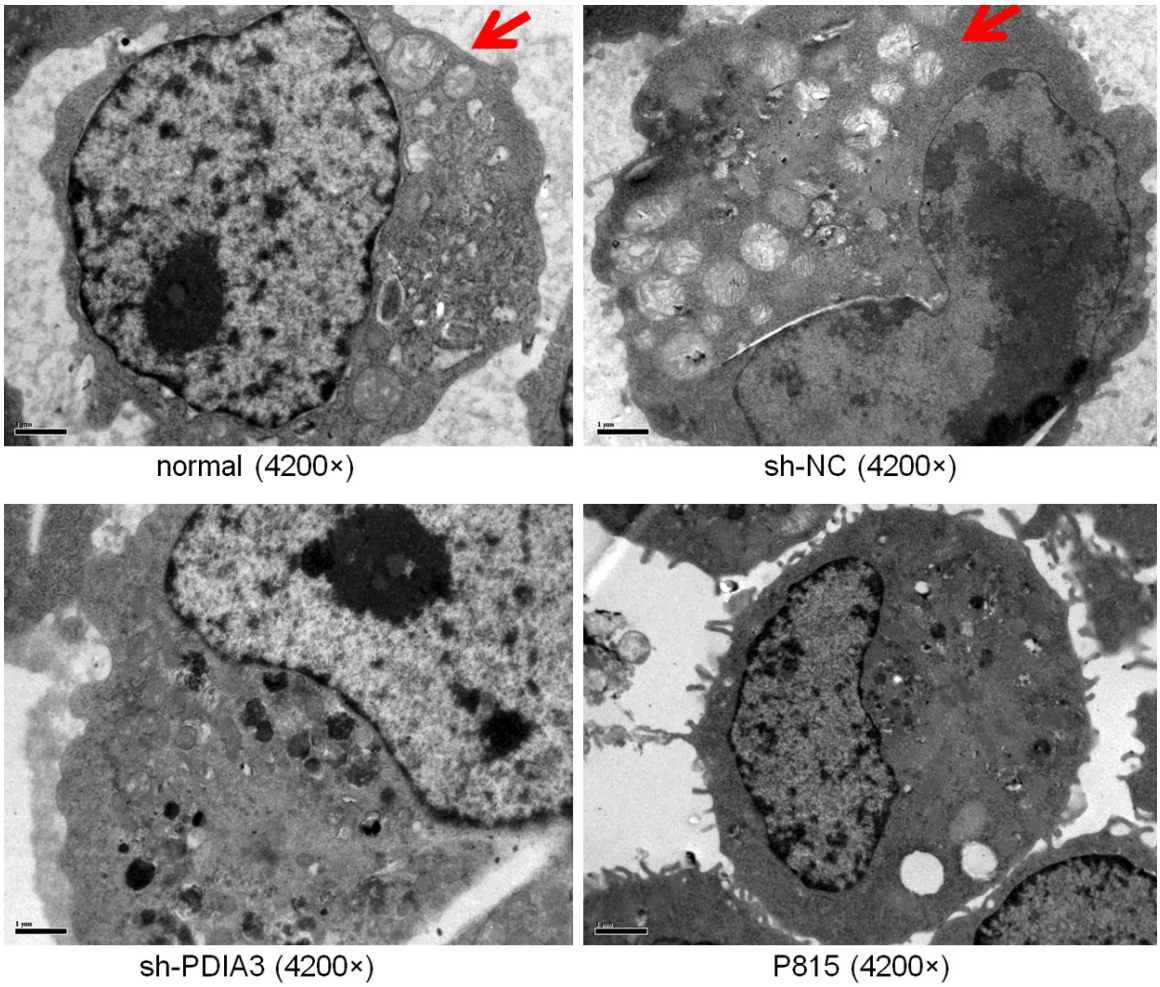

Figure 5. MC ultrastructure following stimulation with co-culture lysates induced by dendritic cell line cells with different PDIA3 status. Depicted in this figure are the results from cells that were not transduced (labeled as normal), cells transduced with a scrambled sh-RNA lentivirus (labeled as sh-NC), and a cell transduced with sh-PDIA3 (labeled as sh-PDIA3). Shown are transmission electron microscope pictures having a final magnification of 4200 times. The ultrastructural network changes are indicated by an arrow.

mune status of dendritic cell lines. Also, in co-culturing or in subsequent MC activation experiments with co-culture supernatants, transduction is not a factor and effects strictly depend on knockdown of PDIA3. Thus, the current approach in arriving at the above-mentioned conclusion appears valid. Indeed, the notion that aberrant MC activation is instrumental in IBS has quickly gathered momentum [2, 23, 24].

In this context, it is important to note that MC can be activated by multiple routes. The most classic pathway is initiated by the interaction of IgE and its receptor FcεRI [25]. Indeed, MC activation by antigen-specific IgE relates to upregulation of IL-4 [26]. A previous study observed that IL-4 provoked marked intestinal mastocytosis in a murine mode of food allergy. IL-4 stimulated proliferation and enhanced survival in cultured bone marrow-derived mast cells (BMMC) *in vitro*. Thus, IL-4-signaling may play an important role in MC expansion [27]. IL-9 is a cytokine with pleiotropic functions that can mediate allergic inflammation and immunity. Recent studies have indicated a central role for IL-9 as an early mast cell activating effector cytokine during intestinal helminth infections [28]. The current study adds to this momentum.

In a rat model of visceral hypersensitivity, the number of colonic MCs and expression of IL-4 and IL-9 in colonic mucosa were all higher than those observed in the control group. This abnor-

mal immune response of intestinal DC stimulates the secretion of IL-4 [7]. The present research shows that DC-lymphocyte co-culture supernatant is characterized by a predominance of IL-4 secretion. This predominance critically depends on the presence of PDIA3 in stimulating DCs. This research also shows that substantial secretion of IL-4 evokes more MC activation and results in higher levels of secreted trypsin. Hence, the view that DCs may stimulate the degranulation of MC and generate visceral hypersensitivity in IBS is supported by current observations. This study did not observe any significant differences in IL-9 production, but co-cultivation periods employed in this study may have precluded adequate detection.

Activated MCs secrete a variety of highly biologically active substances, including histamine, serotonin, and tryptase, which can activate sensory neurons within the gastrointestinal tract, resulting in IBS symptoms [2]. Elevated trypsin levels may conceivably have provoked more PAR2 activation, finally resulting in increased paracellular permeability or sensitization of neuronal endings in the periphery, evoking visceral hypersensitivity [29]. Thus, it was hypothesized that, in IBS, DCs may promote T-cell activation to stimulate IL-4 secretion, resulting in MC activation and visceral hypersensitivity. Knockdown of PDIA3 can block this process and reduce DC-induced T cell stimulation and MC activation, eventually reducing visceral sensitivity. *In toto*, this indicates that increased expression of PDIA3 changes the cytokine milieu to a phenotype of favoring MC activation. PDIA3 expression in dendritic cell lines influences the production of interleukin-4 in co-culture, albeit at low levels, arguing for the involvement of other factors being involved as well. Disregarding the exact nature of these factors, current results support a potential role for targeting PDIA3 in rational approaches dealing with IBS. The current study does not establish the exact mechanisms through which PDIA3 expression and IBS provoke changes in cytokine profiles. Further investigations are necessary to correlate these findings with the effects observed in the present study. Disregarding this qualifier, however, this study identified possible effects of PDAI3 expression on the MC compartment through modulation of the DC compartment. This observation provides new insight into the function of PDIA3 in the interaction between DCs and MCs and their relationship to experimental IBS. Further research is needed to assess the usefulness of these observations.

Conclusion

The current study showed that lower expression of PDIA3 in dendritic cell lines *in vitro* induced an alternative immune response, characterized by reduced secretion of IL-4 and a reduced capacity for subsequent MC activation. Thus, intestinal mucosal immune abnormalities associated with IBS may well involve alternative activation of PDIA3-mediated DC-MC relay pathways.

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Disclosure of conflict of interest

None.

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