

Epigenetic Modification of Enhancer of Zeste Homolog 2 Modulates the Activation of Dendritic Cells in Allergen Immunotherapy

Hang Li^a Yihui Wen^a Shulian Wu^a Dehua Chen^a Xi Luo^b Rui Xu^a
Renqiang Ma^a Weiping Wen^a

^aDepartment of Otorhinolaryngology Head and Neck Surgery, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China; ^bDepartment of Otolaryngology, Affiliated Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, China

Keywords

Enhancer of zeste homolog 2 · Allergic rhinitis · Allergen immunotherapy · Dendritic cells

Abstract

Background: Allergen immunotherapy (AIT) is the only etiological and potentially curative therapy for allergic rhinitis (AR). **Objectives:** We sought to investigate the role of epigenetic regulator enhancer of zeste homolog 2 (EZH2) in the activation of dendritic cells (DCs) in AIT. **Method:** In this study, EZH2 expression in circulating myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) were evaluated using flow cytometry. Clinical information from 56 AR patients receiving AIT was collected, including 30 subjects with subcutaneous immunotherapy (SCIT) and 26 subjects with sublingual immunotherapy (SLIT). In vitro, the effect of EZH2 inhibitor, 3 Deazaneplanocin A (DZNep), on the phenotypic and functional activation of monocyte-derived DCs (moDCs) was evaluated. **Results:** EZH2 expression in circulating mDCs and pDCs were both negatively correlated to treatment time of AIT ($r = -0.39, p = 0.003$ and $r = -0.47, p = 0.0002$, respectively). Furthermore, there was a higher correlation between EZH2 expression and AIT treatment time in the SCIT group compared to that of the SLIT group in mDCs ($r = -0.42,$

$p = 0.02$ vs. $r = -0.23, p = 0.26$) and pDCs ($r = -0.52, p = 0.003$ vs. $r = -0.33, p = 0.10$). In vitro, the co-stimulatory molecules on moDCs, such as CD80, CD86, and CD83, were significantly inhibited by DZNep in a dose-dependent manner. The DC-driven T-cell proliferation was suppressed by DZNep (MD = 22.88, 95% CI 7.809–37.96, $p < 0.05$). **Conclusions:** Our study shows that EZH2, which is required in the activation of DCs, mediates the epigenetic modification in AIT and stresses the importance of patient adherence during AIT.

© 2019 S. Karger AG, Basel

Introduction

The increasing prevalence of allergic rhinitis (AR) causes a higher individual and social burden [1, 2]. AR is defined as the symptomatic nasal inflammation induced by allergen inhalation in sensitized individuals [3]. AR patients show the immune features of the infiltration of eosinophils, Th2 polarization, and enhanced allergen-specific IgE [4]. Up to now, allergen immunotherapy

H.L., Y.W., and S.W. contributed equally to this work as first authors.
Edited by: H.-U. Simon, Bern.

(AIT), including subcutaneous and sublingual immunotherapy (SCIT and SLIT), is the only etiological and potentially curative therapy for AR [5]. The mechanism of AIT is to induce immunological tolerance and maintain Th1/Th2 immune balance with a long-term, low dose of allergen exposure [6–9]. However, the cellular and molecular mechanisms have not been fully elucidated.

Dendritic cells (DCs), as the most potent antigen-presenting cells, play essential roles in regulating immune response and immune tolerance [10–12]. There are 2 major DC subsets in human peripheral blood, including conventional or myeloid DCs (mDCs, Lin[−]HLA-DR⁺CD11c⁺CD123[−]) and plasmacytoid DCs (pDCs, Lin[−]HLA-DR⁺CD11c[−]CD123⁺) [13–15]. After capturing allergen, immature DCs acquire phenotypic and functional characteristics of mature DCs, including upregulated costimulatory molecules and major histocompatibility complex (MHC), migrate to draining lymph nodes and present antigens to T cells [16]. Increasing evidence suggests that AR is not only the inflammation of nasal tissue but also the systemic immune imbalance [17].

Enhancer of zeste homolog 2 (EZH2), the catalytic component of polycomb repressive complex 2, is known as the histone-lysine N-methyltransferase and catalyzes the trimethylation of lysine 27 on histone H3 [18–21]. EZH2 has been revealed as an epigenetic regulator in various immune cells, including B cells, T cells, DCs, and so on [22–25]. EZH2 participates in the development and activation of lymphocytes [18]. Gunawan et al. [25] showed that EZH2, mediating the post-translational methylation of Talin1, disrupts the binding of Talin1 and F-actin and regulate the adhesion and migration of DCs. The role of EZH2 in the activation of DCs remains to be determined.

In this study, we hypothesized that EZH2 plays a role in regulating the activation of DCs and participates in the epigenetic mechanism of AIT. We show that the levels of EZH2 in circulating mDCs and pDCs were negatively correlated with AIT treatment time in AR patients. In vitro, we applied the inhibitor of EZH2, 3 Deazaneplanocin A (DZNep), and found that EZH2 deficiency impaired the phenotypic and functional activation of DCs. These findings reveal the novel role of EZH2 in the epigenetic mechanism of AIT and stress the importance of patient adherence during AIT.

Materials and Methods

Subjects

Blood specimens from 56 HDM-sensitive AR patients receiving AIT were drawn and processed to evaluate the expression of

Table 1. Clinical characteristics of tested subjects

	SCIT (n = 30)	SLIT (n = 26)	p value
Age, years	21.07±2.057	23.88±3.036	>0.05
Gender, male/female	21/9	11/15	>0.05
Treatment time, days*	368.4±72.38	468.2±55.96	>0.05

* Values are presented as means ± SEMs.

SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy.

EZH2 in circulating DCs. The clinical characteristics of the tested subjects were shown in Table 1. The diagnosis of HDM-sensitive AR was based on the AR and its Impact on Asthma guidelines, including clinical symptoms of nasal obstruction, rhinorrhea, sneezing, nasal pruritus, itchy eyes, and watery eyes and sensitization to HDM (Der p, Der f, or both), as determined by specific IgE [26]. Blood specimens from healthy volunteers were drawn and processed to explore the role EZH2 in the activation of monocyte-derived DCs (moDCs) in vitro. Every volunteer in this study signed and gave their informed consent. This study was approved by the Ethics Committee of The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China (Approval No. 2016197).

Flow Cytometry and Antibodies

To determine the expression of EZH2 in human circulating DCs, the following anti-human monoclonal antibodies were used: FITC anti-human Lineage Cocktail (CD3/14/16/19/20/56) (Biolegend), Brilliant Violet 421™ anti-human HLA-DR (Biolegend), PE anti-human CD11c (Biolegend), PE/Cy7 anti-human CD123 (Biolegend), Alexa Fluor® 647 anti-human/mouse EZH2 (BD). For the in vitro study, moDCs were harvested and stained with the following antibodies: Anti-Human CD1a PE-Cyanine7, Anti-Human CD83 FITC, Anti-Human CD80 (B7–1) PE-Cy5, Anti-Human CD86 (B7–2) PE, Anti-Human HLA-DR/MHC II-eFluor®450 (All from eBioscience). Samples were analyzed using a flow cytometer (CytoFLEX S by Beckman Coulter, CA, USA), and analyzed using the CytExpert Software (Beckman Coulter).

In vitro Induction of moDCs and EZH2 Inhibitor DZNep Treatment

CD14⁺ cells and CD4⁺ cells were isolated from PBMCs using CD14 Microbeads and CD4 Microbeads (Miltenyi Biotec, Bergisch Gladbach Germany). The cells were seeded into a 24-well plate at a density of 5 × 10⁵ cells/well. The complete medium that was made up of RPMI-1640 (Gibco), penicillin/streptomycin/L-glutamine (Gibco), and 10% fetal bovine serum (Gibco, US origin) were used to feed CD4⁺ T cells. To induce DCs from CD14⁺ cells monocytes, complete medium supplemented with granulocyte-macrophage colony-stimulating factor (GM-CSF, working concentration 1,000 U/mL, Peprotech, London, UK), and interleukin 4 (IL-4, working concentration 1,000 U/mL, Peprotech, London, UK) was used for 5 days. The cells were further stimulated by lipopolysaccharides (LPS, 100 ng/mL, Sigma, L4516, Germany) with or without EZH2 inhibitor DZNep (Selleck Chemicals, S7120, USA) for an additional 48 h. Different doses of DZNep (1, 3, 5, and 10 μM) were added to explore the role of EZH2 in DCs. Cells were

harvested on day 7 for phenotype analysis and functional detection. Culture supernatants were collected and stored at -80°C before cytokine measurement.

Autologous Mixed Leukocyte Reactions

moDCs on day 7 were collected, washed with PBS, and co-cultured with autologous CD4^{+} T cells and the proliferation of CD4^{+} T cells was measured by carboxyfluorescein diacetate succinimidyl ester (CFSE; eBioscience, San Diego, CA, USA). moDCs on day 7 were counted and seeded in a 24-well plate at a density of 5×10^4 cells/well in 1 mL RPMI-1640 complete medium and co-cultured with autologous CD4^{+} T cells at a ratio of 1:10. Soluble anti-CD3 (OKT3, 1 $\mu\text{g/mL}$, eBioscience) and anti-CD28 (CD28.2, 1 $\mu\text{g/mL}$, eBioscience) monoclonal antibodies were added for activating T cells. Cells were harvested and analyzed by flow cytometry after 72 h of co-culture. CFSE data were analyzed by using the ModFit software (Verity Software House, Topsham, ME, USA).

Measurement of Cytokine Levels

To explore the effect of EZH2 inhibitor DZNep on cytokine secretion of moDCs, IL-6, and IL-10 were measured in the cell culture supernatant by Enzyme-Linked Immune Sorbent Assay. All Enzyme-Linked Immune Sorbent Assay kits were bought from RayBiotech (Norcross, GA, USA).

Statistical Analysis

Results were expressed as means $\pm$ SEM. Statistical analysis was performed with GraphPad Prism 6 software (La Jolla, CA, USA). The correlation analyses were conducted with Spearman's rank test. Considering the individual variations, the data of cell culture experiments were analyzed with RM one-way ANOVA with the Greenhouse-Geisser correction. We considered a p value of <0.05 to be statistically significant.

Results

EZH2 Expression Levels in Circulating DCs Are Negatively Correlated with AIT Treatment Time in AR Patients

In this cross-sectional study, we evaluated the expression of EZH2 in circulating DCs subsets and its correlation with AIT treatment time in AR patients ($n = 56$). Gating strategies for flow cytometry analysis are shown in Figure 1a. The results are shown as the relative median fluorescence intensity (normalized to isotype control staining). The proportions of mDCs and pDCs subsets in PBMCs were not significantly correlated with AIT treatment time ($r = 0.18$, $p = 0.19$ and $r = 0.21$, $p = 0.12$, respectively; Fig. 1b, c). The expression of EZH2 in circulating mDCs was negatively correlated with AIT treatment time ($r = -0.39$, $p = 0.003$; Fig. 1d). A similar correlation was found between EZH2 expression in circulating pDCs and AIT treatment time ($r = -0.47$, $p = 0.0002$; Fig. 1e). There was no significant correlation between EZH2 expression

in circulating DC subsets and clinical symptom (online suppl. Fig. S1A, S1B; for all online suppl. material, see www.karger.com/doi/10.1159/000500882). These results infer that AIT can epigenetically modulate circulating DCs in AR patients.

The Correlation between EZH2 Expression in DCs Subsets and AIT Treatment Time in the SCIT and SLIT Group

The efficacy comparison SCIT and SLIT has been widely discussed. Overall, SCIT is more effective than SLIT in controlling the clinical symptoms [27, 28]. In this study, EZH2 expression in mDCs and AIT treatment time showed higher correlation in the SCIT group than that in the SLIT group ($r = -0.42$, $p = 0.02$ vs. $r = -0.23$, $p = 0.26$; Fig. 2a). Similar results were found in the correlation between EZH2 expression in pDCs and AIT treatment time ($r = -0.52$, $p = 0.003$ vs. $r = -0.33$, $p = 0.10$; Fig. 2b). These results infer that the epigenetic modulation efficacy of AIT treatment may differ from SCIT to SLIT.

EZH2 Inhibition Causes a Dose-Dependent Downregulation of Co-Stimulatory Molecules on moDCs

In vitro, immature moDCs on day 5 were stimulated with LPS for 48 h with or without indicated concentration of EZH2 inhibitor DZNep (including 1, 3, 5, and 10 μM). Cells were harvested on day 7, and the expression of co-stimulatory molecules was assessed by flow cytometry. The expression of co-stimulatory molecules on moDCs, such as CD80, CD86, and CD83, were significantly suppressed after DZNep treatment in a dose-dependent manner (Fig. 3a, b). MHC II was significantly inhibited after high dose (10 μM) of DZNep treatment (Fig. 3a, b). The detailed results for each marker at each dose are shown in online supplementary Table S1. We also measured the levels of IL-6 and IL-10 in the supernatants of moDCs, but there were no significant changes after DZNep treatment (for IL-6 between LPS treatment vs. LPS+ DZNep treatment, MD = 3.791, 95% CI -6.144 to 13.73, $p > 0.05$, for IL-10, MD = -28.22 , 95% CI -86.63 to 30.20, $p > 0.05$; Fig. 3c, d). These results indicate that EZH2 inhibitor DZNep suppresses the activation of DCs through downregulating the expression of co-stimulatory molecules on DCs.

EZH2 Inhibitor DZNep Restrains DCs-Induced T-Cell Proliferation

To evaluate the effect of DZNep on the proinflammatory function of DCs, we co-cultured LPS-treated moDCs,

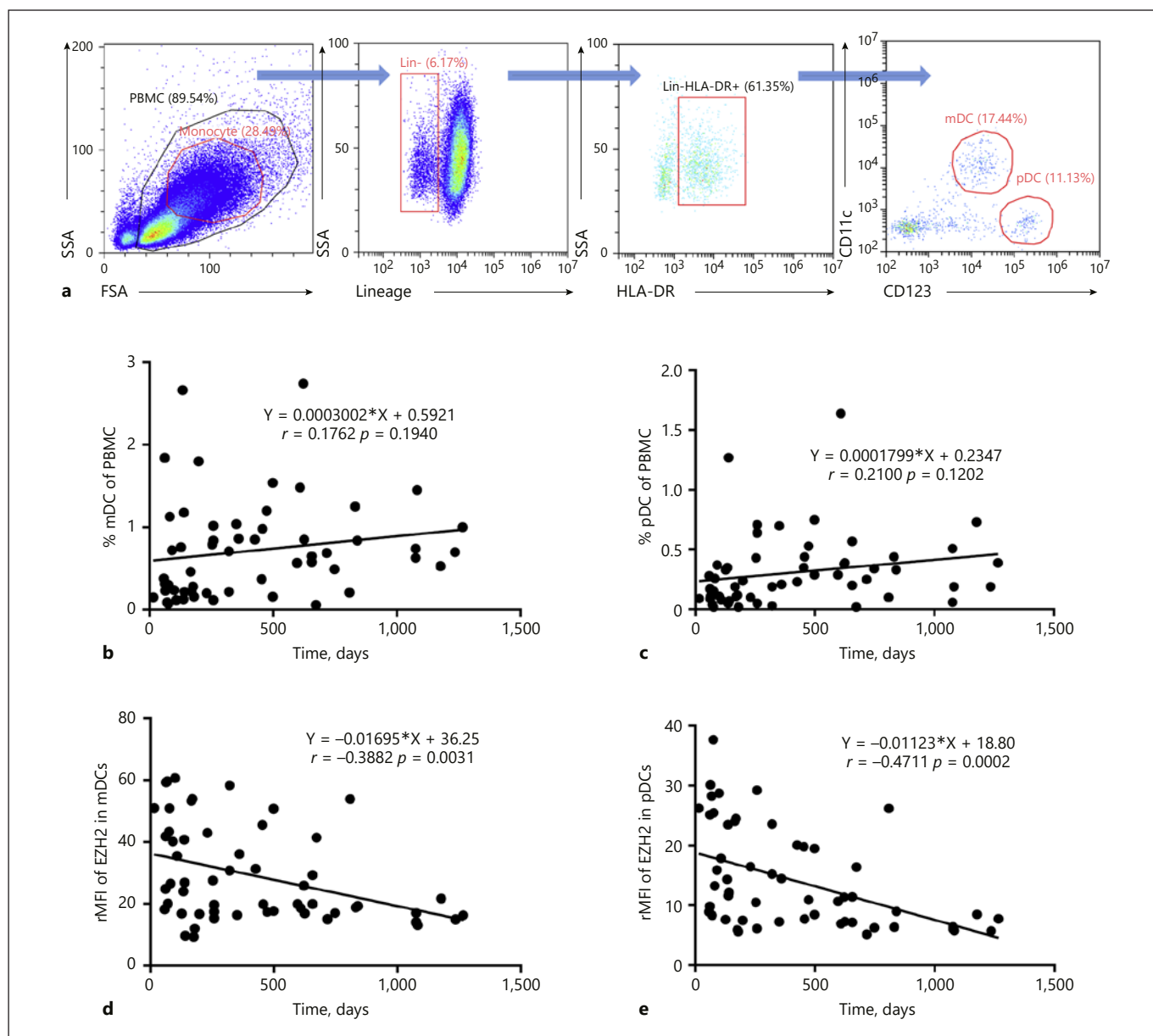


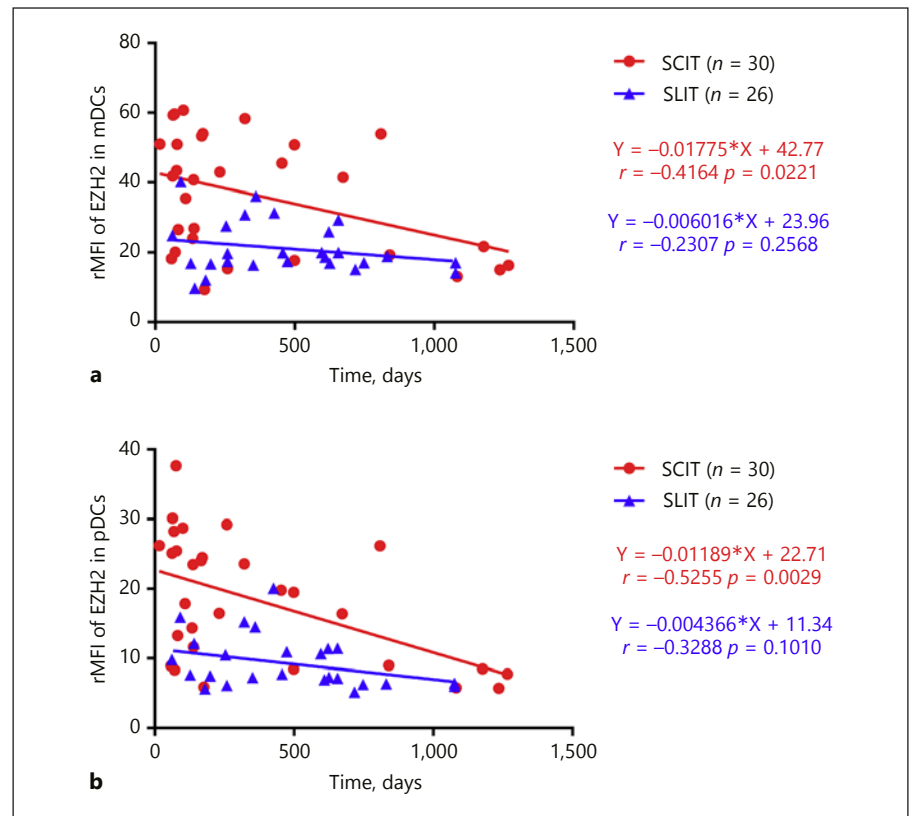
Fig. 1. The levels of EZH2 in circulating DCs are negatively correlated with treatment time of the AIT in AR patients. **a** Representative flow cytometric gating strategy to define Lin-HLA-DR + CD11c + CD123[−] mDCs and Lin-HLA-DR + CD11c[−] CD123⁺ pDCs. **b, c** The correlation analyses between the proportion of circulating DCs subsets, including mDCs (**b**) and pDCs (**c**), and AIT treatment time are shown. **d, e** The correlation analyses between

EZH2 expression in circulating DCs subsets, including mDCs (**d**) and pDCs (**e**), and AIT treatment time are shown. The rMFI shows the expression of EZH2 in DCs subsets normalized to isotype control staining $n = 56$. mDCs, myeloid dendritic cells; pDCs, plasmacytoid DCs; rMFI, relative median fluorescence intensity; EZH2, enhancer of zeste homolog 2.

LPS+ DZNep-treated moDCs, or medium control moDCs with CFSE-labeled CD4⁺ T cells at a ratio of 1:10 (moDCs: CD4⁺ T cells). After 72 h of co-culture, cells were collected for analysis. As shown in Figure 4, the proliferation of CD4⁺ T cells is stimulated by LPS (MD = −12.46, 95% CI

−20.97 to −3.943, $p < 0.05$). And the increased proliferation by LPS could be rescued by EZH2 inhibitor DZNep (MD = 22.88, 95% CI 7.809–37.96, $p < 0.05$). The results above show that EZH2 inhibitor DZNep suppresses the capacity of DCs to induce T-cell proliferation.

Fig. 2. EZH2 expression in circulating DC subsets and AIT treatment time show a higher correlation in SCIT group than SLIT group. The correlation analyses between EZH2 expression in circulating DC subsets, including mDCs (a) and pDCs (b), and AIT treatment time in SCIT and SLIT subgroup are shown. Red line and dots show the SCIT group ($n = 30$) and blue line and triangles show the SLIT group ($n = 26$). rMFI, relative median fluorescence intensity; EZH2, enhancer of zeste homolog 2; mDCs, myeloid dendritic cells; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; pDCs, plasmacytoid DCs.



Discussion

This study aimed to investigate the role of EZH2 in the activation of DCs and its epigenetic mechanism in AIT. AIT is a long-term treatment with poor patient compliance. Lemberg et al. [29] show that the dropout rate of AIT was up to 39.0% in SLIT patients and most patients who drop out quit during the first year of therapy. The evidence is needed to encourage the patients to dedicate to the therapy even without significant symptom improvement in a limited time. Our study provides epigenetic evidence for the necessity for medication adherence. We found that even in a cross-sectional study, EZH2 expression in the circulating DC subsets was highly correlated with the AIT treatment time, which suggests that the role of EZH2 in the epigenetic mechanism of AIT is worth further research.

DCs are specialized antigen-presenting cells, which play a vital role during the process of allergic inflammation as well as the course of AIT [30–32]. mDCs from HDM-allergic children who had received 1 year of SLIT showed decreased capacity to be activated by LPS [30]. Consistently, C1Q, known as a regulatory DC marker, is increased after AIT and correlates with the clinical effi-

cacy of SLIT [31]. Nevertheless, the previous studies investigated the changes of DCs during AIT based on CD14⁺ monocytes and mDCs, the effect of AIT on circulating mDCs and pDCs has not been clarified yet. In this study, we found that the expression of EZH2 in circulating mDCs and pDCs were both negatively correlated with the treatment time of AIT. The proportion of mDCs and pDCs in the PBMCs was not significantly correlated with treatment time in this cross-sectional study. However, the weak tendencies in Figure 1b, c infer that prospective studies with a larger cohort are needed to conclude.

EZH2 was first found overexpressed in various cancers, but its roles in the differentiation of immune cells and the development of inflammatory diseases have become an area of active interest [25, 33–35]. EZH2 was proved to be involved in the T helper (Th) cells differentiation [36–38]. However, the function of EZH2 in the differentiation of Th1 and Th2 cells and its role in inflammatory diseases remain controversial. Tumes et al. [36] showed that EZH2 deficiency in CD4⁺ T cells caused the accumulation of Th2 cells and resulted in the exacerbation of allergic asthma in a mice study. Consistent with this study, Hou et al. [38] reported that the

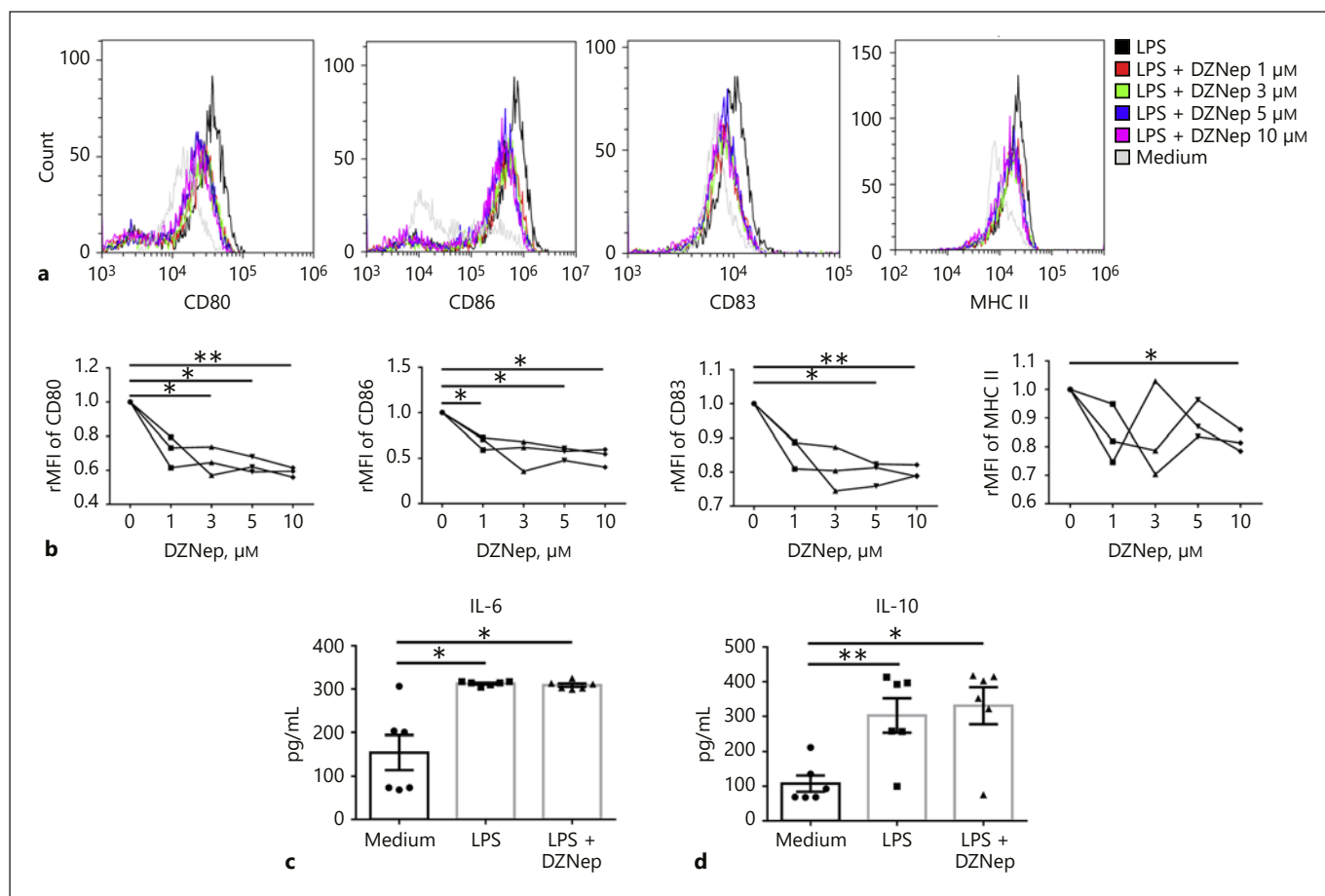


Fig. 3. EZH2-inhibitor DZNep is shown to cause a dose-dependent suppression of co-stimulatory molecules on moDCs, but no changes on the secretion of IL-6 or IL-10. The moDCs from healthy donors on day 5 were treated with indicated concentration of DZNep (including 0, 1, 3, 5, and 10 μ M) for 48 h and the expression of co-stimulatory molecules was determined on day 7 with flow cytometry. **a, b** The expression of co-stimulatory molecules on DCs, such as CD80, CD86, CD83, and MHC II, were suppressed

in a dose-dependent manner after 48 h treatment of DZNep. Representative data (**a**) and statistical analysis (**b**) are shown ($n = 3$). **c, d** IL-6 (**c**) and IL-10 (**d**) were evaluated in supernatants collected on day 7 with ELISA ($n = 6$). Values are presented as means $\pm$ SEMs. * Denotes $p < 0.05$ and ** denotes $p < 0.01$. MHC, major histocompatibility complex; LPS, lipopolysaccharides; DZNep, 3 Deazaneplanocin A; rMFI, relative median fluorescence intensity; IL, interleukin.

levels of EZH2 in both Th1 and Th2 subsets were lower in PBMCs in AR patients than healthy volunteers and EZH2 negatively mediated the differentiation of Th2 cells. These studies suggested the suppressive effect on Th2 response of EZH2. In contrast, Dobenecker et al. [39] reported that inhibition of EZH2 precluded the activation of T cells and attenuated the T-cell-driven responses. EZH2 deficiency impaired the migration of DCs and restricted the progression of multiple sclerosis in a murine model [25]. In agreement with these findings, our in vitro study showed the phenotypic and functional activation of moDCs were significantly suppressed after EZH2 inhibition. These results indicate

that EZH2 plays an essential role in the activation of both DCs and T cells, and the development of inflammation. Further research is needed to elaborate on the molecular mechanisms of EZH2 regulation during allergic disease development.

There are several limitations in this study. First, the role of EZH2 in the nasal tissues may offer a more detailed picture, but we did not include it due to the unnecessary surgical intervention for AR patients and the importance of nasal mucosa in nasal function protection. Second, a prospective study with a larger cohort is needed to verify the findings. In this cross-sectional study, EZH2 expression in DC subsets was not correlated with

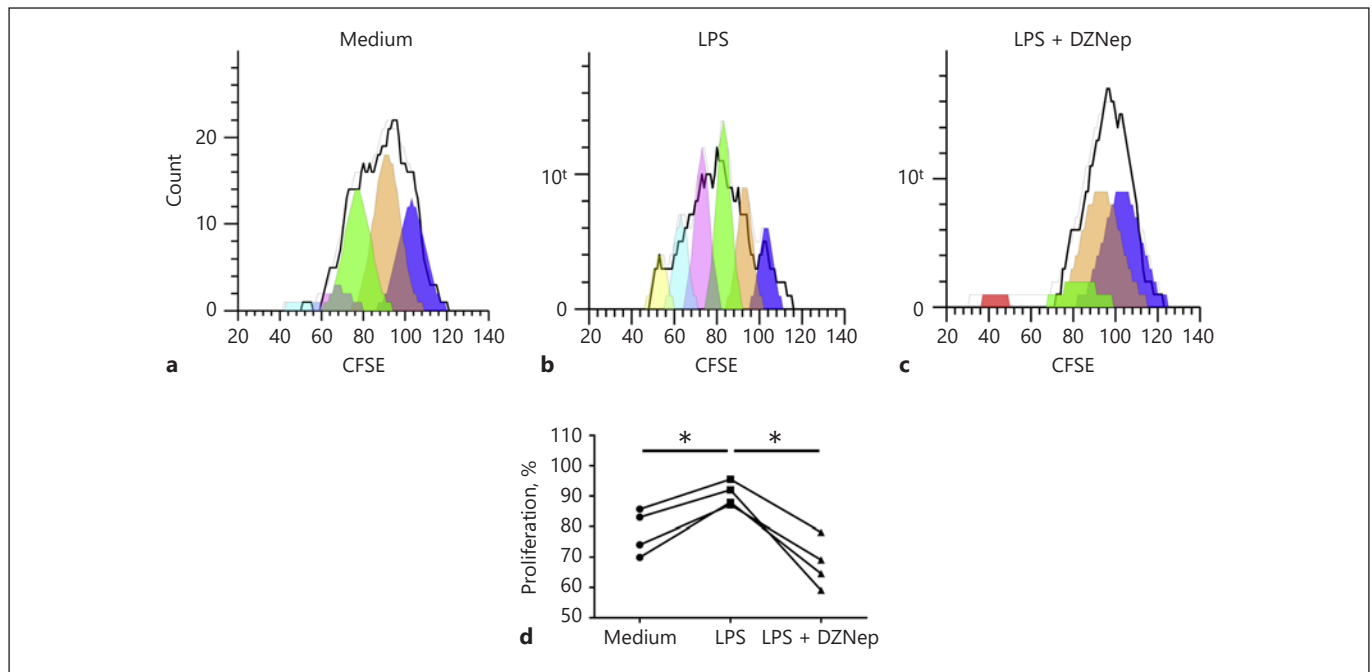


Fig. 4. EZH2 inhibitor DZNep restrains DCs-induced T-cell proliferation. Immature moDCs on day 5 were treated with LPS (100 ng/mL) with or without DZNep (3 μ M) for 48 h and then co-cultured with autologous CFSE-labeled CD4⁺ T cells (at a DC/T-cell ratio of 1:10) for another 3 days. **a–c** CFSE-labeled CD4⁺ T cells

showed the proliferation respectively stimulated by control moDCs (**a**), LPS-stimulated moDCs (**b**), and LPS + DZNep-treated moDCs (**c**). **d** Statistical analyses among the 3 groups are shown ($n = 4$). * Denotes $p < 0.05$. CFSE, carboxyfluorescein diacetate succinimide ester; LPS, lipopolysaccharides; DZNep, 3 Deazaneplanocin A.

the symptom score. However, a prospective study will be helpful to explore the role of EZH2 in clinical outcome during AIT.

In the future, there are many intriguing questions to be explored and answered. First, considering the negative correlation between EZH2 expression in DC subsets and AIT treatment time and the suppression effect of EZH2 deficiency on the maturation of DCs, EZH2 inhibitor could be exploited as a complementary therapy during AIT to improve the efficacy or even shorten the therapy time. Second, EZH2 may be a biomarker to predict and monitor the efficacy of AIT, but this hypothesis needs to be explored in prospective studies. Last but not the least, the underlying molecular mechanism of EZH2 modulating the activation of DCs is worth investigation.

In conclusion, our study indicates that EZH2 is required in the activation of DCs and participates in the epigenetic modification in AIT. The results provide evidence for the significance of patient adherence during AIT. Future prospective cohort studies will help verify and further explore how EZH2 participates in the epigenetic mechanism of AIT.

Statement of Ethics

This study was approved by the Ethics Committee of The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China (Approval No. 2016197).

Disclosure Statement

The authors have no conflicts of interest to declare.

Funding Sources

This study was supported by the National Natural Science Foundation of China (grant number 81600780, 81670902, 81870696), the Natural Science Foundation of Guangdong Province, China (grant number 2016A030310167, 2018A030313667, 2017A030310362).

Author Contributions

W.W. and R.M. designed this study. D.C. and X.L. collected the clinical specimens. H.L., Y.W., and S.W. conducted the experiment, analyzed all of the data, and prepared the figures. H.L. drafted the manuscript. R.M., R.X., and W.W. revised the manuscript.

References

- Zuberbier T, Lötval J, Simoens S, Subramanian SV, Church MK. Economic burden of inadequate management of allergic diseases in the European Union: a GA(2) LEN review. *Allergy*. 2014 Oct;69(10):1275–9.
- Kulthanan K, Chusakul S, Recto MT, Gabriel MT, Aw DC, Prepageran N, et al. Economic Burden of the Inadequate Management of Allergic Rhinitis and Urticaria in Asian Countries Based on the GA²LEN Model. *Allergy Asthma Immunol Res*. 2018 Jul;10(4):370–8.
- Greiner AN, Hellings PW, Rotiroli G, Scadding GK. Allergic rhinitis. *Lancet*. 2011 Dec;378(9809):2112–22.
- Naclerio RM. Allergic rhinitis. *N Engl J Med*. 1991 Sep;325(12):860–9.
- Muraro A, Roberts G, Halken S, Agache I, Angier E, Fernandez-Rivas M, et al. EAACI guidelines on allergen immunotherapy: executive statement. *Allergy*. 2018 Apr;73(4):739–43.
- Pelaia C, Vatrella A, Lombardo N, Terracciano R, Navalesi P, Savino R, et al. Biological mechanisms underlying the clinical effects of allergen-specific immunotherapy in asthmatic children. *Expert Opin Biol Ther*. 2018 Feb;18(2):197–204.
- Zieglmayer P, Focke-Tejkl M, Schmutz R, Lemell P, Zieglmayer R, Weber M, et al. Mechanisms, safety and efficacy of a B cell epitope-based vaccine for immunotherapy of grass pollen allergy. *EBioMedicine*. 2016 Sep;11:43–57.
- Schulten V, Tripple V, Aasbjerg K, Backer V, Lund G, Würtzen PA, et al. Distinct modulation of allergic T cell responses by subcutaneous vs. sublingual allergen-specific immunotherapy. *Clin Exp Allergy*. 2016 Mar;46(3):439–48.
- Cosmi L, Santarlasci V, Angeli R, Liotta F, Maggi L, Frosali F, et al. Sublingual immunotherapy with Dermatophagoides monomeric allergoid down-regulates allergen-specific immunoglobulin E and increases both interferon-gamma- and interleukin-10-production. *Clin Exp Allergy*. 2006 Mar;36(3):261–72.
- Steinman RM. The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol*. 1991;9(1):271–96.
- Meredith MM, Liu K, Darrasse-Jeze G, Kamphorst AO, Schreiber HA, Guernonprez P, et al. Expression of the zinc finger transcription factor zDC (Zbtb46, Btbd4) defines the classical dendritic cell lineage. *J Exp Med*. 2012 Jun;209(6):1153–65.
- Loschko J, Schreiber HA, Rieke GJ, Esterházy D, Meredith MM, Pedicord VA, et al. Absence of MHC class II on cDCs results in microbial-dependent intestinal inflammation. *J Exp Med*. 2016 Apr;213(4):517–34.
- Mohty M, Jarrossay D, Lafage-Pochitaloff M, Zandotti C, Briere F, de Lamballeri XN, et al. Circulating blood dendritic cells from myeloid leukemia patients display quantitative and cytogenetic abnormalities as well as functional impairment. *Blood*. 2001 Dec;98(13):3750–6.
- Vuckovic S, Fearnley DB, Gunningham S, Spearing RL, Patton WN, Hart DN. Dendritic cells in chronic myelomonocytic leukaemia. *Br J Haematol*. 1999 Jun;105(4):974–85.
- Donaghy H, Pozniak A, Gazzard B, Qazi N, Gilmour J, Gotch F, et al. Loss of blood CD11c(+) myeloid and CD11c(-) plasmacytoid dendritic cells in patients with HIV-1 infection correlates with HIV-1 RNA virus load. *Blood*. 2001 Oct;98(8):2574–6.
- Hartgers FC, Figdor CG, Adema GJ. Towards a molecular understanding of dendritic cell immunobiology. *Immunol Today*. 2000 Nov;21(11):542–5.
- Borish L. Allergic rhinitis: systemic inflammation and implications for management. *J Allergy Clin Immunol*. 2003 Dec;112(6):1021–31.
- Hobert O, Jallat B, Ullrich A. Interaction of Vav with ENX-1, a putative transcriptional regulator of homeobox gene expression. *Mol Cell Biol*. 1996 Jun;16(6):3066–73.
- Czermin B, Melfi R, McCabe D, Seitz V, Imhof A, Pirrotta V. Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. *Cell*. 2002 Oct;111(2):185–96.
- Müller J, Hart CM, Francis NJ, Vargas ML, Sengupta A, Wild B, et al. Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. *Cell*. 2002 Oct;111(2):197–208.
- Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, et al. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science*. 2002 Nov;298(5595):1039–43.
- Su IH, Basavaraj A, Krutchinsky AN, Hobert O, Ullrich A, Chait BT, et al. Ezh2 controls B cell development through histone H3 methylation and Igh rearrangement. *Nat Immunol*. 2003 Feb;4(2):124–31.
- Raaphorst FM, Otte AP, van Kemenade FJ, Blokzijl T, Fieret E, Hamer KM, et al. Distinct BMI-1 and EZH2 expression patterns in thymocytes and mature T cells suggest a role for Polycomb genes in human T cell differentiation. *J Immunol*. 2001 May;166(10):5925–34.
- Wu C, Chen Z, Kuchroo VK. Ezh2 lines up the chromatin in T regulatory cells. *Immunity*. 2015 Feb;42(2):201–3.
- Gunawan M, Venkatesan N, Loh JT, Wong JF, Berger H, Neo WH, et al. The methyltransferase Ezh2 controls cell adhesion and migration through direct methylation of the extranuclear regulatory protein talin. *Nat Immunol*. 2015 May;16(5):505–16.
- Brozek JL, Bousquet J, Agache I, Agarwal A, Bachert C, Bosnic-Anticevich S, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines-2016 revision. *J Allergy Clin Immunol*. 2017 Oct;140(4):950–8.
- Di Bona D, Plaia A, Leto-Barone MS, La Piana S, Di Lorenzo G. Efficacy of subcutaneous and sublingual immunotherapy with grass allergens for seasonal allergic rhinitis: a meta-analysis-based comparison. *J Allergy Clin Immunol*. 2012 Nov;130(5):1097–1107.e2.
- Bauer CS, Rank MA. Comparative efficacy and safety of subcutaneous versus sublingual immunotherapy. *J Allergy Clin Immunol*. 2014 Sep;134(3):765–765.e2.
- Lemberg ML, Berk T, Shah-Hosseini K, Kasche EM, Mösges R. Sublingual versus subcutaneous immunotherapy: patient adherence at a large German allergy center. *Patient Prefer Adherence*. 2017 Jan;11:63–70.
- Angelini F, Pacciani V, Corrente S, Silenzi R, Di Pede A, Polito A, et al. Dendritic cells modification during sublingual immunotherapy in children with allergic symptoms to house dust mites. *World J Pediatr*. 2011 Feb;7(1):24–30.
- Zimmer A, Bouley J, Le Mignon M, Pliquet E, Horiot S, Turfkruyer M, et al. A regulatory dendritic cell signature correlates with the clinical efficacy of allergen-specific sublingual immunotherapy. *J Allergy Clin Immunol*. 2012 Apr;129(4):1020–30.
- Piconi S, Trabattini D, Rainone V, Borgonovo L, Passerini S, Rizzardini G, et al. Immunological effects of sublingual immunotherapy: clinical efficacy is associated with modulation of programmed cell death ligand 1, IL-10, and IgG4. *J Immunol*. 2010 Dec;185(12):7723–30.
- Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, et al. The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature*. 2002 Oct;419(6907):624–9.
- Kleer CG, Cao Q, Varambally S, Shen R, Ota I, Tomlins SA, et al. EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc Natl Acad Sci USA*. 2003 Sep;100(20):11606–11.
- Breuer RH, Snijders PJ, Smit EF, Sutedja TG, Sewalt RG, Otte AP, et al. Increased expression of the EZH2 polycomb group gene in BMI-1-positive neoplastic cells during bronchial carcinogenesis. *Neoplasia*. 2004 Nov-Dec;6(6):736–43.
- Tumes DJ, Onodera A, Suzuki A, Shinoda K, Endo Y, Iwamura C, et al. The polycomb protein Ezh2 regulates differentiation and plasticity of CD4(+) T helper type 1 and type 2 cells. *Immunity*. 2013 Nov;39(5):819–32.
- Yang XP, Jiang K, Hirahara K, Vahedi G, Afzali B, Sciume G, et al. EZH2 is crucial for both differentiation of regulatory T cells and T effector cell expansion. *Sci Rep*. 2015 Jun;5(1):10643.
- Hou TY, Chen MR, Chou YC, Kan PC, Tsai YT, Cha TL. Impact of Enhancer of Zeste Homolog 2 on T Helper Cell-Mediated Allergic Rhinitis. *Front Immunol*. 2017 Jul;8:790.
- Dobenecker MW, Park JS, Marcello J, McCabe MT, Gregory R, Knight SD, et al. Signaling function of PRC2 is essential for TCR-driven T cell responses. *J Exp Med*. 2018 Apr;215(4):1101–13.

Fig S1

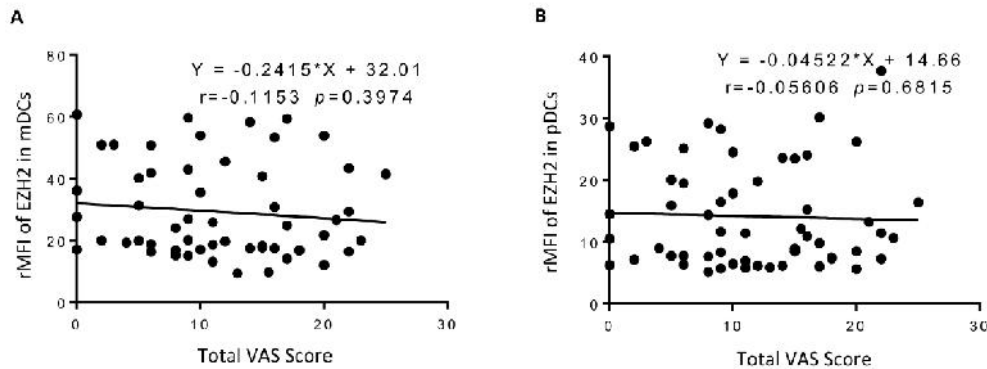


Figure S1. The correlation between EZH2 expression in circulating DC subsets and clinical symptom in allergic rhinitis patients receiving allergen immunotherapy.

The correlation analyses between EZH2 expression in circulating DC subsets, including mDCs (A) and pDCs (B), and total visual analog scale (VAS) Score are shown. The total VAS score rated 6 symptoms (including nasal obstruction, rhinorrhea, sneezing, nasal pruritus, itchy eyes, and watery eyes) from absent (0) to very severe (10). The total VAS score ranged from 0 to 60 for all combined symptoms.[1, 2] The relative median fluorescence intensity (rMFI) shows the expression of EZH2 in DCs subsets normalized to isotype control staining. n=56.

1. Brozek JL, Bousquet J, Agache I, Agarwal A, Bachert C, Bosnic-Anticevich S, Brignardello-Petersen R, Canonica GW, Casale T, Chavannes NH *et al*: **Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines-2016 revision**. *The Journal of allergy and clinical immunology* 2017, **140**(4):950-958.
2. Bousquet PJ, Combescurre C, Neukirch F, Klossek JM, Mechin H, Daures JP, Bousquet J: **Visual analog scales can assess the severity of rhinitis graded according to ARIA guidelines**. *Allergy* 2007, **62**(4):367-372.

Table S1. Test statistics of co-stimulatory molecules expression at indicated doses of EZH2 inhibitor DZNep

Co-stimulatory molecule	Comparison	Mean Difference	95% CI of difference	<i>p</i> -value
CD80	0 vs 1 μ M	0.2863	-0.05319 to 0.6259	>0.05
	0 vs 3 μ M	0.3484	0.03459 to 0.6622	<0.05
	0 vs 5 μ M	0.3694	0.1971 to 0.5417	<0.05
	0 vs 10 μ M	0.4102	0.3048 to 0.5155	<0.01
CD86	0 vs 1 μ M	0.3254	0.05201 to 0.5989	<0.05
	0 vs 3 μ M	0.4465	-0.2031 to 1.096	>0.05
	0 vs 5 μ M	0.4449	0.1824 to 0.7074	<0.05
	0 vs 10 μ M	0.4842	0.1052 to 0.8632	<0.05
CD83	0 vs 1 μ M	0.1381	-0.03227 to 0.3085	>0.05
	0 vs 3 μ M	0.1925	-0.04939 to 0.4344	>0.05
	0 vs 5 μ M	0.2014	0.07007 to 0.3328	<0.05
	0 vs 10 μ M	0.2004	0.1294 to 0.2713	<0.01
MHC II	0 vs 1 μ M	0.1620	-0.2232 to 0.5472	>0.05
	0 vs 3 μ M	0.1604	-0.4765 to 0.7974	>0.05
	0 vs 5 μ M	0.1109	-0.1386 to 0.3604	>0.05
	0 vs 10 μ M	0.1811	0.03952 to 0.3226	<0.05