

Tolerance-like mediated suppression by mesenchymal stem cells in patients with dust mite allergy-induced asthma

Simi Kapoor, MD, Shyam A. Patel, PhD, Saritha Kartan, MD, David Axelrod, MD, Eugenio Capitle, MD, and Pranela Rameshwar, PhD Newark, NJ

Background: Mesenchymal stem cells (MSCs) can suppress and enhance immune functions. MSCs show promise as off-the-shelf cellular therapy for several disorders, including inflammation.

Objective: We investigated the effects of MSCs on the proliferation of PBMCs to allergic subjects (dust mite [DM]), allergic asthmatic subjects, or both.

Methods: Proliferation was studied by using tritiated thymidine uptake with or without MSCs. The refractoriness of PBMCs to DM was examined after preconditioning with MSCs and after repeated challenge with low-dose DM. Flow cytometry was used to study regulatory T cells and dendritic cells (DCs), and ELISA was used to study cytokine production.

Results: Seven subjects with allergic asthma met the inclusion/exclusion criteria. MSCs significantly ($P < .05$) reduced the proliferation of 6 subjects with allergic asthma but not those with allergy alone. The effect was specific to the allergen because MSCs did not affect challenges to tetanus toxoid. There was no change in CD4/CD25/forkhead box protein 3–positive cells, although there were decreased IFN- γ and increased IL-10 levels. Numbers of mature DCs were increased 6-fold.

Refractoriness to DM was achieved by means of repeated exposure to low-dose DM and MSCs and also MSC-preconditioned MSC.

Conclusion: MSCs suppressed the proliferation of DM-challenged PBMCs from allergic asthmatic subjects but not from allergic subjects without asthma. MSCs blunted the maturation of DCs but not regulatory T cells. Repeated exposure to low-dose DM and MSCs, as well as preconditioning of PBMCs with MSCs, caused refractoriness to DM. These findings have implications for the use of MSCs in attenuation of the inflammatory responses to allergic triggers in asthmatic patients with off-the-shelf MSCs. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: Mesenchymal stem cells, proliferation, T cells, dust mite, allergic asthma, dendritic cells

Abbreviations used

DC: Dendritic cell
DM: Dust mite
MSC: Mesenchymal stem cell
SI: Stimulation index
Treg: Regulatory T

Mesenchymal stem cells (MSCs) are ubiquitous multipotent cells that are abundant in adult bone marrow and adipose tissue.¹ MSCs can be found in fetal organs and amniotic fluid.¹ Over the last decade, MSCs have gained significant interest because of their pliability to develop into almost any cell type,² making them attractive for regenerative medicine, including the fields of orthopedic surgery, rheumatology, gastroenterology, and transplant surgery.³⁻⁵ MSCs also have potential as cellular immune-suppressive therapy for inflammatory disorders, such as systemic lupus erythematosus,^{6,7} rheumatoid arthritis,^{8,9} inflammatory bowel disease,¹⁰⁻¹² and graft-versus-host disease.¹³⁻¹⁶

Despite extensive information on the immune functions of MSCs and their potential to treat inflammatory diseases, MSC therapy has not reached the clinic for the treatment of allergic inflammation. Because of the presence of several receptors on MSCs, including those for cytokines, the functions of MSCs can be influenced by the tissue microenvironment through cross-communication with specific ligands.¹⁷ Because allergic asthma is a heterogeneous disorder,¹⁸⁻²⁰ it would be difficult to predict how MSCs would respond *in vivo*. To this end, we designed *in vitro* studies to dissect the effects of MSCs. We established an experimental model of inflammation to recapitulate allergic asthma. We also studied a potential role for MSCs in achieving refractoriness to allergic responses.

Allergic asthma is an antigen-driven disorder in subjects with aberrant T-cell regulation.¹ It was difficult to enroll monosensitized subjects because most subjects are likely to be sensitized to more than 1 antigen; however, we were able to enroll 2 monosensitized subjects (Table I). We therefore selected dust mite (DM) (Der p 1) because it was the most common allergen in the allergy clinic. Furthermore, because DM is a perennial allergen, seasonal variation would have minimal to no effect in the *in vitro* studies. The overarching hypothesis of this study is that MSCs would suppress the proliferative responses of PBMCs from allergic asthmatic subjects to DM. Furthermore, we proposed that after repeated exposure to low doses of DM and MSCs, PBMCs from allergic asthmatic subjects should become refractory when re-exposed to DM in an anergic-type response.

This article reports on the suppressive effect of MSCs on DM-activated PBMCs in allergic asthmatic subjects but not in

From the Department of Medicine, University of Medicine and Dentistry of New Jersey, New Jersey Medical School.

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Corresponding author: Pranela Rameshwar, PhD, UMDNJ–New Jersey Medical School, 185 South Orange Ave, MSB, Room E-579, Newark, NJ 07103. E-mail: rameshwa@umdnj.edu.

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TABLE I. Demographics of the patient population in the group with DM allergy and asthma

Subject no.	Age (y)	Ethnicity	FEV ₁ /FVC ratio (%)	FEV ₁ (%)	Atopic diseases	Total IgE	Monosensitized/polysensitized	BMI	Controller medications
1	38	AA	111	100	AR/AC	NA	Monosensitized	38.6	Fluticasone, 220 µg
2	21	AA	95	103	AR/AC eczema	NA	Polysensitized	38.3	Budesonide
3	40	AA	98	91	AR/AC	NA	Polysensitized	28.3	Budesonide
4	23	H	91	88	AR/AC	1187	Polysensitized	23.9	Albuterol
5	33	H	75	100	AR chronic sinusitis	NA	Monosensitized	40.6	Ciclesonide
6	39	W	83	86	AR smoker	NA	Polysensitized	34.3	Fluticasone/salmeterol, 500/50 µg Montelukast Levocetirizine
7	23	M	98	96	AR/AC	NA	Polysensitized	25.1	Albuterol (as needed)

AA, African American; AC, allergic conjunctivitis; AR, allergic rhinitis; BMI, body mass index; FVC, forced vital capacity; H, Hispanic; M, mixed; NA, not available; W, white.

DM-sensitive nonasthmatic subjects. The suppressive effect could not be explained by an increase in CD4/CD25/forkhead box protein 3–positive cells, which is consistent for regulatory T (Treg) cells, but MSCs and DM-challenged PBMCs resulted in decreased IFN- γ levels and a concomitant increase in IL-10 levels. MSCs and DM caused a decrease in mature dendritic cell (DC) numbers in allergic asthmatic subjects. The suppressive effect of MSCs was specific for DM because MSCs did not suppress challenges to tetanus toxoid. The experimental evidence indicated that MSCs might be involved in resetting the immune response to make the cells more refractory to challenge with DM antigen, which is similar to other reports for MSCs on macrophages and sepsis.¹⁵

METHODS

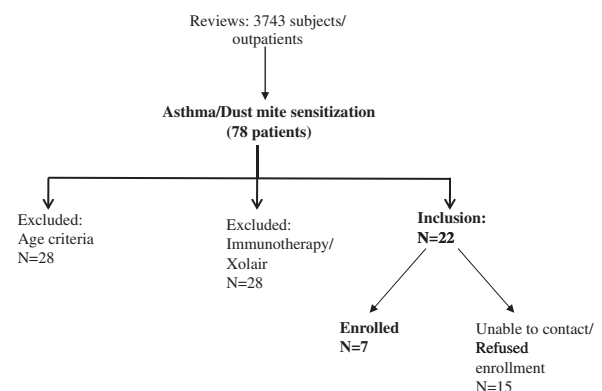
Reagents

PBS, Ficoll-Hypaque, RPMI 1640, and Dulbecco modified Eagle medium were purchased from Sigma (St Louis, Mo). FCS was purchased from Hyclone Laboratories (Logan, Utah). DM antigen was obtained from Hollister-Stier (Spokane, Wash). The solution contains 15,000 AU/mL *Dermatophagoides farinae* and 15,000 AU/mL *Dermatophagoides pteronyssinus*. Tetanus toxoid was obtained from Bristol-Myers Squibb (New York, NY) and distributed by Antigen Supply House (Northridge, Calif).

Study subjects

Subjects were recruited from the Allergy and Immunology Clinic, New Jersey Medical School (Newark, NJ), and voluntarily donated 10 mL of blood. The subjects were retrospectively reviewed for the last 3 years (Fig 1) by using the diagnostic code for allergic asthma. Inclusion criteria included age of 18 to 40 years and DM sensitivity, as determined based on history and skin prick test responses. Exclusion criteria include the following comorbid conditions: heart disease, diabetes, cancer, pregnancy, HIV, immunodeficiency, and autoimmune disease; use of immune modulator; use of systemic steroids within 3 months before the blood draw; or asthma flare within 1 month before the blood draw.

As shown in Fig 1, among the 3743 screened subjects, 78 met the criteria for allergic asthma and DM sensitivity. Of these, 28 subjects were not within the acceptable age range, and 28 subjects were excluded because of requirements for immune therapy and treatment with omalizumab (Xolair; Genentech and Novartis Pharmaceuticals, East Hanover, NJ). Of the remaining 22 subjects who met the inclusion criteria, 15 either could not be contacted or refused enrollment in the study. Finally, 7 subjects were recruited for the allergic asthma group. Most of the subjects were allergic to more than 1 antigen, but 2 were monosensitized to DM. Four healthy control subjects with negative skin test for a panel of allergens and 4 DM-sensitive nonasthmatic control subjects volunteered for the study. All control subjects were evaluated for history of allergy, had physical examinations, and underwent allergy skin tests. The Institutional Review Board of the University of Medicine and Dentistry of

**FIG 1.** Flow diagram on inclusion/exclusion criteria. Shown are the methods used in enrolling subjects for the study.

New Jersey, Newark Campus, approved the study protocol. We obtained informed consent from all subjects enrolled in the study.

MSC culture

Bone marrow aspirates were obtained from the posterior iliac crests of healthy donors (age, 18–30 years). Use of human subjects was adherent to the guidelines approved by the Institutional Review Board of the University of Medicine and Dentistry of New Jersey. MSCs were cultured from bone marrow aspirates, as previously described.^{21,22} Unfractionated aspirates were added to Dulbecco modified Eagle medium with 10% FCS (D10 medium) and transferred to plasma-treated, tissue-culture Falcon 3003 Petri dishes (Fisher Scientific, Waltham, Mass). Plates were incubated, and at day 3, the red blood cells and granulocytes were removed by using Ficoll-Hypaque density gradient. The mononuclear fraction was replaced in the dishes with fresh D10 medium. Fifty percent of the media was replaced weekly until the adherent cells were approximately 80% confluent. At this time, the adherent cells were serially passaged at least 4 times. At this time, the cells were symmetric: CD14⁺, CD29⁺, CD44⁺, CD45⁺, CD105⁺, and prollyl-4-hydroxylase negative.

Proliferation

PBMCs were isolated from blood by using Ficoll-Hypaque gradient. PBMCs were studied in proliferation assays by using tritiated thymidine incorporation. PBMCs (2×10^5) were placed in 96-well flat-bottom plates in 200 µL of RPMI 1640 containing 10% FCS. The addition of FCS was necessary to maintain viability after 3 days. Wells were stimulated with DM antigen (5 µg/mL) or tetanus toxoid (1:100 final dilution)^{21,23} or unstimulated. Dose-response and time-course studies indicated 5 µg as the optimum concentration. In parallel, MSCs (2×10^3) were added to similar cultures, both stimulated and unstimulated. At day 4, each well was pulsed with 1 µCi/mL

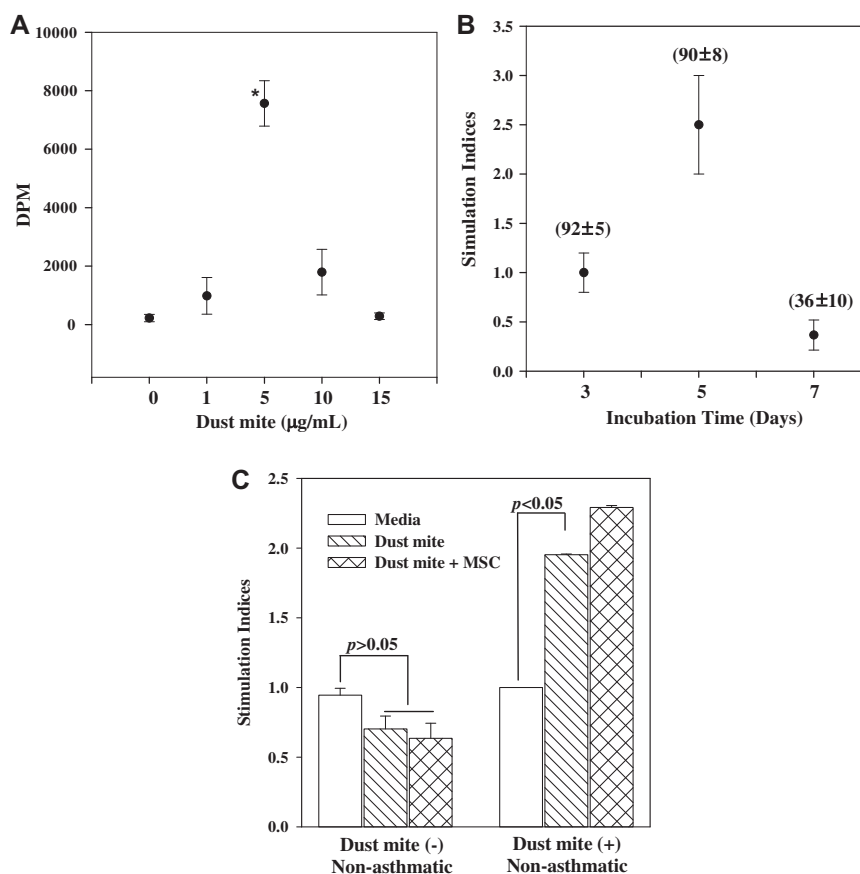


FIG 2. Specificity of DM antigen. **A**, Dose-response studies were performed with PBMCs from DM-sensitive subjects in proliferation assays. The data (disintegrations per minute [DPM]) are presented for 3 subjects, each tested in independent studies (mean $\pm$ SD, $n = 9$). * $P < .01$ versus other concentrations of DM. **B**, Time-course studies were performed with the optimum (5 μ g/mL) DM level, as described in Fig 2, A. **C**, Proliferation assays were performed in the presence of DM, MSCs with optimum DM, or both. Four subjects were studied in each category, and each was studied in 3 independent experiments. The data are presented as means $\pm$ SDs ($n = 12$).

tritiated thymidine. On day 5, the cells were harvested with a PhD cell harvester (Cambridge Technologies, Cambridge, Mass) onto glass-fiber filters (Cambridge Technologies). Radioactive incorporation was acquired on a liquid scintillation counter (Beckman Coulter, Fullerton, Calif). The stimulation indices (SIs) were calculated as disintegrations per minute of experimental points/disintegrations per minute of unstimulated PBMCs.

Preconditioned PBMCs

The proliferation studies above were repeated but with PBMCs preincubated (preconditioned) with MSCs. PBMCs in 3 independent studies with cells from allergic asthmatic subjects and subjects with only DM sensitivity were preincubated with MSCs. On day 5, the MSC-free PBMCs were analyzed for responses to optimal DM in proliferation assays, as described above.

Repeated exposure of PBMCs to DM and MSCs

PBMCs (10^6 /mL) were resuspended in RPMI 1640 with 10% FBS and then exposed to repeated doses of DM as follows: 0.5 μ g on day 0, 0.25 μ g on day 3, 0.25 μ g on day 5, 0.125 μ g on day 7, and 0.125 μ g on day 10. MSCs (10^5) were added on days 0, 5, and 10 to the flasks. In parallel, flasks contained optimal DM or media alone. On day 12, the PBMCs were thoroughly washed and then studied in proliferation assays in the absence of MSCs, as described above.

Flow cytometry for Treg cell and DC/cytokine quantitation

Flow cytometry for Treg cells was performed as previously described²² by using the Human Regulatory T Cell Staining Kit from eBioscience (San Diego, Calif). The kit included fluorescence isothiocyanate-anti-CD4, allophycocyanin-anti-CD25, and phycoerythrin-anti-forkhead box protein 3. DC analyses were performed by using 4-color flow cytometry with V450-CD86 (Becton Dickinson, Franklin Lakes, NJ), phycoerythrin-CD14 (Becton Dickinson), allophycocyanin-HLA-DR (Caltag Laboratories, Burlingame, Calif), and fluorescence isothiocyanate-anti-CD64 (Becton Dickinson). After labeling, cells were analyzed with the LSRII system (BD Biosciences, San Jose, Calif). Parallel studies contained isotype controls, all of which were from Becton Dickinson. Positive control BD Compbeads (BD Bioscience) were labeled with the all fluorochrome-tagged antibodies. The labeled cells were analyzed with the FACSCalibur system (BD Biosciences).

Media from PBMCs cultured alone or stimulated with DM, MSCs, or both after 5 days were quantitated for IFN- γ and IL-10 at the Cytokine Core Laboratory (University of Maryland, Baltimore, Md).

Statistical analyses

Data were analyzed by using ANOVA and the Tukey-Kramer multiple comparisons test. We considered a P value of less than or equal to .05 significant.

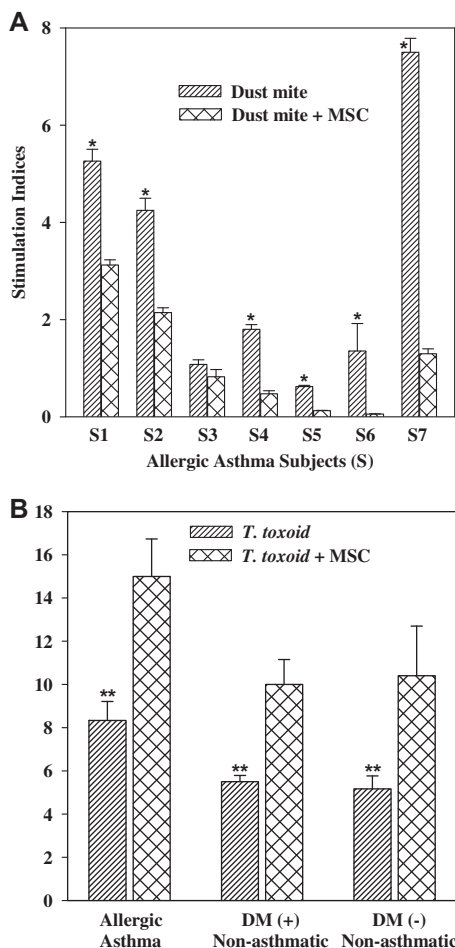


FIG 3. Effects of MSCs on the proliferation of DM/tetanus toxoid-challenged PBMCs. PBMCs were assessed for proliferation in the presence or absence of DM ($n = 7$, **A**) or tetanus toxoid ($n = 4$, **B**), MSCs, or both. Each subject was assessed in 3 independent experiments, and the data are presented as SIs $\pm$ SDs. * $P < .05$ versus DM plus MSCs. ** $P < .05$ versus tetanus toxoid plus MSCs.

RESULTS

Antigenic stimulation of DM on responsive and nonresponsive PBMCs

We first studied the specificity of DM antigen in the proliferation of PBMCs. We compared 4 healthy control subjects with negative skin test results for DM with 7 allergic asthmatic subjects, each studied in 3 independent experiments. The subjects with positive skin test results and who declared no history of asthma were designated DM-sensitive nonasthmatic control subjects and were also included in the studies. The SIs of DM-stimulated PBMCs from the subject with negative skin test results were comparable ($P > .05$) with those of the media control (Fig 2, C, left group), indicating insensitivity/lack of proliferation of the PBMCs to DM challenge. In contrast, the subjects with positive skin test results had significant ($P < .05$) proliferation compared with that seen in the media control (Fig 2, C, right group). The addition of MSCs did not show a significant ($P > .05$) effect on the proliferation of PBMCs (Fig 2, C, right group, diagonal vs hatched bars). In summary, this section shows the specificity of DM in the proliferation of PBMCs.

Effects of MSCs on the proliferation of DM-challenged PBMCs from allergic asthmatic subjects

The studies described in Fig 2 were repeated with the group with DM allergy and asthma, with each subject studied in 3 independent experiments. In all subjects DM induced the proliferation of PBMCs (Fig 3, A, diagonal bars). MSCs significantly ($P < .05$) reduced the proliferation for all subjects except subject 3 (Fig 3, A, hatched bars). The decreases for subjects 1 and 2 were 1.6- and 1.7-fold, respectively. These suppressions were significantly ($P < .05$) less than those seen in the other subjects, with subject 6 showing a 19-fold decrease. In summary, MSCs significantly ($P < .01$) decreased the proliferation of PBMCs to DM from 6 of 7 allergic asthmatic subjects. Our subjects all received a boost of tetanus toxoid. To determine whether the effect on MSCs is antigen specific rather than allergen specific, we repeated the above studies with tetanus toxoid. In all cases proliferation was enhanced, although further with MSCs (Fig 3, B). In summary, this section showed the specificity of DM in the suppression of MSCs but only for PBMCs from allergic asthmatic subjects.

Treg cells and DCs in DM-challenged PBMCs from allergic asthmatic subjects

MSCs have been reported to cause immune suppression through secondary induction of Treg cells.^{22,24} We therefore studied PBMCs challenged for 5 days in the presence or absence of MSCs for Treg cells. Flow cytometry for CD4, Foxp3, and CD25 revealed no significant ($P > .05$) change in Treg cell numbers (Fig 4, A), although there appeared a trend for an increase in Treg cell numbers in the allergic asthmatic subjects compared with the nonallergic DM(-)/nonasthmatic subjects (Fig 4, A, left panels). Because Foxp3 and CD25 do not always prove the presence or absence of Treg cells, we quantitated the media for IFN- γ and IL-10. The results showed significant increases in IL-10 but not IFN- γ levels for both allergic asthmatic subjects and allergic nonasthmatic subjects (Fig 4, C). The IL-10 levels in the nonasthmatic subjects were significantly ($P < .05$) less than similar conditions from the asthmatic subjects. Also, IFN- γ levels remained low in the nonasthmatic subjects, despite stimulation with DM. In summary, the results showed no significant induction of Treg cells based on phenotype in the challenged allergic asthmatic subjects regardless of MSCs but showed an increase in IL-10 levels, suggesting another mechanism of suppression.

Because it is unclear whether Treg cells are involved in the suppressive effects of MSCs (Fig 4, A), we studied the cultures for mature DCs. MSCs have been shown to inhibit monocyte-derived differentiation of DCs.²⁵ Using the same experimental condition as for Treg cell analyses, we performed multicolor flow cytometry in mature DCs. We gated the CD64⁺ fraction to eliminate granulocytes and then performed further analyses based on the expressions of CD14, HLA-DR, and the mature costimulatory molecule CD86. In DM-treated PBMCs there was about a 6-fold increase in CD14⁺/CD86⁺ cell numbers, whereas no change was observed for the CD14⁺ fraction (Fig 4, B), indicating that MSCs blunted DM-induced maturation of DCs.

Nonresponsiveness to DM by MSC-preconditioned PBMCs

This set of studies describes the response of PBMCs preconditioned with MSCs to DM. PBMCs were preincubated with MSCs,

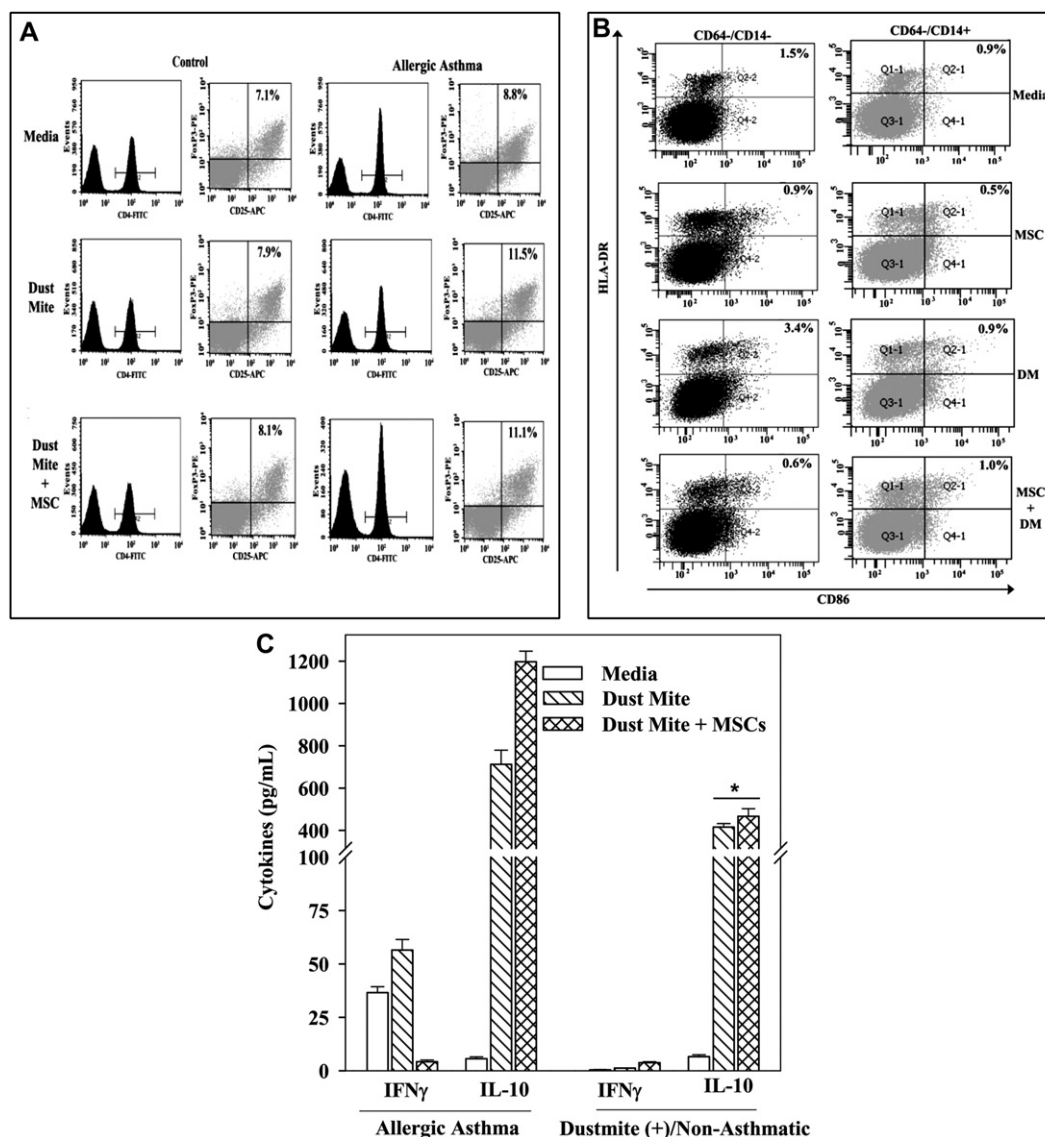


FIG 4. Treg cells and DCs in DM-challenged PBMCs and MSCs. Multicolor flow cytometry was performed for Treg cells (**A**) and DCs (**B**) with PBMCs from subjects with allergic asthma and healthy subjects challenged with DM in the presence or absence of MSCs for 5 days. In the case of DCs, cells negative for CD64 were analyzed for CD86 and HLA-DR expression within the CD14⁺ and CD14⁻ subsets. The studies in Fig 4, A were repeated in 2% FCS. The cells were analyzed for Tregs (not shown) and the media were quantitated for cytokines (**C**). The results are shown for allergic asthma, representing 3 experiments of all subgroups. * $P > .05$.

and at day 5, the MSC-free PBMCs were studied in proliferation assays with DM. As expected, the unconditioned PBMCs responded to DM (Fig 5, open bars). Interestingly, the preconditioned PBMCs from allergic asthmatic subjects proliferated when studied alone (Fig 5, middle bar of right group), but this was significantly ($P < .05$) reduced when rechallenged with DM (Fig 5, left group, hatched bar). Similar treatment with the DM-sensitive nonasthmatic subjects did not show a similar refractory response to rechallenged DM (Fig 5, left group, hatched bars).

We next asked whether preconditioning requires direct contact between PBMCs and MSCs. This was addressed in Transwell cultures, with MSCs in the inner wells and PBMCs in the outer wells, in the presence or absence of optimal DM. After 5 days, the

PBMCs were studied in proliferation assays, as above. The PBMCs responded to DM with SIs of 6 ± 0.8 , indicating that preconditioning of PBMCs from allergic asthmatic subjects requires direct contact with MSCs.

Refractoriness of PBMCs by means of repeated challenge with DM antigen and MSCs

Refractoriness of the preconditioned PBMCs, together with a blunting effect on DC maturation (Figs 4, B, and 5), led us to ask whether we can induce refractoriness with repeated low doses of DM in the presence of MSCs. Four different subjects, each studied in 3 independent experiments, were rechallenged with optimal

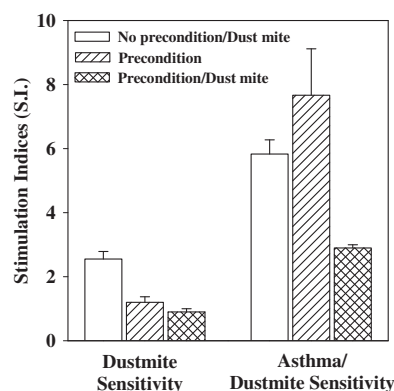


FIG 5. Nonresponsiveness to DM by preconditioned PBMCs from allergic asthmatic subjects. PBMCs from subjects with DM sensitivity ($n = 4$), asthma ($n = 4$), or both were preincubated with MSCs. At day 5, the MSC-free PBMCs were studied in DM-challenged mitogen assays. Each subject was studied in 3 independent experiments. The results are presented as SIs $\pm$ SDs ($n = 12$). * $P < .05$ versus preconditioned PBMCs without DM.

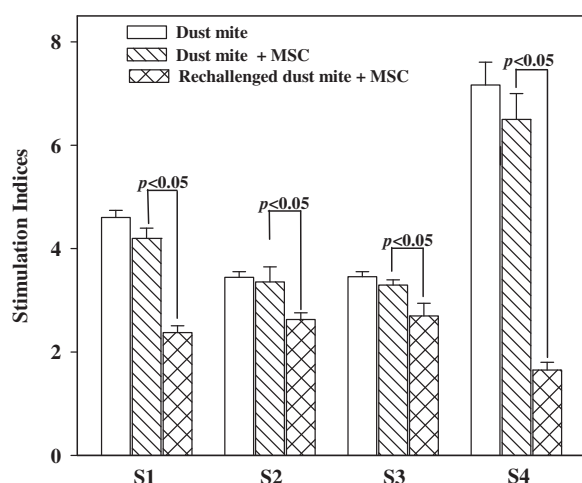


FIG 6. Refractoriness of PBMCs from allergic asthmatic subjects after repeated challenge with low-dose DM and MSCs. PBMCs were challenged with low-dose DM in the presence of MSCs, as outlined in the Methods section. Parallel studies were performed in which PBMCs were challenged once with optimal DM in the presence or absence of MSCs. Cells with repeated DM challenges were washed and then re-exposed to optimal DM in proliferation assay. The results represent studies with 4 subjects, each performed in 3 independent experiments.

DM levels. Indeed, the SIs of PBMCs were significantly decreased, indicating refractoriness of the repeatedly challenge cells, which is similar to what would be expected of anergy (Fig 6). The suppression could not be due to the FCS in the cultures because parallel cultures with DM alone were able to respond to DM (Fig 6, open bars).

DISCUSSION

The literature has reported on the dual immune function of MSCs: immune suppression and enhancement.^{23,26} Each type of response appears to occur during a narrow window, depending on the milieu.^{21,23} In this study we observed a veto-like response because MSCs showed preference by suppressing PBMCs from allergic asthmatic subjects that are expected to be hyperactivated

compared with those from subjects with only allergic responses, both monosensitized and polysensitized (Figs 2, C, and 3, B). The results are not antigen specific but rather allergen specific, as denoted by studies with tetanus toxoid (Fig 3, B). In the presence of MSCs, tetanus toxoid showed enhanced proliferation (Fig 3, B). We expect that this can be explained by the antigen-presenting properties of MSCs.²³

PBMCs from subjects with allergic asthma showed a significantly greater response to DM compared with similar challenges of PBMCs from DM-sensitive nonasthmatic subjects (Figs 2, C, and 3). This type of outcome mimics a veto-like response because it is expected that PBMCs from allergic asthmatic subjects would be more sensitive to stimulation with an allergen.²³ In contrast to the inhibitory effect of MSCs on the proliferation of PBMCs from allergic asthmatic subjects, MSCs could not suppress DM-activated PBMCs from nonasthmatic DM-sensitive subjects (Fig 2, C). These findings are consistent with other studies in which MSCs could not suppress tetanus toxoid-challenged PBMCs.²¹ In fact, the addition of MSCs to the PBMCs from nonasthmatic DM-sensitive subjects showed a trend toward increased proliferation of the PBMCs (Fig 2, right group, hatched bar). We explain this finding using other studies showing an antigen-presenting role of MSCs when IFN- γ levels are low.²³ It is possible that the relatively low proliferative response to DM prevented a high level of IFN- γ for antigen-presenting function.

The suppressive effect of MSCs on the proliferation of PBMCs from allergic asthmatic subjects recapitulates inflammation, which would cause decreases in cytokine production. These findings are relevant to the translation of MSCs for allergic asthma in light of the deleterious secondary effects of cytokines in allergic asthmatic subjects.²⁷

MSC-mediated immune suppression has been linked to increases in Treg cell numbers and related cytokines.^{2,28,29} In this study the level of Treg cells was unchanged with MSCs and DM (Fig 4, A). We correlated these findings with specific cytokines and found an increase in IL-10 levels and no change in IFN- γ levels in the DM-sensitive nonasthmatic group (Fig 4, C). In other studies Treg cell induction has been shown to reduce airway inflammation caused by DM.³⁰ Because these studies were done *in vivo*, the authors can examine the mice for long periods. It is possible that our 5-day model might be inadequate to induce Treg cells. It is difficult to retain the cultures for longer periods without a high rate of cell death. The studies by Leech et al³⁰ were done only with DM, which caused reduced responses in nonasthmatic subjects, indicating differences in the 2 models. We believe that the inability of MSCs to induce Treg cell numbers, despite their suppressive effect, is an advantage for translation in allergic asthma. If MSCs induce Treg cells, this could cause untoward effects, such as those linked to immune-suppressive drugs. Treg cells have been reported to suppress T-cell responses by absorbing the proinflammatory IL-2.³¹ Because this method could not occur in our model, the effect of MSCs is through other mechanisms. In our studies we have observed a blunt effect of MSCs in the maturation of DCs (Fig 4, B).

The mechanisms and consequences of reduced mature DCs in the presence of DM and MSCs require further exploration (Fig 4, B). Although the literature overwhelmingly showed evidence of MSC-mediated suppression through impediment of DC maturation,^{29,32-34} this model is different because the blunting effect is within the milieu of an allergen. MSCs can downregulate the expression of activating receptors on natural killer cells.³⁵ Although

it is unclear whether the natural killer effect is pertinent to our model, these cells will require further examination because activated T cells in allergic asthmatic subjects might be perceived as foreign to natural killer cells. In light of MSCs exerting a suppressive effect through mechanisms other than Treg cells, future studies should examine other roles of MSCs, such as cell-cycle arrest^{36,37} and production of nitric oxide and indoleamine 2,3-dioxygenase, which inactivate T cells.³⁸⁻⁴³

This study also provides a novel method through which tolerance to allergen could be achieved. It will be interesting to perform animal models to determine whether MSCs can provide long-lasting tolerance to allergens other than DM. We have shown refractoriness of PBMCs to rechallenged DM using 2 methods: preconditioning with MSCs and repeated exposure to low-dose DM and MSCs (Figs 5 and 6). Other studies have reported on killing of T cells by tolerogenic DCs,⁴⁴ which could be explored in future studies.

The varied responses of DM-challenged PBMCs to MSCs could not be explained by the subjects' body mass indices, although the answer to this question requires a larger cohort. This question is pertinent because adipose tissues are a rich source of MSCs.¹⁶ If PBMCs from obese patients are exposed to adipose-derived MSCs, this might influence the allergic response and perhaps asthmatic effects. In this study, grouping of the subjects' body mass indices (between 20 and 30 kg/m² and between 31 and 40 kg/m²) as a function of fold decrease in PBMC proliferation showed no conclusive outcome.

In summary, this pilot study showed a complex role for MSCs in patients with allergic disease. We recognize that further studies with more subjects are needed to confirm our findings and to clarify the questions raised by this initial study. The findings are significant to allergic asthma. Although we used DM as a model system, the findings can be extrapolated to other allergens. These studies add to the literature on stem cell therapy for allergic asthma.

Key messages

- MSCs, through intercellular contact with PBMCs from allergic asthmatic subjects, suppressed the response of PBMCs to DM challenge.
- The suppressive effects of MSCs correlated with an increase in mature DCs, a decrease in IFN- γ levels, and an increase in IL-10 levels.
- PBMCs gained refractoriness through preconditioning with MSCs and through repeated exposure to low-dose DM and MSCs, indicating that MSCs have the potential to reset the immune system for allergic asthma.

REFERENCES

1. Rasmussen I. Immune modulation by mesenchymal stem cells. *Exp Cell Res* 2006; 312:2169-79.
2. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 2008;8:726-36.
3. Prockop DJ, Kota DJ, Bazhanov N, Reger RL. Evolving paradigms for repair of tissues by adult stem/progenitor cells (MSCs). *J Cell Mol Med* 2010;14:2190-9.
4. Maitra B, Szekely E, Gjini K, Laughlin MJ, Dennis J, Haynesworth SE, et al. Human mesenchymal stem cells support unrelated donor hematopoietic stem cells and suppress T-cell activation. *Bone Marrow Transplant* 2004;33:597-604.
5. Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC. Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. *Transplantation* 2003;75:389-97.
6. Liang J, Gu F, Wang H, Hua B, Hou Y, Shi S, et al. Mesenchymal stem cell transplantation for diffuse alveolar hemorrhage in SLE. *Nat Rev Rheumatol* 2010;6: 486-9.
7. Sun L, Akiyama K, Zhang H, Yamaza T, Hou Y, Zhao S, et al. Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans. *Stem Cells* 2009;27:1421-32.
8. Gonzalez-Rey E, Gonzalez MA, Varela N, O'Valle F, Hernandez-Cortes P, Rico L, et al. Human adipose-derived mesenchymal stem cells reduce inflammatory and T cell responses and induce regulatory T cells in vitro in rheumatoid arthritis. *Ann Rheum Dis* 2010;69:241-8.
9. Zheng ZH, Li XY, Ding J, Jia JF, Zhu P. Allogeneic mesenchymal stem cell and mesenchymal stem cell-differentiated chondrocyte suppress the responses of type II collagen-reactive T cells in rheumatoid arthritis. *Rheumatology* 2008;47:22-30.
10. Zhang Q, Shi S, Liu Y, Uyanne J, Shi Y, Shi S, et al. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. *J Immunol* 2010;184:1656.
11. Garcia-Olmo D, Garcia-Arnan M, Herreros D, Pascual I, Peiro C, Rodriguez-Montes JA. A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. *Dis Colon Rectum* 2005;48:1416-23.
12. Parekkadan B, Tilles AW, Yarmush ML. Bone marrow-derived mesenchymal stem cells ameliorate autoimmune enteropathy independently of regulatory T cells. *Stem Cells* 2008;26:1913-9.
13. Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, et al. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol* 2002;30:42-8.
14. Ringden O, Uzunel M, Rasmussen I, Remberger M, Sundberg B, Lonnies H, et al. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation* 2006;81:1390-7.
15. Tyndall A, Pistoia V. Mesenchymal stem cells combat sepsis. *Nat Med* 2009;15:18-20.
16. Salem HK, Thiemermann C. Mesenchymal stromal cells: current understanding and clinical status. *Stem Cells* 2010;28:585-96.
17. Helmy KY, Patel SA, Silverio K, Pliner L, Rameshwar P. Stem cells and regenerative medicine: accomplishments to date and future promise. *Ther Deliv* 2010;1: 693-705.
18. Lun S, Wong C, Ko F, Hui D, Lam C. Expression and functional analysis of Toll-like receptors of peripheral blood cells in asthmatic patients: implication for immunopathological mechanism in asthma. *J Clin Immunol* 2009;29:330-42.
19. Park HK, Cho KS, Park HY, Shin DH, Kim YK, Jung JS, et al. Adipose-derived stromal cells inhibit allergic airway inflammation in mice. *Stem Cells Dev* 2010; 19:1811-8.
20. Kim HY, DeKruyff RH, Umetsu DT. The many paths to asthma: phenotype shaped by innate and adaptive immunity. *Nat Immunol* 2010;11:577-84.
21. Potian JA, Aviv H, Ponzio NM, Harrison JS, Rameshwar P. Veto-like activity of mesenchymal stem cells: functional discrimination between cellular responses to alloantigens and recall antigens. *J Immunol* 2003;171:3426-34.
22. Patel SA, Meyer JR, Greco SJ, Corcoran KE, Bryan M, Rameshwar P. Mesenchymal stem cells protect breast cancer cells through regulatory T cells: role of mesenchymal stem cell-derived TGF- β . *J Immunol* 2010;184:5885-94.
23. Chan JL, Tang KC, Bonilla LM, Pierobon N, Ponzio NM, et al. Antigen-presenting property of mesenchymal stem cells occurs during a narrow window at low levels of interferon- γ . *Blood* 2006;107:4817-24.
24. Di Ianni M, Del Papa B, De Ioanni M, Moretti L, Bonifacio E, Cecchini D, et al. Mesenchymal cells recruit and regulate T regulatory cells. *Exp Hematol* 2008;36: 309-18.
25. Spaggiari GM, Abdelrazik H, Becchetti F, Moretta L. MSCs inhibit monocyte-derived DC maturation and function by selectively interfering with the generation of immature DCs: central role of MSC-derived prostaglandin E2. *Blood* 2009;113: 6576-83.
26. Francois M, Romieu-Mourez R, Stock-Martineau S, Boivin MN, Bramson JL, Gailpeau J. Mesenchymal stromal cells cross-present soluble exogenous antigens as part of their antigen-presenting cell properties. *Blood* 2009;114:2632-8.
27. Bullens DM. Measuring T cell cytokines in allergic upper and lower airway inflammation: can we move to the clinic? *Inflamm Allergy Drug Targets* 2007;6:81-90.
28. Nouri-Aria KT, Durham SR. Regulatory T cells and allergic disease. *Inflamm Allergy Drug Targets* 2008;7:237-52.
29. Ramasamy R, Fazekasova H, Lam EW, Soeiro I, Lombardi G, Dazzi F. Mesenchymal stem cells inhibit dendritic cell differentiation and function by preventing entry into the cell cycle. *Transplantation* 2007;83:71-6.
30. Leech MD, Benson RA, deVries A, Fitch PM, Howie SEM. Resolution of Der p1-induced allergic airway inflammation is dependent on CD4+CD25+Foxp3+ regulatory cells. *J Immunol* 2007;179:7050-8.
31. Busse D, de la Rosa M, Hobiger K, Thurley K, Flossdorf M, Scheffold A, et al. Competing feedback loops shape IL-2 signaling between helper and regulatory T

- lymphocytes in cellular microenvironments. *Proc Natl Acad Sci U S A* 2010;107:3058-63.
32. Jiang XX, Zhang Y, Liu B, Zhang SX, Wu Y, Yu XD, et al. Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. *Blood* 2005;105:4120-6.
 33. Nauta AJ, Kruisselbrink AB, Lurvink E, Willemze R, Fibbe WE. Mesenchymal stem cells inhibit generation and function of both CD34+-derived and monocyte-derived dendritic cells. *J Immunol* 2006;177:2080-7.
 34. Li YP, Paczesny S, Lauret E, Poirault S, Bordigoni P, Mekhloufi F, et al. Human Mesenchymal stem cells license adult CD34+ hemopoietic progenitor cells to differentiate into regulatory dendritic cells through activation of the Notch pathway. *J Immunol* 2008;180:1598-608.
 35. Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. *Blood* 2006;107:1484-90.
 36. Glennie S, Soeiro Is, Dyson PJ, Lam EWF, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. *Blood* 2005;105:2821-7.
 37. Benvenuto F, Ferrari S, Gerdoni E, Gualandi F, Frassoni F, Pistoia V, et al. Human mesenchymal stem cells promote survival of T cells in a quiescent state. *Stem Cells* 2007;25:1753-60.
 38. Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* 2008;2:141-50.
 39. Munn DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A, Mellor AL. Inhibition of T cell proliferation by macrophage tryptophan catabolism. *J Exp Med* 1999;189:1363-72.
 40. Hwu P, Du MX, Lapointe R, Do M, Taylor MW, Young HA. Indoleamine 2,3-dioxygenase production by human dendritic cells results in the inhibition of T cell proliferation. *J Immunol* 2000;164:3596-9.
 41. Frumento G, Rotondo R, Tonetti M, Damonte G, Benatti U, Ferrara GB. Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. *J Exp Med* 2002;196:459-68.
 42. Krampera M, Cosmi L, Angeli R, Pasini A, Liotta F, Andreini A, et al. Role for Interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. *Stem Cells* 2006;24:386-98.
 43. Ryan JM, Barry F, Murphy JM, Mahon BP. Interferon-gamma does not break, but promotes the immunosuppressive capacity of adult human mesenchymal stem cells. *Clin Exp Immunol* 2007;149:353-63.
 44. Luckey U, Maurer M, Schmidt T, Lorenz N, Seebach B, Metz M, et al. T cell killing by tolerogenic dendritic cells protects mice from allergy. *J Clin Invest* 2011;121:3860-71.