

5. Kusel MM, de Klerk NH, Keadze T, et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J Allergy Clin Immunol*. 2007;119(5):1105-1110.
6. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med*. 1995;332(3):133-138.
7. Virkud YV, Kelly RS, Croteau-Chonka DC, et al. Novel eosinophilic gene expression networks associated with IgE in two distinct asthma populations. *Clin Exp Allergy*. 2018;48(12):1654-1664.
8. Trump S, Bieg M, Gu Z, et al. Prenatal maternal stress and wheeze in children: novel insights into epigenetic regulation. *Sci Rep*. 2016;6:28616.
9. Tischer C, Standl M, Lehmann I, Schaaf B, von Berg A, Heinrich J. Sleeping on animal fur is related to asthma outcomes in later childhood. *Eur Respir J*. 2015;46(1):107-114.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

DOI: 10.1111/all.13838

Differential effects of mesenchymal stem cells on T cells isolated from childhood allergies and autoimmune diseases

To the Editor,

Mesenchymal stem cells (MSCs) are multipotent stromal cell precursors and are involved in immunoregulation. They can suppress the maturation, proliferation, or effector function of a wide range of innate and adaptive immune cells, including T cells.¹ Numerous studies have reported that MSCs can inhibit Th1 and Th17 cells and ameliorate Th1- or Th17-mediated autoimmune diseases.^{2,3} However, the effects of MSCs on Th2 cells and Th2-mediated immune responses are not well understood. In addition, the therapeutic potential of MSCs in children with autoimmune diseases is rarely studied. In this study, we investigated the effect of MSCs on cultured Th2 cells and T cells isolated from children with allergic asthma and examined the therapeutic potential of MSCs in autoimmune disease by using blood samples from pediatric patients.

To study the effect of MSCs on the development of Th2 cells in vitro, CD4 T cells were cultured in Th2-polarizing conditions in the presence or absence of MSCs and assayed Th2 cell function after 5 days of culture. MSCs were isolated from chorionic decidua tissues of human placenta (denoted as pcMSCs) as described in our previous study.⁴ pcMSCs markedly suppressed the production of interleukin (IL)-4, IL-5, and IL-13 and proliferation of Th2 cells (Figure S1A-C). We further investigated the suppression of Th cells already differentiated to the Th2 phenotype by pcMSCs. We demonstrated that pcMSCs downregulated CD25, IL-4, IL-5, and IL-13 expression in Th2 cells (Figure S1D-F) and therefore suppressed the activation of Th2 cells.

Further, we examined whether MSCs exerted a similar effect in Th2-mediated asthma. We collected peripheral blood mononuclear cells (PBMCs) from children with allergic asthma. PBMCs were activated using anti-CD3 and anti-CD28 antibodies in the presence or

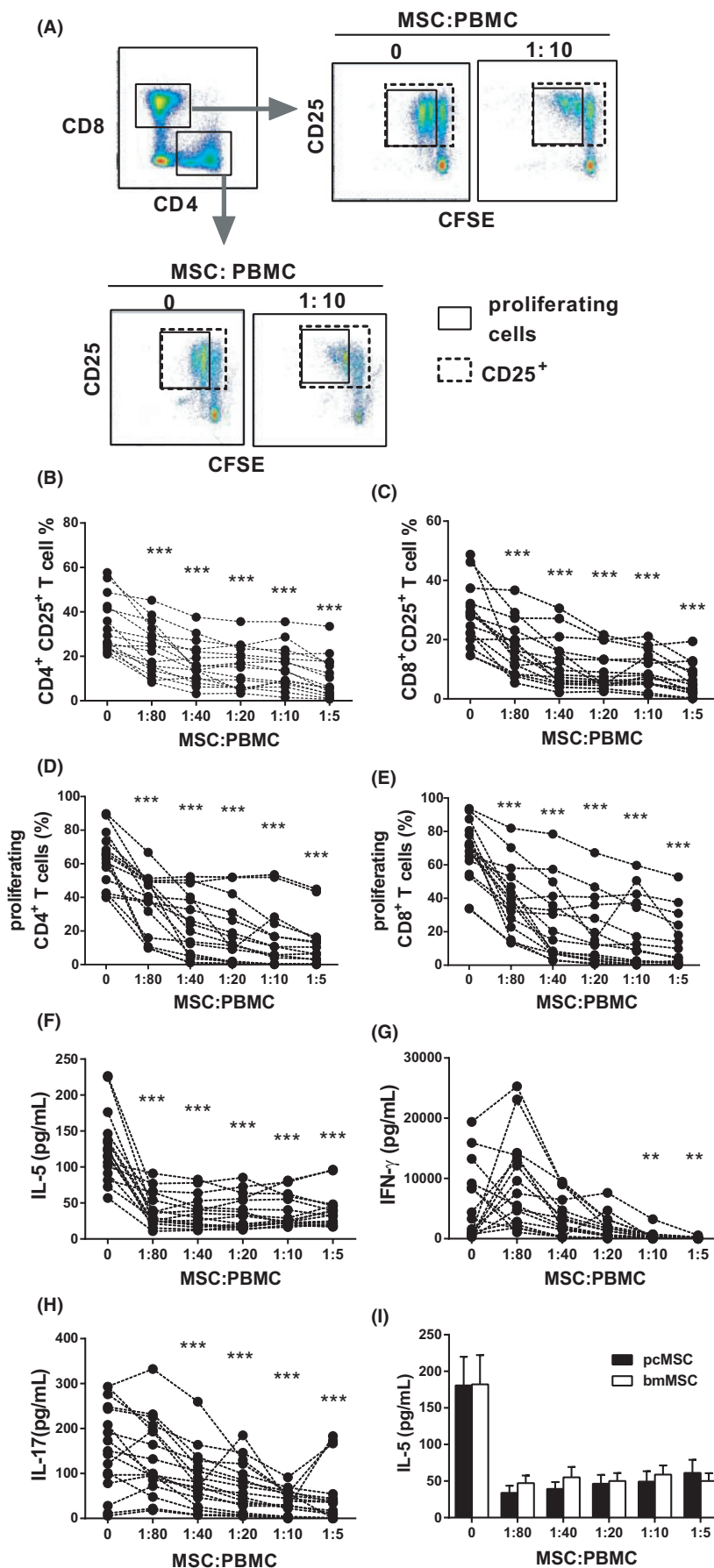
absence of pcMSCs. We determined the activation, proliferation, and cytokine production of stimulated T cells. A representative flow cytometry analysis of PBMCs is shown in Figure 1A. The suppressive effect of pcMSCs on the activation and proliferation of both CD4⁺ and CD8⁺ T cells was dose-dependent (Figure 1B-E). Remarkably, the Th2-cytokine IL-5 was dramatically reduced in the conditioned supernatant of PBMCs by pcMSC coculture and the suppression was found even at low MSC-to-PBMC ratio (1:80) (Figure 1F). Th1 and Th17 cytokines (interferon (IFN)- γ and IL-17, respectively) were also downregulated by pcMSCs (Figure 1G and 1H). In addition, bone marrow-derived MSCs (bmMSCs)⁵ also suppressed IL-5 production (Figure 1I). The direct effect of MSCs on CD4⁺ T cells was defined by using purified CD4⁺ T cells (Figure S2). Hence, MSCs suppressed the activation, proliferation, and cytokine (especially IL-5) production of T cells isolated from children with allergic asthma. Our results were consistent with the previous study,⁶ which showed that bmMSCs suppress the proliferation of PBMCs isolated from adult allergic asthmatic patients and this effect was allergen-specific. However, we also observed a small number of patients whose T cells were not reactive under anti-CD3 and anti-CD28 antibody stimulation (Figure S3, MSC: PBMC = 0). Coculturing these nonreactive T cells with pcMSCs promoted their activation, proliferation, and cytokine production (Figure S3). Hence, apart from this exception, we inferred that MSCs could suppress the activation, proliferation, and cytokine (especially IL-5) production of T cells isolated from children with allergic asthma.

We then studied the therapeutic potential of MSCs in pediatric autoimmune diseases. We isolated PBMCs from children with Henoch-Schönlein purpura (HSP), juvenile idiopathic arthritis (JIA), and systemic lupus erythematosus (SLE). We found that pcMSCs did not have a consistent influence on the activation, proliferation, and cytokine production of T cells isolated from children with autoimmune diseases (Figure 2). This was not consistent with previous

Abbreviations: CFSE, carboxyfluorescein succinimidyl ester; MSCs, mesenchymal stem cells; PBMCs, peripheral blood mononuclear cells; pcMSCs, placenta chorionic decidua tissue MSCs.

Yih-Mei Liou and Teng-Yuan Wei contributed equally to this work.

FIGURE 1 pcMSCs suppressed activation, proliferation, and cytokine production of T cells from childhood allergic asthma. PBMCs from children with allergic asthma were labeled with CFSE, activated with anti-CD3 and anti-CD28 Abs, and cocultured with pcMSCs for 72 h. (A-E) Activation (represented as CD25⁺) (B, C) and proliferation (represented as dilution of CFSE) (D, E) of CD4⁺ and CD8⁺ T cells were assessed by flow cytometry. A representative analysis of flow cytometry. (A). Supernatant (F) IL-5, (G) IFN- γ , and (H) IL-17 were detected by ELISA. Each symbol represents an individual patient (n = 16). (I) PBMCs from five patients were activated and cocultured with pcMSCs or bmMSCs for 72 h. Supernatant IL-5 was detected. ** $P < 0.01$ and *** $P < 0.001$ compared to 0 group



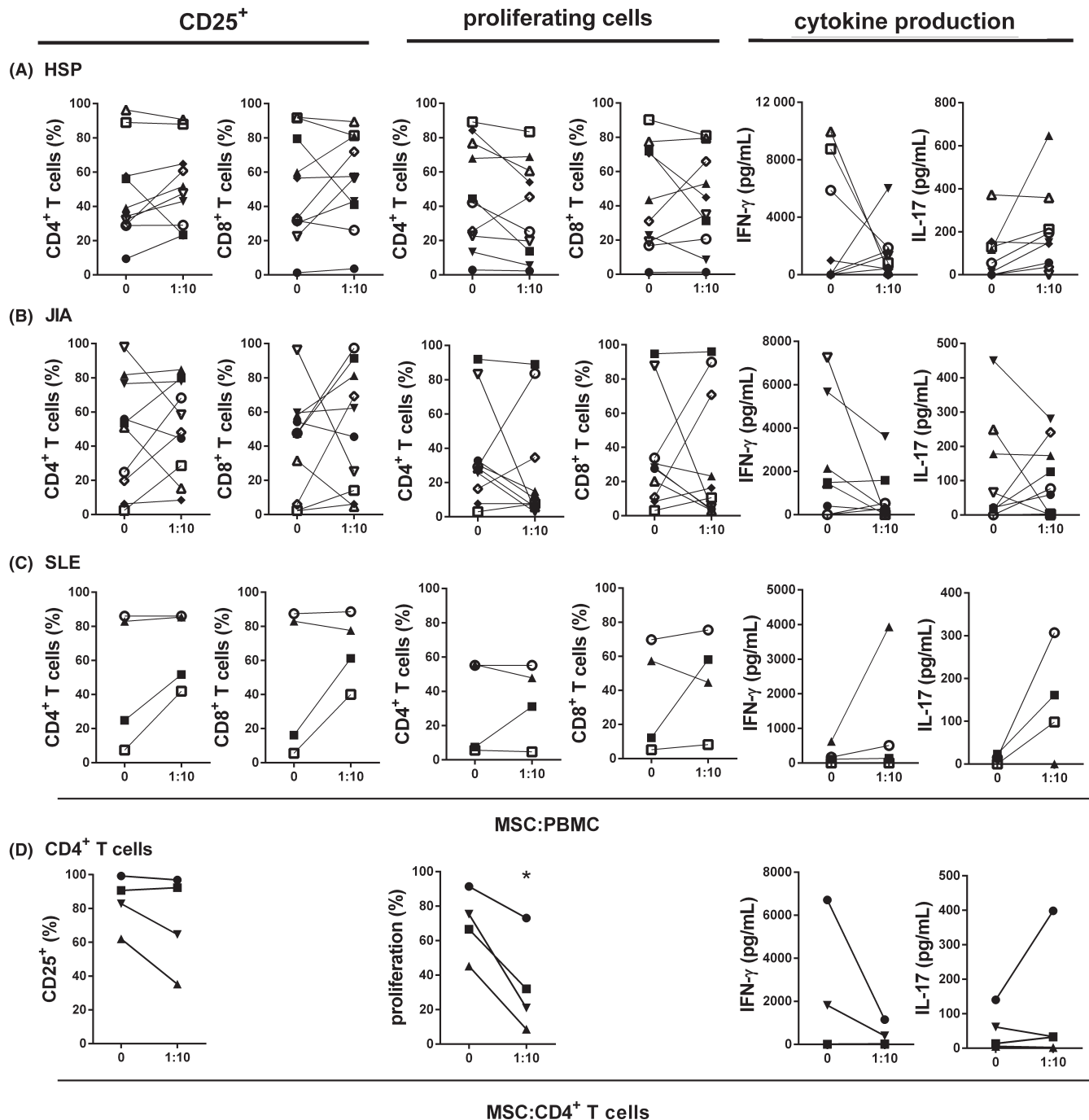


FIGURE 2 pcMSCs did not display apparent immunosuppressive effect on T cells in children with autoimmune diseases. CFSE-labeled PBMCs (A–C) or purified CD4⁺ T cells (D) from patients with Henoch-Schönlein purpura (HSP), juvenile idiopathic arthritis (JIA), or systemic lupus erythematosus (SLE) were activated with anti-CD3 and anti-CD28 Abs and cocultured with pcMSCs for 72 h. Activation and proliferation of CD4⁺ and CD8⁺ T cells as well as production of cytokines IFN-γ and IL-17 were determined. Each symbol represents an individual patient. n = 10 for HSP; n = 10 for JIA; and n = 4 for SLE. *P < 0.05 compared to 0 group

studies which reported that MSCs would abrogate a variety of autoimmune diseases,^{2,3} although the patients in these studies were adults. There are limited reports on usage of MSC for treating autoimmune diseases in children. One study stated that MSCs induce and not suppress the polyclonal expansion and differentiation of B cells isolated from pediatric SLE patients.⁵ We assumed that the

differential nature of autoimmune diseases in children and adults might contribute to the difference in immunomodulatory effects of MSCs.

To test the effect of pcMSCs on low-reactive T cells above commented, we obtained low-reactive T cells by reducing the plating density of PBMCs from healthy subjects. We observed that the

proliferation, activation, and IFN- γ production of low-reactive T cells were markedly elevated after coculturing with pcMSCs (Figure S4A). These results revealed that the immune-enhancing effect of pcMSCs was solely dependent on the low-reactive status of T cells and not on the specific disease of patients. Moreover, pcMSCs could not enhance the function of resting PBMCs which were not stimulated with anti-CD3 and anti-CD28 antibodies (data not shown). As reported previously, MSCs could promote T-cell proliferation at reduced stimulation strength.⁷ In addition, low levels of environmental IFN- γ enhance the antigen-presenting ability of MSCs and exacerbate inflammatory responses.⁸ Hence, the immune-enhancing effect of MSCs is dependent on the reactive status of T cells and not on the defective T cells in patients.

Previous reports suggested that IFN- γ and tumor necrosis factor (TNF)- α pretreatment strengthens the immunosuppressive capability of MSCs.⁹ We speculated whether the IFN- γ and TNF- α pretreatment could counter the immune-enhancing effect of pcMSCs on low-reactive T cells. We found that IFN- γ and TNF- α -pretreated pcMSCs did not abrogate the immune-enhancing effect of pcMSCs. In contrast, notably increase in activation and proliferation of T cells was observed at 1:10 of IFN- γ and TNF- α -pretreated pcMSC-to-PBMC ratio (Figure S4B).

We then investigated the possible mechanism of T-cell suppression by pcMSCs. We found pcMSCs did not induce the apoptosis of T cells (Figure S5A). Neither cell-cell interactions nor IL-10 was critical for the suppressive function of pcMSCs (Figure S5B and S5C). These results suggested the involvement of other factors in the immunosuppressive function of pcMSCs on T cells.

In summary, we demonstrated that pcMSCs could suppress in vitro the differentiation and function of Th2 cells. Moreover, pcMSCs suppressed the activation, proliferation, and cytokine production of T cells isolated from children with allergic asthma. In contrast, pcMSCs could not suppress T cells isolated from pediatric autoimmune disease patients. However, pcMSCs displayed an unexpected immune-enhancing effect on low-reactive T cells, which could not be neutralized by IFN- γ and TNF- α pretreatment. Hence, pcMSCs might have the potential to treat high-reactive T cells from children with allergic asthma, but their immune-enhancing effect should be carefully evaluated. The limitation of this study was we have not performed the study directly on the patients.

ACKNOWLEDGEMENTS

We thank Meng-Shiue Wu (Department of Pharmacology, College of Medicine, National Taiwan University) for preparation of pcMSCs. This work was supported by grants from National Taiwan University College of Medicine, 100R311002, 101R39012, 102R39012, and 103-L001 (YHC).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

Keywords

allergic asthma, autoimmune disease, children, mesenchymal stem cells, T cells

Yih-Mei Liou^{1,2}

Teng-Yuan Wei¹

Thai-Yen Ling³

Sheng-Chieh Lin^{4,5}

Yao-Hsu Yang⁶

Bor-Luen Chiang^{6,7}

Ya-Hui Chuang^{1,2} 

¹Department of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, National Taiwan University, Taipei, Taiwan

²Department of Laboratory Medicine, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan

³Department of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan

⁴Department of Pediatrics, Shuang Ho Hospital, Taipei Medical University, Taipei, Taiwan

⁵Department of Pediatrics, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

⁶Department of Pediatrics, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan

⁷Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan

Correspondence

Ya-Hui Chuang, Department of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, National Taiwan University, No. 1, Chang-Te Street, 10016 Taipei, Taiwan.
Email: yahuichuang@ntu.edu.tw

ORCID

Ya-Hui Chuang  <https://orcid.org/0000-0003-0857-0035>

REFERENCES

- Keating A. How do mesenchymal stromal cells suppress T cells? *Cell Stem Cell* 2008;2(2):106-108.
- Xu J, Wang D, Liu D, et al. Allogeneic mesenchymal stem cell treatment alleviates experimental and clinical Sjogren syndrome. *Blood* 2012;120(15):3142-3151.
- Liu R, Li X, Zhang Z, et al. Allogeneic mesenchymal stem cells inhibited T follicular helper cell generation in rheumatoid arthritis. *Sci Rep* 2015;5:12777.
- Su L-J, Wu M-S, Hui YY, et al. Fluorescent nanodiamonds enable quantitative tracking of human mesenchymal stem cells in miniature pigs. *Sci Rep* 2017;7:45607.
- Traggiai E, Volpi S, Schena F, et al. Bone marrow-derived mesenchymal stem cells induce both polyclonal expansion and differentiation of B cells isolated from healthy donors and systemic lupus erythematosus patients. *Stem Cells* 2008;26(2):562-569.

6. Kapoor S, Patel SA, Kartan S, Axelrod D, Capitle E, Rameshwar P. Tolerance-like mediated suppression by mesenchymal stem cells in patients with dust mite allergy-induced asthma. *J Allergy Clin Immunol*. 2012;129(4):1094-1101.
7. Li W, Ren G, Huang Y, et al. Mesenchymal stem cells: a double-edged sword in regulating immune responses. *Cell Death Differ*. 2012;19(9):1505-1513.
8. Chan JL, Tang KC, Patel AP, et al. Antigen-presenting property of mesenchymal stem cells occurs during a narrow window at low levels of interferon-gamma. *Blood* 2006;107(12):4817-4824.
9. Duijvestein M, Wildenberg ME, Welling MM, et al. Pretreatment with interferon-gamma enhances the therapeutic activity of mesenchymal stromal cells in animal models of colitis. *Stem Cells* 2011;29(10):1549-1558.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

DOI: 10.1111/all.13858

Eliciting allergens and treatment of anaphylaxis: Report of the finnish national anaphylaxis registry

To the Editor

Anaphylaxis is the most severe form of an allergic reaction. The Finnish National Anaphylaxis Registry has been collecting data of severe allergic reactions since year 2000.¹ Here, we summarize a total of 1442 reported anaphylactic cases between 2000 and 2017 and identify and report changes in provoking agents as well as characterize the symptoms and treatments over the full Registry period.

The data are based on voluntary reports of systemic allergic reactions filed by hospital personnel and covering the Finnish population of 5.5 million people. A questionnaire or an online electronic form was used for reporting. Children (<16 years) represented 663 (46%) cases (Table 1) with the highest frequency of reports (336 cases, 23%) accumulating from small children under school age (<6 years) (Figure S1). Males accounted the majority (61%) of anaphylactic reports in children, whereas females dominated among adults (64%) (Figure S1).

The most frequently reported provoking allergens were foods (811 cases, 56%), drugs (365 cases, 25%), and insect venoms (90 cases, 6%) in concordance with earlier reports of anaphylactic elicitors.^{2,3} However, the Finnish Anaphylaxis Registry revealed significantly less reported cases due to insect stings both in children and adults compared to the European Anaphylaxis Registry (Figure 1A).² Furthermore, severe allergic reactions due to immunotherapy were reported in 121 (8%) cases. Other causative agents were involved in 48 (3%) patient cases, and without knowledge of a potential triggering allergen anaphylaxis affected 21 (1.5%) patients.

Food triggered 502 (76%) anaphylaxis in children, of which the six most frequent elicitors remained the same over the Registry period (ie milk, wheat, eggs, nuts, fruits, and fish). Intriguingly, the amount of anaphylaxis due to nuts significantly increased during the past 5 years, mostly due to increase in reported cases with peanut and cashew nut (Figure S2A,B). The increase in nut exposure and in particularly cashew could be explained by increase in importation and simultaneous decrease in retail price.⁴ Furthermore, cashew has become a substituting nut for more expensive nuts or seeds in many foods, hence, providing cause for accidental exposures. Nonetheless,

no increase in nut-provoked anaphylaxis in adults was observed. A total of 134 (27%) cases of food-triggered anaphylaxis in children were due to accidental exposure to a known allergen. To that end, we analyzed the places of accidental food exposures and noticed decrease in events in daycare centers or schools and increase at home and leisure time (Figure S2C). Overall, the food-triggered anaphylaxis among pediatric patients occurred mostly at private homes (47%), daycare centers and schools (16%), and leisure time (14%). These data indicate slightly higher frequency of anaphylaxis in daycare centers and schools compared to earlier European reports.⁵ Most likely, the differences in warm meal catering services for children between European countries play a significant role in the observation.

In adults, the most common causes of anaphylaxis were foods (39%) and drugs (39%) (Table 1). Nuts, fruits, and wheat were the most reported food elicitors, and antibiotics, contrast media, and analgesics were the most frequent drug elicitors (Table 1). No significant changes in eliciting allergens, location of anaphylaxis events, or symptoms were observed among adult patients during the Registry period.

Insect venom caused 38 (6%) anaphylaxis in children and 53 cases (7%) in adults. Anaphylaxis due to bee sting was rare (0% in children and 0.5% in adults). The lower frequency of hymenoptera anaphylaxis compared to European reports⁵ may be due to shorter summers or differences in the colliding habitats of the insects and humans in countries with low population density. A recent registry-based report on anaphylaxis associated deaths in Finland, however, identified hymenoptera venoms as major cause of fatal cases.⁶ Only one report of fatal hymenoptera anaphylaxis was collected to the Registry, indicating a significant lack of reports from the fatal cases.

Therapeutic allergen products comprised 8% of the reported cases in the Registry. Immunotherapy (IT) to birch pollen was better tolerated than timothy (Table 1). Only 2 cases of anaphylaxis due to hymenoptera IT were reported. No reports of Sublingual IT were received.

Symptoms in skin and in respiratory system were involved in most cases (87% and 79%, respectively). Most frequent allergic

ONLINE SUPPLEMENT

Differential Effects of Mesenchymal Stem Cells on T Cells Isolated from Childhood Allergies and Autoimmune Diseases

Yih-Mei Liou^{1,7,#}, Teng-Yuan Wei^{1,#}, Thai-Yen Ling², Sheng-Chieh Lin^{3,4}, Yao-Hsu Yang⁵, Bor-Luen Chiang^{5,6}, and Ya-Hui Chuang^{1,7,*}

#These authors contributed equally to this work.

¹Department of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, National Taiwan University; ²Department of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan; ³Department of Pediatrics, Shuang Ho Hospital, Taipei Medical University, Taipei, Taiwan. ⁴Department of Pediatrics, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan. ⁵Department of Pediatrics, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan. ⁶Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan. ⁷Department of Laboratory Medicine, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan.

CORRESPONDING AUTHOR

Ya-Hui Chuang, Ph. D.,

Department of Clinical Laboratory Sciences and Medical Biotechnology

College of Medicine, National Taiwan University

No. 1, Chang-Te Street, 10016, Taipei, Taiwan

TEL: 886-2-2312-3456 Ext. 66906

FAX: 886-2-2371-1574

E-mail: yahuichuang@ntu.edu.tw

Methods

MSCs

MSCs were isolated from choriodecidual tissues of human placentas, denoted as pcMSCs, as described in our previous study ¹. To isolate pcMSCs, choriodecidual tissues were digested with SMEM medium supplemented with 0.5 mg/mL protease, 0.5 mg/mL collagenase B, and 1 mg/mL DNase I at 4 °C overnight. The digested tissue was filtered through a 100-µm nylon membrane and cells were collected by centrifugation. The cells were subsequently re-suspended in culture medium (MCDB201 supplemented with 1% insulin transferrin selenium, 10 ng/mL epidermal growth factor, and 1% penicillin/streptomycin) and planted in culture dishes coated with human collagen type IV. Non-adherent cells were removed and the adherent cells were kept in the culture medium changed every 3–4 days. pcMSCs were displayed fibroblast-like morphology after attachment and showed positive surface staining for CD29, CD44, CD73 and CD90 but negative surface staining for CD14, CD34, CD45 and HLA-DR. *In vitro* assays for osteogenic, chondrogenic, and adipogenic differentiation of the cells all showed positive signals when stained with Alizarin Red S, Alcian Blue, and Oil Red O, respectively ¹.

Study subjects

Heparinized peripheral blood samples from 31 patients with allergic asthma (16 boys and 15 girls, mean age 5.9 years, serum total IgE 651.3 ± 234.6 KU/L), 10 patients with Henoch Schönlein purpura (HSP) (7 boys and 3 girls, mean age 7.2 years), 10 patients with juvenile idiopathic arthritis (JIA) (7 boys and 3 girls, mean age 12.6 years), 4 patients with systemic lupus erythematosus (SLE) (4 girls, mean age 10.3 years) and 19 healthy individuals (8 males and 11 females, mean age 23 years) were studied. The blood samples of patients were obtained at the acute stage and before steroid or other immunosuppressants treatment. Studies were performed with approval from the Institutional Review Board on human subjects (N201801037 at Taipei Medical University and 201412133RINC at National Taiwan University) and conducted in accordance with the Declaration of Helsinki. All persons gave their informed consent prior to their inclusion in the study. Human peripheral blood mononuclear cells (PBMCs) was isolated from heparinized whole blood by density gradient centrifugation using Histopaque (Sigma-Aldrich, St. Louis, MO, USA).

Mouse Th2 cell differentiation assay

BALB/c mice were obtained from the National Laboratory Animal Center, Taiwan and maintained in the Animal Center of the College of Medicine, National Taiwan University. Experiments were performed following approval of The Institutional Animal Care and Use Committee (IACUC) of National Taiwan University College of Medicine and College of Public Health (No. 20150298). CD4⁺ T cells were isolated from lymph nodes of 6-8 weeks old female BALB/c mice by positive selection with a microbead conjugated mAb to CD4 (clone GK1.5) by IBD (BD Biosciences, San Diego, CA, USA). CD4⁺ cells (1×10^6 cells/well) were stimulated with coating anti-mouse CD3 Ab (2 µg/ml, BD Biosciences) and soluble anti-mouse CD28 Ab (2 µg/ml, BD Biosciences) in the presence of recombinant IL-2 (100 U/ml) (Peprotech Inc., Rocky Hill, NJ, USA), recombinant IL-4 (10 ng/ml) (BD Biosciences) and anti-mouse IFN-γ Abs (10 µg/ml, Biolegend, San Diego, CA, USA) on a 48-well plate for the Th2 cell differentiation. After 5 days of culturing in Th2-polarizing conditions, cells became Th2-polarizing cells². According to the experimental design, pcMSCs were added before or after Th2 cell culture protocol. Proliferation, activation, and cytokine production of Th2 cells cultured with or without pcMSCs were analyzed, as described below.

T cell functional assay

Cells were labeled with 5 µM carboxyfluorescein succinimidyl ester (CFSE) for 5 min prior to culture for visualizing cell proliferation. CFSE is retained in the cytoplasm and diluted out with each cell division, and thus allows tracking of cell divisions in cultured cells by flow cytometry. CFSE labeled mouse Th2-polarizing cells, human PBMCs or human CD4⁺ T cells (2×10^5 /well in 96 flat well) were stimulated with anti-mCD3 Abs and anti-mCD28 Abs for mouse T cells or anti-hCD3 Abs and anti-hCD28 Ab for human T cells (0.5 µg/ml, Biolegend) in the presence or absence of different numbers of pcMSCs. Three days later, CFSE dilution and CD25 expression of T cells were determined by flow cytometry for proliferation and activation status of T cells. Stained cells were assessed on the BD FACSVers instrument (BD Biosciences) and FlowJo software (Tree Star, Inc., Ashland, OR, USA). 30000-50000 gated lymphocytes were analyzed. A representative analysis of flow cytometry was shown in Figure 1A. Supernatant cytokines were assayed by commercially available ELISA kits (Duoset; R&D Systems, Minneapolis, MN, USA). For the study of IL-10, 5 µg/ml anti-human IL-10R Abs (Biolegend) were used.

Intracellular cytokines staining

For the assay of IL-4 production of mouse Th2-polarizing cells, Th2-polarizing cells were treated with 50 ng/ml phorbol-myristate acetate (PMA, Sigma Aldrich) and 1 µg/ml ionomycin (Sigma Aldrich) in the presence of brefeldin A (10 µg/ml) (BD Biosciences) for 4 hours, followed by staining with fluorescence-labeled anti-CD3 and anti-CD4 Abs (Biolegend), permeabilized with Cytofix/Cytoperm reagent (BD Biosciences), and stained with anti-IL-4-PE Abs. Stained cells were assessed on the BD FACSVerse instrument (BD Biosciences) and FlowJo software (Tree Star).

Statistical analysis

Results are expressed as the mean $\pm$ standard error of the mean (SEM). Statistical significance between two groups was determined by the paired Student t test, whereas statistical differences between multiple groups were determined by repeated measured one way ANOVA with Tukey post-test analysis. Differences were considered significant when P values were <0.05 .

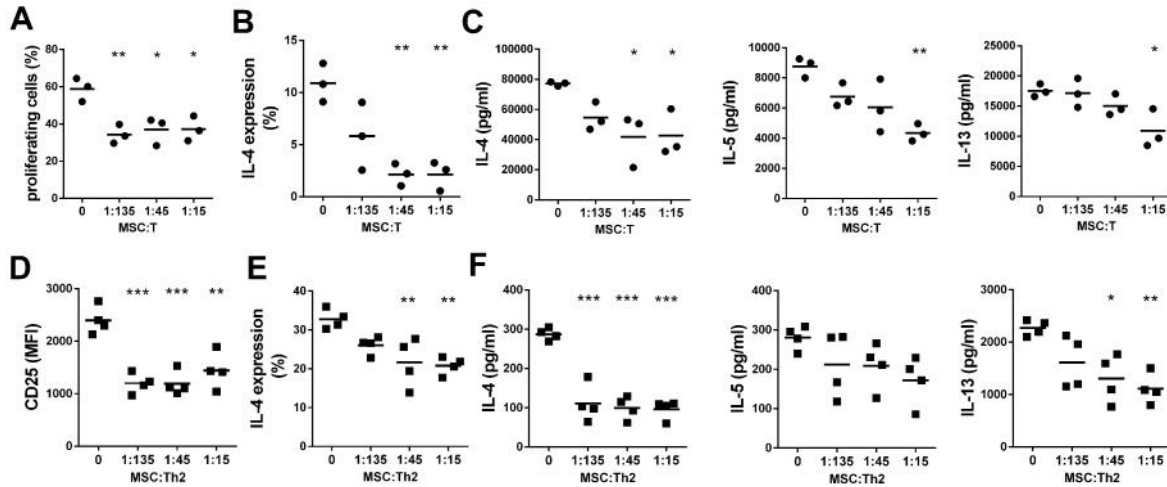
Figure S1

Figure S1. *pcMSCs inhibit differentiation and function of Th2 cells.* (A-C) CD4⁺ T cells isolated from BALB/c mice were labeled with CFSE, and cocultured with pcMSCs in Th2-polarizing conditions for 5 days. (A) Proliferation of Th2-polarizing cells was assessed by flow cytometry at the end of the 5-day culture. (B) Th2-polarizing cells were collected and stimulated with phorbol-myristate acetate (PMA) and ionomycin for 4 hours, and IL-4 expression was measured by intracellular flow cytometry. (C) Th2-polarizing cells were collected and stimulated with anti-CD3 and anti-CD28 Abs for 24 hours, and supernatants IL-4, IL-5, and IL-13 were measured by ELISA. (D-F) CD4⁺ T cells isolated from BALB/c mice were cultured in Th2-polarizing conditions for 5 days. Th2-polarizing cells were then activated with anti-CD3 and anti-CD28 Abs and cocultured with pcMSCs for 3 days. (D) CD25 expression was measured by flow cytometry. (E) Cells were further stimulated with PMA and ionomycin for 4 hours, and IL-4 expression in T cells was measured by flow cytometry. (F) Supernatants IL-4, IL-5, and IL-13 were assessed by ELISA. Individual symbols represent an independent experiment with 1-3 mice per experiment and the horizontal lines represent the mean values of 3-4 independent experiments. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ compared to 0 group.

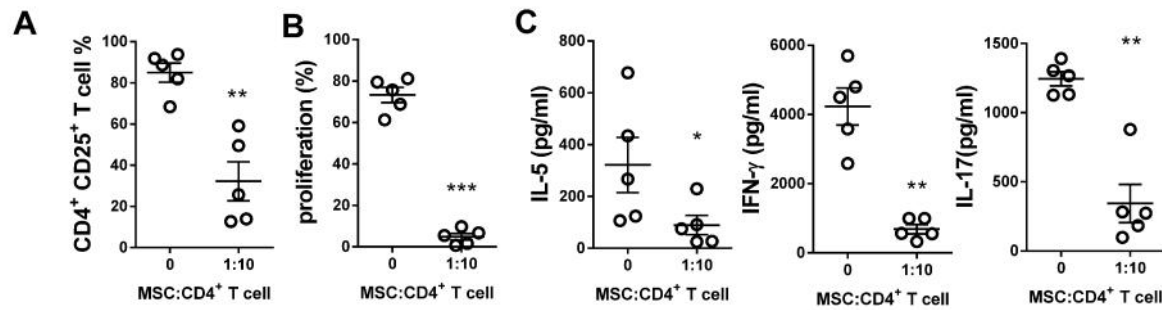
Figure S2

Figure S2. *pcMSCs directly suppressed the function of CD4⁺ T cells from patients with allergic asthma.* CD4⁺ cells were purified by magnetic beads, stimulated with anti-CD3 and anti-CD28 Abs in the presence or absence of pcMSCs for 72 hrs. (A) Activation and (B) proliferation of CD4⁺ T cells were assessed by flow cytometry. (C) Cytokines IL-5, IFN-γ, and IL-17 were detected by ELISA. Symbols represent individual subjects, and horizontal lines represent means of 5 subjects. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ compared to 0 group.

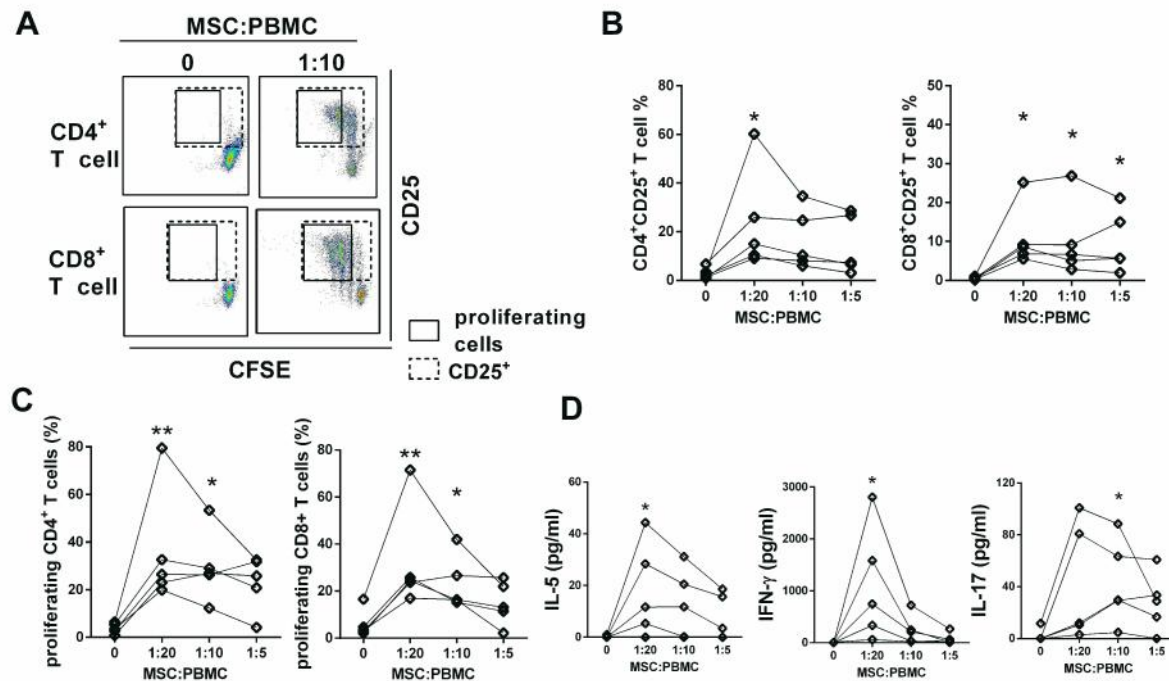
Figure S3

Figure S3. *pcMSCs enhanced the function of low responder T cells from children patients with allergic asthma.* CFSE-labeled PBMCs from pediatric patients with allergic asthma were

activated with anti-CD3 and anti-CD28 Abs and cocultured with pcMSCs for 72 hrs. (A) A representative analysis of flow cytometry. (B) Activation and (C) proliferation of CD4⁺ and CD8⁺ T cells were assessed by flow cytometry. (D) Cytokines IL-5, IFN- γ , and IL-17 were detected by ELISA. Each symbol represents an individual patient (n=5). *, p<0.05; **, p<0.01 compared to 0 group.

Figure S4

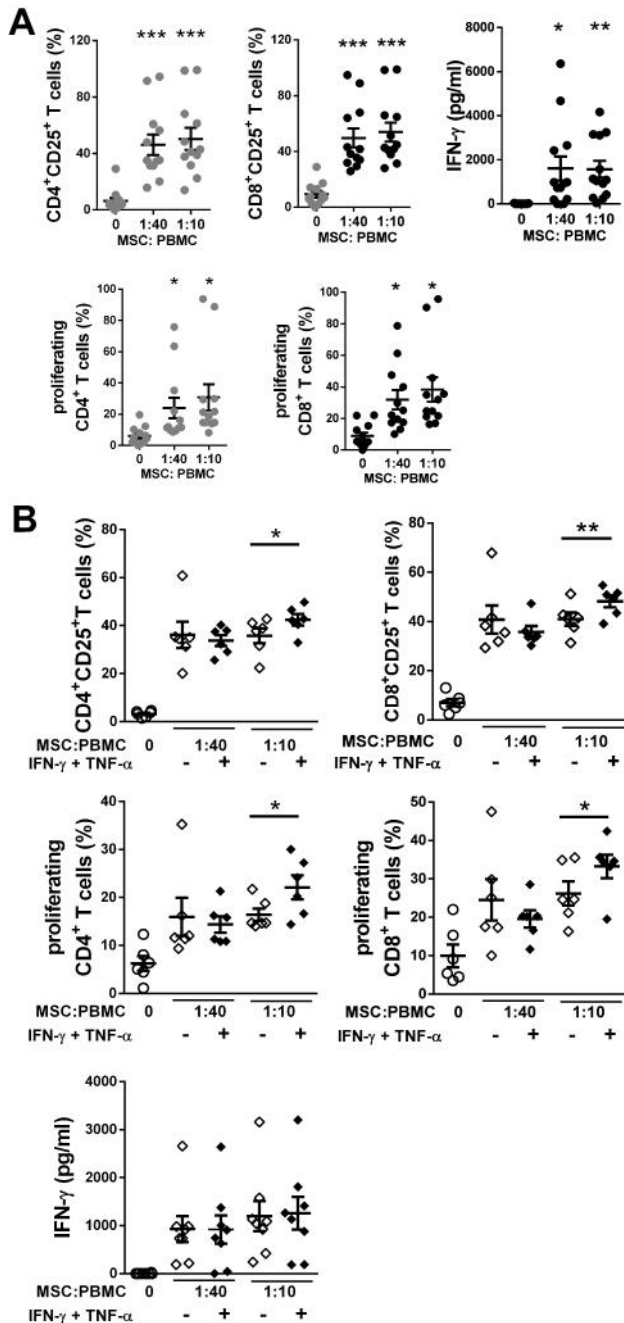


Figure S4. *pcMSCs enhanced low-reactive T cells and the effect could not be prevented by IFN- γ and TNF- α pretreatment.* (A) PBMCs from healthy donors in 1/4 cell density (5×10^4 /well in 96 well plate) were activated with anti-CD3 and anti-CD28 Abs and cocultured with pcMSCs for 72 hrs. Proliferation and activation of CD4⁺ and CD8⁺ T cells and IFN- γ were assayed. *, p<0.05; **, p<0.01; ***, p<0.001 compared to 0 group. (B) pcMSCs were pretreated with IFN- γ and TNF- α (10ng/mL each) for 8 hours. PBMCs were then cocultured with pretreated pcMSCs in the condition described as in (A). Each symbol represents an individual subject. n=13 (A) and n=6 (B). *, p<0.05; **, p<0.01; ***, p<0.001 compared to 0 group.

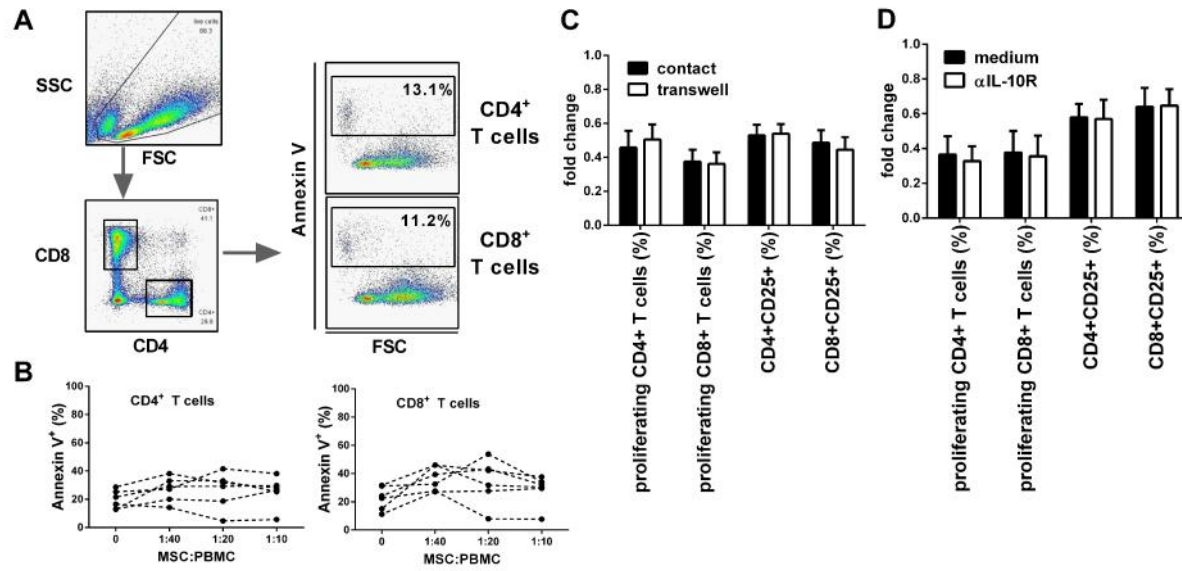
Figure S5

Figure S5. Suppression of T cells by pcMSCs was not dependent on cell-cell contact, apoptosis, or IL-10. PBMCs isolated from children with allergic asthma were activated with anti-CD3 and anti-CD28 Abs and cocultured with pcMSCs for 72 hrs. (A and B) Apoptotic CD4⁺ and CD8⁺ T cells were stained with annexin V and determined by flow cytometry. A representative analysis of flow cytometry was shown in (A). Each symbol represents an individual subject. (C and D) (C) PBMCs and pcMSCs were cocultured in the same well (labeled contact) or separated by a transwell (labeled transwell). N=10 subjects. (D) Anti-IL-10R blocking Abs were added in the culture platform. N=4 subjects. pcMSCs /PBMCs ratio was 1:10. Activation and proliferation of CD4⁺ and CD8⁺ T cells were assessed by flow cytometry. The data represents the fold change comparing the results with PBMCs only control.

Reference

1. Su LJ, Wu MS, Hui YY, et al. Fluorescent nanodiamonds enable quantitative tracking of human mesenchymal stem cells in miniature pigs. *Sci Rep.* 2017;7:45607.
2. Lin PY, Jen HY, Chiang BL, Sheu F, Chuang YH. Interleukin-21 suppresses the differentiation and functions of T helper 2 cells. *Immunology.* 2015;144(4):668-676.