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Dp2-Induce Autoantigen/Autoantibody Production from Patients with Systemic Lupus Erythematosus and Its Modulation by Cppecp

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Abstract

Epidemiology studies have shown that the prevalence of allergies in patients with Systemic Lupus Erythematosus (SLE) was higher than in those of normal, healthy subjects. Although many patients with SLE also have allergies, the immunological events triggering the onset and progression of the clinical manifestations of SLE by allergens have yet to be clarified. Our previous report showed that three autoantigens, Phosphoglycerate Kinase 1 (PGK-1), Triosephosphate Isomerase (TIM) and enolase were identified through the use of autologous serum in B cell lysate. This was derived from House Dust Mites (HDM) allergic SLE patients after Dp2 stimulation, where the concentration of anti-PGK-1 was significantly up-regulated after Dp2 stimulation, when compared to HDM allergic without SLE patients and healthy subjects. In this study, we further investigated Dp2 stimulated autoantigens (PGK-1 and TRIM-21) and autoantibodies (anti-PGK-1 and TRIM-21) production and their relationship with inflammasomal activation IL-1β production. Both Peripheral Blood Mononuclear Cell (PBMC) and B cell lines derived from patients with Dermatopgagoides pteronyssinus (Dp)-allergic SLE was included in this study. CPPecp and siRNA knockdown were added to evaluate their effect on cell activation. Our results showed that Dp2 could up-regulate PGK-1,TRIM -21,anti-PGK-1 and anti-TRIM-21 in B cell lines, and also up-regulate enolase and PGK-1 in PBMC. The expression of IL-1β and NLRP3 was also increased through Dp2 stimulation. Although both autoantigens and autoantibodies were upregulated, the increment of autoantigen and autoantibodies were abolished after being co-cultured with CPPecp. In the siRNA NLRP3 study only the increment of TRIM-21 and anti-PGK-1 was abolished. In conclusion, our study demonstrated that allergen exposure in the patients with HDM-sensitive SLE may play a role in the incremental expression of autoantigens and autoantibodies. In patients with Dp allergic SLE, Dp2 could upregulate the production of auto-antigen and auto-antibodies, while contributing to the pro-inflammatory cytokine secretion and disease activation. Dp2-induced auto-antigen and auto-antibody production could be abolished by being co-cultured with Dp2 and CPPecp, indicating that CPPecp has the potential to be a new immune-modulatory agent for the treatment of autoimmune diseases in the future.

Introduction

Systemic Lupus Erythematosus (SLE) is a multifactorial systemic autoimmune disorder which affects multiple organs including the skin, lungs, kidneys, and heart [1]. In some SLE patients, autoantibodies may target specific proteins in the cytosol or nucleus. These proteins are called autoantigens. Since these self-antigens are normally covered from the extracellular space by both the nuclear membrane and the cell membrane, the major issue is how these self-antigens are exposed or upregulated, and then detected by the immune system.

In recent years, bystander activation of the immune system in SLE patients has been related to environment factors. Bystander activation means there is an indirect or non-specific activation of autoimmune cells which is caused by the inflammatory environment present during infection [2]. This can lead to an enhanced processing and presentation of self antigens, which induces the expansion or spreading of the immune response towards different self-antigens [3, 4]. House Dust Mites (HDM) is the most commonly found aeroallergens worldwide. Dust mite allergens similar to the environmental pathogen, Lipopolysaccharide (LPS), can combine with Toll Like Receptors (TLR) to cause an inflammatory cell-mediate immune response. Its major allergen, Dermatophagoides pteronyssinus 2 (Dp2), can internalize to epithelium and augment toll-like receptor-mediated proinflammatory signaling with IL-6 and IL-8 secretion [5]. Dp2 is able to both trigger human B lymphocyte activation, and induce B cell proliferation. Dp2 can induce expressionof IL-1β, TNF-α and IL-8, and up-regulate MD2, TLR4 induction in B cells, which enhance the host's immune response [6]. Dp2 can induce bystander activation of B cells derived from patients with SLE [7]. Whether SLE patients with hypersensitivity to Dp2 were more susceptible to inflammasome activation and thus prone to autoantibody production remains unclarified.

In recent years, the correlation of inflammasome activation of NLRP3 and disease activity of SLE has been reported. Kahlenberg, et al demonstrated an upregulation of NLRP3 and caspase-1 in lupus nephritis biopsies [8]. Furthermore, immune complexes such as complement and autoantigen/autoantibody complex have been demonstrated to induce inflammasome activation through the stimulation of TLR dependent activation of NFκB, which then causes the NLRP3 inflammasome activation [9, 10]. Neutrophil extracellular traps have been recently demonstrated, and could activate caspase-1, causing IL-1β and IL-18 over expression in macrophages derived from SLE patients [11]. The importance of Dp2 in inflammasomal activation has also been demonstrated by our group [12]. Dp2 can induce expression of NLRP3, ASC, Caspase-1, IL-1β and IL-6 activity in THP-1 cells. The pro-inflammatory cytokines IL-1β and IL-6 were also upregulated in the Peripheral Blood Mononuclear Cells (PBMCs) derived from HDM allergic patients after Dp2 stimulation. The Dp2-induced inflammasomal activation can be down regulated by cell penetration peptides derived from human eosinophil cationic proteins (CPPecp) [12].

The aim of this study was to investigate whether Dp2 could induce inflammasomal activation with autoantigen/ autoantibody production in SLE, and to also determine whether it could be modulated by CPPecp.

Materials and Methods

Selection of patients

HDM-sensitive SLE and asthma patients (mite-specific IgE positive in their serum as measured by using the Pharmacia CAP System, Uppsala, Sweden) were selected from the clinic in the Division of Allergy, Immunology and Rheumatology of Taichung Veterans General Hospital. Diagnosis of SLE was made according to the 1997 America College of Rheumatology revised classification criteria for SLE. This study was supported by the National Science Council, Republic of China (MOST 104-2314-B-075A-015-) and Taichung Veterans General Hospital (IRB TCVGH No. CE14019 and CE14027).

Cells culture:

For cell separation and culture, 16-mL blood samples were collected, and the PBMCs were separated by density centrifugation using the Ficoll-Paque Plus density gradient (Pharmacia Biotech, Freiburg, Germany) [13]. Purified B cells from the PBMCs were prepared as previously described [6]. B cell preparations were 95% positive for the CD19 marker as determined by FACS analysis. The cells were maintained in a RPMI-1640 medium containing 10% heat inactivated FBS and 1% streptomycin/penicillin in a humidified 5% CO2 atmosphere.

Peptide synthesis

CPPecp (NYRWRCKNQN, 1381Da) was synthesized at Angene Biotech Co., Ltd., Taiwan, and its purity (>90%) was assessed through analytical high-performance liquid chromatography. Peptide sequences were confirmed with matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry at Angene Biotech Co., Ltd., Taiwan.

Dp 2 preparation

Purified recombinant protein Dp2 (RP-DP2C-1) was purchased from Indoor Biotechnologies (Charlottesville, Virginia, USA).

Enzyme-linked Immunosorbent Assay (ELISA)

Commercially available ELISA kits were used to determine cytokine and autoantibody levels in the cell culture supernatants. The cell supernatants were collected after Dp2 or CPPecp treatment during the indicated times, and the levels of IL-1β,IL-8,IL-6,IL-17,anti-PGK-1 and anti-TRIM-21 were quantified by ELISA. Plates were read on a SpectraMax M2 microplate reader (Molecular Devices, CA, USA), and analyzed with SOFTmax analysis software (Molecular Devices, CA, USA). The means of triplicate ELISA values for each of the dose relationships among these protein expressions were calculated by linear regression.

Western blotting

Whole cell lysates were prepared as previously described [14]. After blocking, the blots were incubated with antibodies for anti-human NLRP3,ASC,PGK-1,Enolase-1,TRIM-21,MD-2 and β-actin, (Cell Signaling, Massachusetts, USA; Santa Cruz Biotechnology; Millipore, Massachusetts, USA) in TBS overnight at 4°C using 0.1% Tween 20, followed by three 10-minute washes in TBS with 0.1% Tween 20. The membranes were then incubated with horseradish peroxidase-conjugated, secondary antibodies (Millipore, Massachusetts, and USA) for one hour. Detection was performed with ECL (Millipore, Massachusetts, USA), and chemiluminescence was detected by LAS 3000. Band intensity was analyzed by Multi Gauge software V 3.0.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). Data is presented as mean ± Standard Error of the Mean (SEM). P-values ≤ 0.05 were considered statistically significant. The pair student's t test was used to determine the statistical differences between groups.

Results

Effect of CPPecp on Dp2-induced autoantigen and autoantibody production from PBMCs

PBMCs derived from patients with Dp-allergic SLE (n=6) were stimulated with Dp2 in conjuction with or without CPPecp, followed by the autoantigen (Figure 1A) and autoantibody (Figure 1B) measurements. The results showed that both TRIM-21 and Enolase-1 had increased after Dp2 stimulation. The increments of both TRIM-21 and Enolase-1 were diminished after Dp2 co-culture with CPPecp. When the anti-PGK-1 and anti-TRIM-21 were measured, they showed to have a trivial effect on

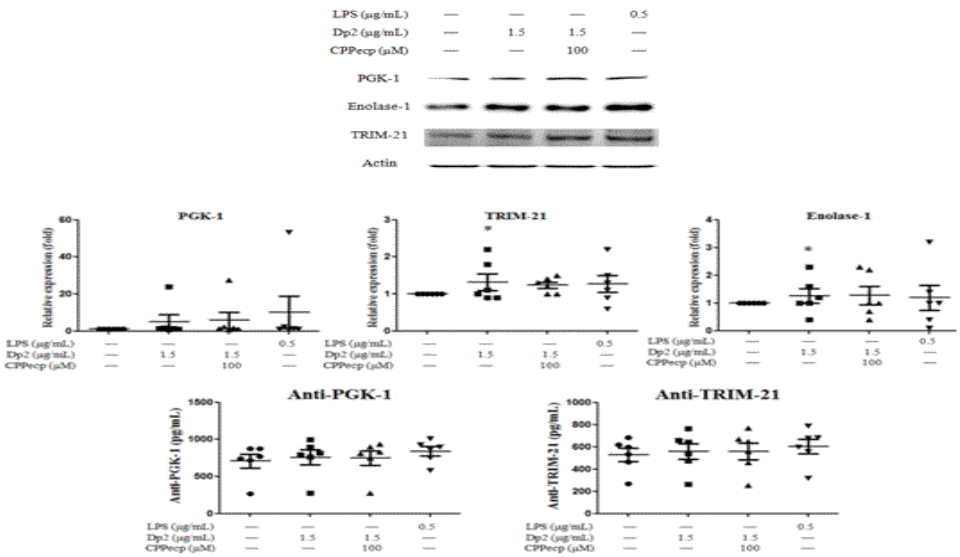

Figure 1: Effect of CPPecp on Dp2-induced autoantigen and autoantibody production from PBMCs. PBMCs derived from patients with Dp-allergic SLE (n=6) were incubated with Dp2 in conjunction with or without CPPecp for 5 days in a CO2 incubator. Cell pellets were collected for the autoantigen determination (A) and supernatant for the autoantibody measurement (B). *p<0.05 in comparison with buffer control.

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Effect of CPPecp on Dp2-induced autoantigen and autoantibody production from B cell lines

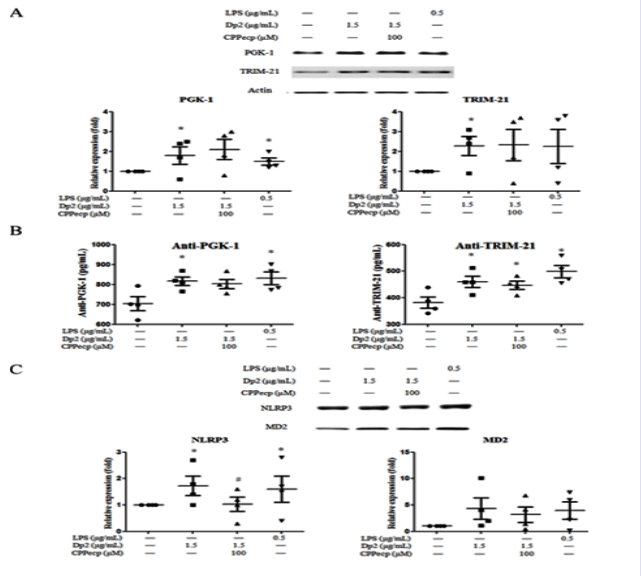

Figure 2: Effect of CPPecp on Dp2-induced autoantigen and autoantibody production from the B cell line. B cells derived from patients with Dp-allergic SLE (n=4) were incubated with Dp2 with or without CPPecp for 5 days in a CO2 incubator. Cell pellets were collected for the autoantigen determination (A) and supernatant for the autoantibody measurement (B). Cell pellets were collected for the NLRP3 and MD2 measurement (C). *p<0.05 in comparison with buffer control. #p<0.05 in comparison with Dp2.

B cell lines derived from patients with Dp-allergic SLE (n=4) were incubated using Dp2 in conjunction with or without CPPecp, followed by the autoantigen and autoantibody measurements. The results showed that both PGK-1 and TRIM-21 had significantly increased after Dp2 stimulation.

The increment of autoantigen was decreased after Dp2 co-culture with CPPecp (Figure 2A). When the anti-PGK-1 and anti-TRIM-21 were measured, there were also significant increases in both of their autoantibody production after Dp2 stimulation. However, the increment of anti-PGK-1 was decreased after Dp2 co-culture with CPPecp (Figure 2B). When both the NLRP3 and MD2 were measured, there showed only a significant increase of NLRP3 production, but not MD2. The increment of NLRP3 was also decreased after Dp2 co-cultured with CPPecp (Figure 2C).

Effect of CPPecp on Dp2-induced on IL-1β production from PBMCs and B cell lines

PBMCs (n=6, Figure 3A) and B cells (n=4, Figure 3C) derived from patients with Dp-allergic SLE and Dp-allergic rhinitis (n=9, Figure 3B) were incubated with Dp2 in conjunction with or without CPPecp for 5 days. The results showed that IL-1β increased after Dp2 stimulation in both groups of Dp-allergic patients in PBMCs and B cells (Figure. 3). The increment of IL-1β decreased after Dp2 was co-cultured with CPPecp (Figure. 3).

Effect of siRNA NLRP3 on the Dp2-induced autoantigen production from B cell lines

B cells derived from patients with Dp-allergic SLE (n=4), were pre-treated with siRNA NLRP3 followed by the stimulation of Dp2. The cell pellets were collected for autoantigen measurement (Figure. 4).

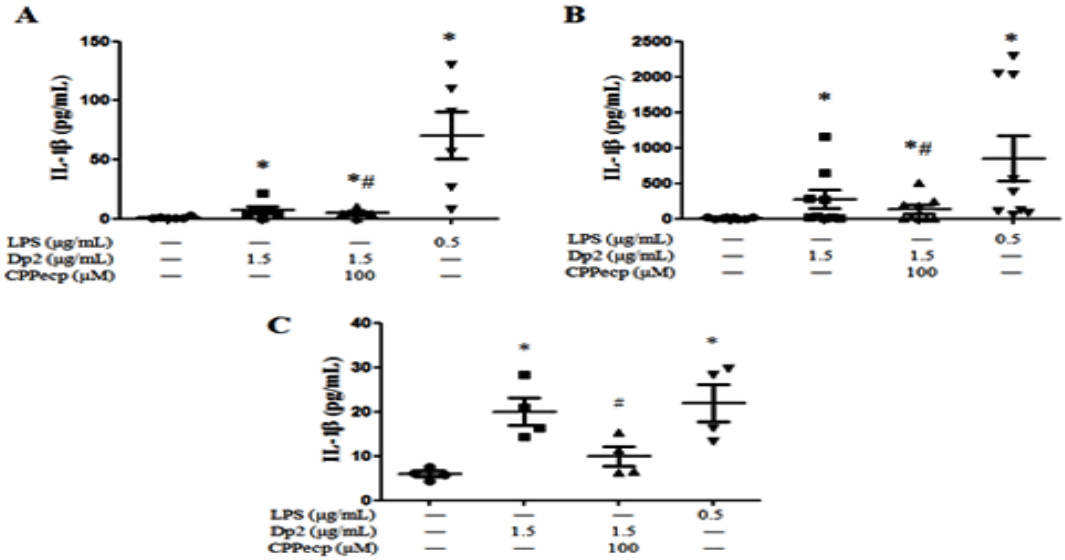

Figure 3: Effect of CPPecp on Dp2-induced on IL-1β production from PBMCs and B cells. PBMCs (n=6, A) and B cells (n=4, C) derived patients with Dp-allergic SLE and PBMCs from Dp-allergic rhinitis (n=9, B) were incubated with Dp2 in conjunction with or without CPPecp for 5 days. Cell supernatant was collected for the measurement of IL-1β. *p<0.05 in comparison with buffer control. #p< 0.05 in comparison with Dp2.

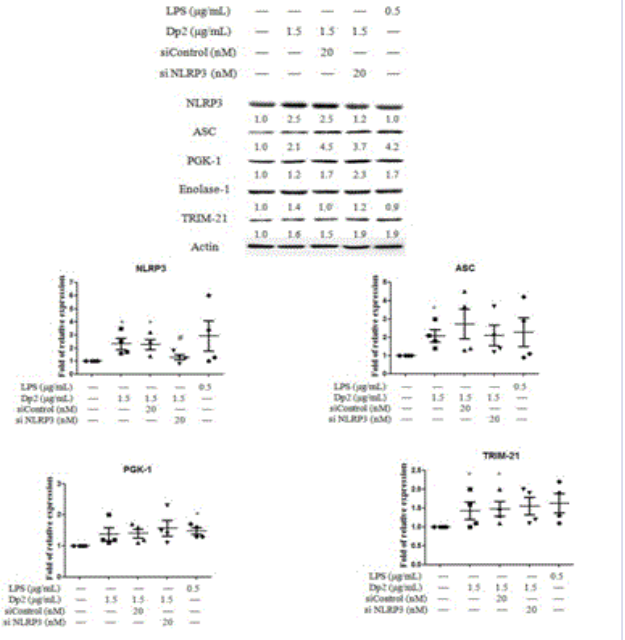

Figure 4: Effect of siRNA NLRP3 on the Dp2-induced autoantigen production from B cells. B cells (n=4) derived from patients with Dp-allergic SLE were pre-treated with or without siRNA NLRP3 for 3 days, followed by the stimulation of Dp2 for 5 days in the CO2 incubator. Cell pellets were collected for the measurement of autoantigen. *p<0.05 in comparison with buffer control. #p<0.05 in comparison with Dp2.

The results showed that both TRIM-21 and PGK-1 had increased after Dp2 stimulation. The increment of TRIM-21 had decreased after pre-treatment with siRNA NLRP3. In the siRNA knockdown study, the production of NLRP3 was specifically decreased after being pre-treated with siRNA NLRP3. There was no effect on the production of ASC after being pre-treated with siRNA NLRP3.

Effect of siRNA NLRP3 on the Dp2-induced autoantibody production from B cell lines

B cells derived from patients with Dp-allergic SLE were pre-treated with siRNA NLRP3 for 3 days, followed by the stimulation of Dp2 for 5 days in the CO2 incubator. Cell cultured supernatant was collected for the measurement of both anti-PGK-1 and anti-TRIM-21. The results showed there were significant increases in anti-PGK-1 and anti-TRIM-21 production through Dp2 stimulation. The Dp2 upregulated increment of anti-PGK-1 (Figure 5A) production had decreased, but not for anti-TRIM-21 (Figure 5B) after pre-treatment with siRNA NLRP3.

Discussion

Although many SLE patients were suffering from HDM allergies, the immunological events triggering the onset and progression of the clinical manifestations of SLE by allergens remains unclarified. In this study, we confirmed that Dp2, a major allergen of HDM, could induce proinflammatory cytokine IL-1βproduction with NLRP3 activation, and also demonstrated the enhanced production of autoantigens and autoantibodies in the same culture cells derived from patients with Dp-allergic SLE. In the autoantibody study, Dp2 could up-regulate the expression of anti-PGK-1 and anti-TRIM-21 production in B cell lines but not PBMC, indicating that there were only a few antibodies producing

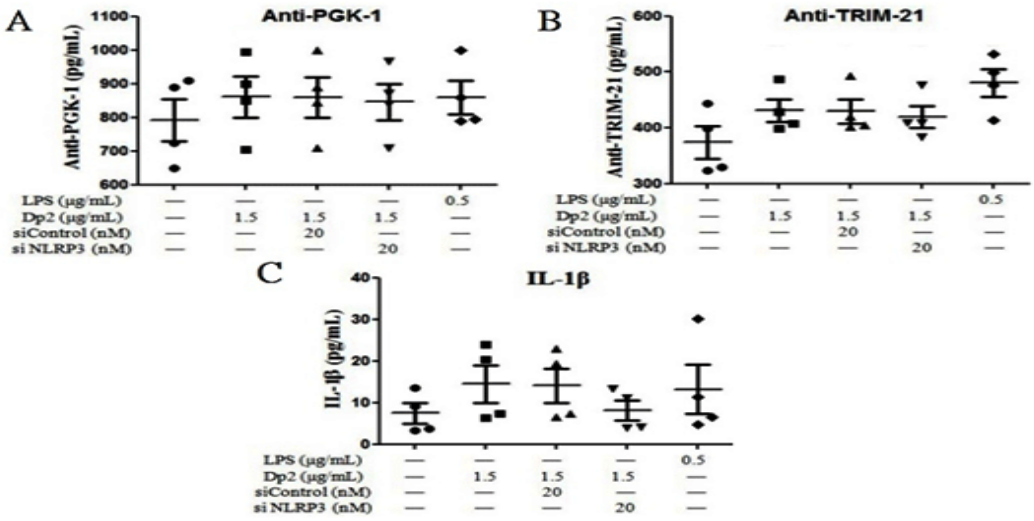

Figure 5: Effect of siRNA NLRP3 on the Dp2-induced autoantibody and IL-1β production from B cells. B cells (n=4) derived from Dp-allergic SLE were incubated with or without siRNA NLRP3 for 3 days, followed by the stimulation of Dp2 for 5 days in the CO2 incubator. Cell cultured supernatant was collected for the measurement of autoantibodies (A,B) and IL-1β (C).

B cells in the PBMC. When B cell lines were cultured with Dp2 in conjunction with CPPecp, the increment of anti-PGK-1 was abolished from being associated with the decreased expression of NLRP3 and IL-1β. These results indicated that CPPecp abolished anti-PGK-1 production at least in part from downregulation of NLRP3.

In the autoantigen study, PBMCs could up-regulate the expression of TRIM-21/enolase through Dp2 stimulation. However when cells were cultured with Dp2 in conjunction with CPPecp, the increment of Dp2-stimulated TRIM-21/enolase could be abolished, with no effect on autoantibody production in the cultured supernatant. These results indicated that while more autoantigens (including monocyes and lymphocytes) were present in PBMCs, there were only limited antibodies producing B cells in the PBMCs. These limited Dp2-stimulated B cells in the PBMC may also not be producing significant amounts of autoantibodies in the culture supernatant. This is unlikely due to the B cells in the PBMCs being unaffected by CPPecp, since CPPecp could down-regulate HDM allergen-induced proinflammatory cytokine production, by inhibiting IL-1 β expression in the same cell culture supernatant.

In our previous study, CPPecp was shown to down-regulate Dp2-induced inflammasome activation, through the production of pro-inflammatory cytokine IL-1β and IL-6 in PBMCs derived from patients with Dp allergic asthma [12]. Similar results obtained in this study showed that CPPecp could down regulate Dp2-stimulated IL-1β production from both PBMCs and B cells derived from Dp-allergic SLE. The Dp2 stimulated NLRP3 expression from B cells could be also down-regulated by CPPecp.

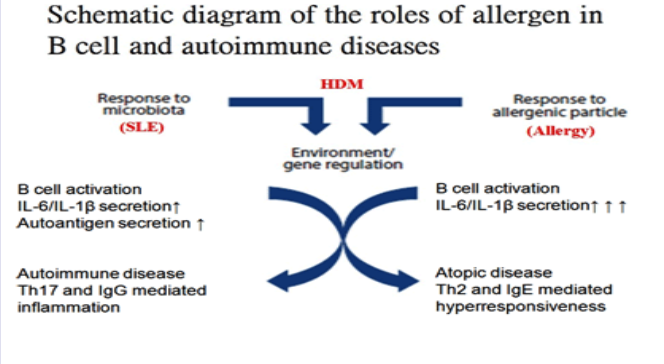

Figure 6

Since CPPecp could down-regulate inflammasome activation, it could also abolish the increment of TRIM-21 and enolase expression in PBMCs, along with the increment of TRIM-21 and PGK-1 in B cell lines after Dp2 co-cultured with CPPecp. These results indicate that CPPecp down-regulated Dp2-induced autoantigen production could be handled through the modulation of inflammation activation. When the NLRP3 in B cells was silenced with siRNA, despite NLRP3 and IL-1β expression possibly being inhibited, there was only a trivial effect on the Dp2-induced autoantigen and autoantibody production. These results suggest that the expression of autoantigen and autoantibodies by B cells are not simply modulated by NLRP3. Since it has been reported, there have been many members in the family of NOD-Like Receptors (NLR) involved in the sensing of pathogens [14]. When molecular mechanisms are involved in the sensing of Dp2 during inflammasomal activation, other cytosolic innate immunity receptors could also be involved, in addition to NLRP3, in the production of autoantigens and autoantibodies.

In summary, the environmental allergen Dp may play a role in the pathogenesis of autoimmune disease through the activation of inflammasome in B cells, and also enhance the expression of autoantibodies. Modulation of inflammasome activation caused by Dp2 may be of importance towards the prevention of the development of autoimmune disease. Both autoantigens and autoantibodies can be downregulated by CPPecp though upregulating NLRP3, although siRNA NLRP3 can only partially inhibit their expression. A detailed pathway towards inflammasome activation by Dp2 remains unclarified until all members of the NOD-Like Receptor (NLR) family have been investigated in any future studies.

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