

# Human umbilical cord derived mesenchymal stem cells promote interleukin-17 production from human peripheral blood mononuclear cells of healthy donors and systemic lupus erythematosus patients

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## Introduction

Mesenchymal stem cells (MSCs) are multi-potent stem cells that can be isolated from a number of different tissues, including bone marrow, peripheral blood [1], cord blood [2], trabecular bone [3], adipose tissue [4] and synovium [5]. Various studies have shown that MSCs have unique immunoregulatory properties and the ability to treat several kinds of autoimmune disease, including systemic lupus erythematosus (SLE). Its immunosuppression has been observed with regard to modulating the balance of T helper type 1 (Th1)/Th2 or regulatory T cells ( $T_{reg}$ )/Th17, which are the subsets of  $CD4^+$  T cells per functional categories. There have been some reports that differently derived MSCs can promote the subset productions of Th2 and  $T_{reg}$  in the balance of Th1/Th2 and  $T_{reg}$ /Th17, respectively [6–8]. Inconsistently, in 2009, bone marrow (BM)-MSCs were shown to promote the expansion of Th17 cells [9]. However, little is known about the immune properties of MSCs derived from human umbilical cords (hUC-MSCs).

## Summary

Inflammation instigated by interleukin (IL)-17-producing cells is central to the development and pathogenesis of several human autoimmune diseases and animal models of autoimmunity. The expansion of IL-17-producing cells from healthy donors is reportedly promoted by mesenchymal stem cells derived from fetal bone marrow. In the present study, human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) were examined for their effects on lymphocytes from healthy donors and from patients with systemic lupus erythematosus (SLE). Significantly higher levels of IL-17 were produced when  $CD4^+$  T cells from healthy donors were co-cultured with hUC-MSCs than those that were cultured alone. Blocking experiments identified that this effect might be mediated partially through prostaglandin  $E_2$  ( $PGE_2$ ) and IL-1 $\beta$ , without IL-23 involvement. We then co-cultured hUC-MSCs with human  $CD4^+$  T cells from systemic lupus erythematosus patients. *Ex-vivo* inductions of IL-17 by hUC-MSCs in stimulated lymphocytes were significantly higher in SLE patients than in healthy donors. This effect was not observed for IL-23. Taken together, our results represent that hUC-MSCs can promote the IL-17 production from  $CD4^+$  T cells in both healthy donor and SLE patients.  $PGE_2$  and IL-1 $\beta$  might also be partially involved in the promotive effect of hUC-MSCs.

**Keywords:** hUC-MSCs, IL-17, prostaglandin  $E_2$ , SLE

Interleukin (IL)-17 was described in 1995–96 as a proinflammatory cytokine that was produced by Th17 cells, which were distinct from Th1 or Th2 [10,11]. Accumulating evidence has since described the differentiation pathway of Th17 cells as using IL-23, transforming growth factor (TGF)- $\beta$ , IL-6, IL-1 $\beta$  and prostaglandin  $E_2$  ( $PGE_2$ ) [12–16]. With the discovery of Th17 cells, a better understanding has become available regarding the physiopathology of a number of chronic inflammatory diseases [17], such as spondyloarthritis, rheumatoid arthritis [18], asthma [19], multiple sclerosis [20], systemic lupus erythematosus (SLE) [21] and psoriasis [22]. In some studies, increased levels of IL-17 and IL-23 have been detected in the serum of SLE patients [23,24].

In the present study, we co-cultured hUC-MSCs with human  $CD4^+$  T cells from SLE patients or healthy donors. We have demonstrated for the first time, to our knowledge, that hUC-MSCs increased the production of IL-17. In addition to IL-1 $\beta$ ,  $PGE_2$  might partially promote the production of IL-17.

## Materials and methods

### *Ex-vivo* induction of cytokines

Human peripheral blood mononuclear cells (hPBMCs) were isolated by Ficoll-Paque (Axis-Shield, Dundee, UK) density gradient centrifugation (density  $1.077 \pm 0.002$  at 2200 rpm/min  $\times$  20 min) from the venous blood of healthy volunteers and SLE patients. A subpopulation of CD4<sup>+</sup> T cells was purified by using relevant magnetic MicroBead kits (Miltenyi Biotec, Bergisch Gladbach, Germany), according to the manufacturer's instructions. The purity of the isolated cells was more than 95%. hPBMCs ( $1 \times 10^5$ ) were then incubated with phytohaemagglutinin (PHA) (Sigma, St Louis, MO, USA), while CD4<sup>+</sup> T cells ( $1 \times 10^5$ ) were incubated with anti-CD3/CD28 Dynabeads (Invitrogen, Carlsbad, CA, USA) in the presence or absence of hUC-MSCs at 37°C in a 5% CO<sub>2</sub> atmosphere. In another series of experiments, we added indicating inhibitors (10  $\mu$ M indomethacin: Biosource, Rochdale, UK; 1  $\mu$ g/ml IL-1RA: R&D Systems, Minneapolis, MN, USA; 10  $\mu$ g/ml anti-IL-6 antibody: Biolegend, San Diego, CA, USA; anti-TGF- $\beta$  antibody (clone 2G7 in ascitic fluid at 1 : 20 dilution) was kindly provided by D. Fradelizi) to stimulated hUC-MSCs/CD4<sup>+</sup> T cells. After incubation, the cell-free supernatant of the *ex-vivo* culture was collected and kept frozen at  $-80^\circ\text{C}$  until assayed for cytokine concentrations by enzyme-linked immunosorbent assay (ELISA).

### Quantification of cytokines by ELISA

Concentrations of IL-23 and IL-17 in plasma and *ex-vivo* culture supernatant were measured by ELISA. PGE<sub>2</sub> was assayed using an ELISA kit from Cayman Chemicals. Interferon (IFN)- $\gamma$ , IL-4 and TGF- $\beta$  were obtained from Jingmei Biotech Co. Ltd (PR China).

### Flow cytometry

After 3 days of culture, CD4<sup>+</sup> T cells were harvested and restimulated for another 5 h with 25 ng/ml phorbol myristate acetate (PMA) and 1 mg/ml ionomycin in the presence of GolgiStop. Upon fixation and permeabilization with Cytotfix/Cytoperm (Becton Dickinson, San Jose, CA, USA), cells were labelled with anti-IFN- $\gamma$  fluorescein isothiocyanate (FITC) and anti-IL-17 phycoerythrin (PE) monoclonal antibodies (mAb). In another experiment the cells were labelled with anti-CD4 PE and anti-CD25 FITC mAb without additional stimulation to indicate T<sub>reg</sub> cells. All the antibodies were purchased from eBioscience (San Diego, CA, USA) and the flow cytometry analysis was performed using the Becton Dickinson fluorescence activated cell sorter (FACS) Calibur using CellQuest software (Becton Dickinson).

### Quantitative analysis of mRNA expression

Total RNA was extracted with TRIzol (Life Technologies, Carlsbad, CA, USA) and then used to synthesize cDNA using murine leukaemia virus reverse transcriptase (MLV RT) (Life Technologies), following the manufacturer's protocol. Polymerase chain reaction (PCR) cycling conditions were: predenature at 95°C for 10 min, denature at 95°C for 15 s and extension at 60°C for 1 min, followed by a final single peak-melting curve program. The ratio was calculated according to the formula: ratio =  $2^{-\text{ddCt}}$  (ddCt = mean Ct gene – mean Ct housekeeping). The primer sequences were as follows: hypoxanthine guanine phosphoribosyl transferase (HPRT) forward: 5'-TGACACTGGCAAAAACAATGCA-3' and reverse: 5'-GTCCTTTTCACCAGCAAGCT-3'; retinoic acid receptor-related orphan receptor C (RORC) forward: 5'-TTTTCCGAGGATGAGATTGC-3' and reverse: 5'-CTTTCCACATGCTGGCTACA-3'.

### SLE patients

Twelve SLE patients (ten females, two males) were recruited at the Beijing Hospital. Diagnosis of SLE was established according to the 1982 revised American Rheumatism Association criteria (ARA) [25]. Active lupus patients were identified according to the SLE Activity Index (SLEDAI) score [26] and informed consent, as specified by the Declaration of Helsinki, was obtained from all participants.

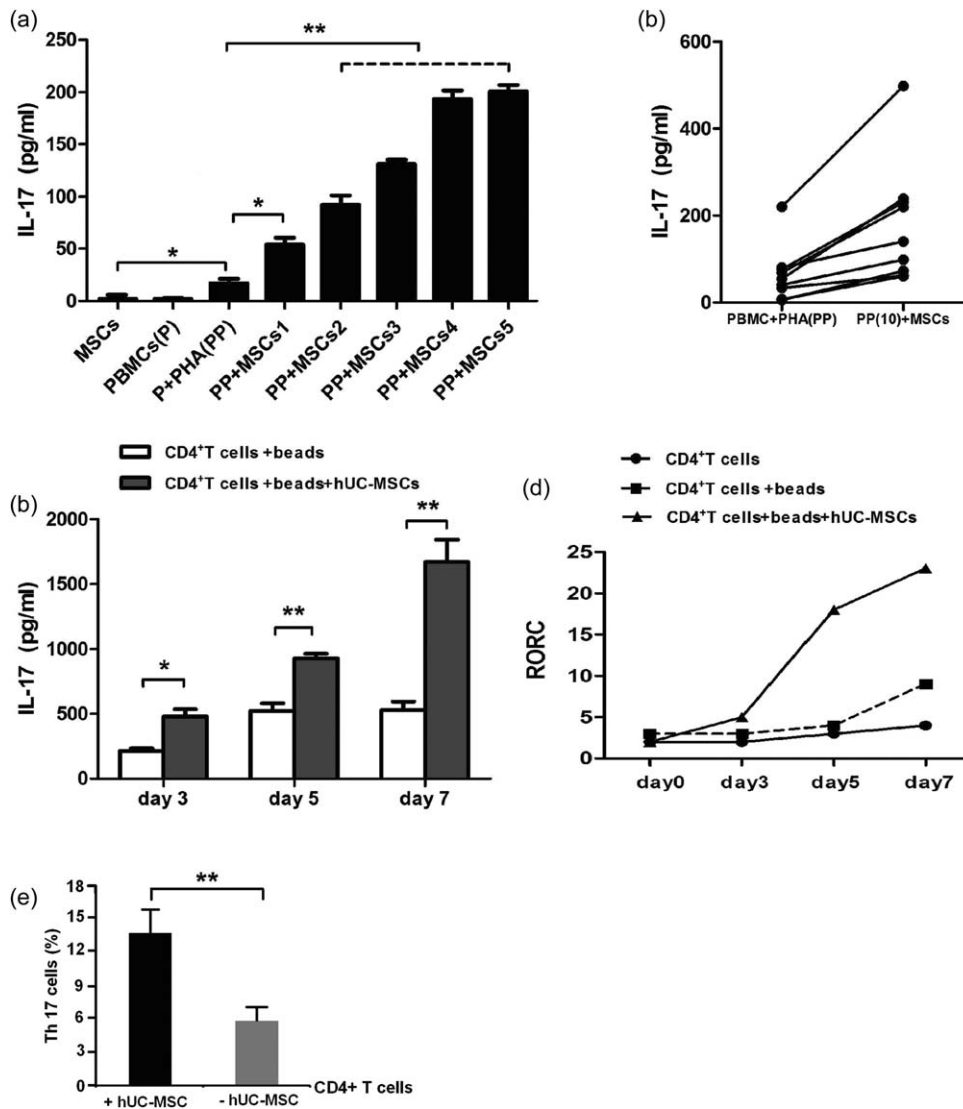
### Statistical analysis

The results were analysed using the GraphPad Prism software package version 4 (GraphPad Software Inc., San Diego, CA, USA). Data are presented as mean  $\pm$  standard error of the mean (s.e.m.). The two-tailed Student's *t*-test was used to determine the significance and  $P < 0.05$  was considered statistically significant.

## Results

### hUC-MSCs promote the production of IL-17 from hPBMCs/CD4<sup>+</sup> T cells of healthy donors

To study the effects of hUC-MSCs on the production of IL-17 from hPBMCs/CD4<sup>+</sup> T cells, hPBMCs were incubated with PHA and were then co-cultured with hUC-MSCs. Our findings demonstrated that the hUC-MSCs promoted the production of IL-17 from hPBMCs in a dose-dependent manner. The best ratio was 2 : 1 (hPBMCs : hUC-MSCs) in our co-culture system. IL-17 production was increased slightly when non-fractionated hPBMCs were activated by PHA ( $2.18 \pm 0.77$  versus  $17.47 \pm 3.81$ , hUC-MSCs only:  $2.43 \pm 0.88$ ;  $P < 0.05$ ), (Fig. 1a). Subsequent experiments therefore used hPBMCs or CD4<sup>+</sup> T cells co-cultured with hUC-MSCs at the ratio of 2 : 1 (hPBMCs or CD4<sup>+</sup> T cells :



**Fig. 1.** Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) promote interleukin (IL)-17 release by human peripheral blood mononuclear cells (hPBMCs)/CD4<sup>+</sup> T cells. (a) hPBMCs ( $1 \times 10^5$ ) were stimulated with 10  $\mu$ g/ml phytohaemagglutinin (PHA), with or without different numbers of hUC-MSCs. (MSCs1:  $5 \times 10^3$ ; MSCs2:  $1 \times 10^4$ ; MSCs3:  $2 \times 10^4$ ; MSCs4:  $5 \times 10^4$ ; MSCs5:  $1 \times 10^5$ ). The data are expressed as the mean  $\pm$  standard error of the mean (s.e.m.) from three different experiments, each performed in triplicate. \* $P < 0.05$ , \*\* $P < 0.01$ . (b) PHA-activated-hPBMCs from 10 donors were co-cultured with one source of hUC-MSCs from eight donors were co-cultured with one source of PHA-activated hPBMCs. IL-17 concentrations in each culture were measured by enzyme-linked immunosorbent assay (ELISA) and the data represent the mean of single experiments, each performed in triplicate. (c) IL-17 secretion is shown from CD4<sup>+</sup> T cells stimulated in the presence or absence of hUC-MSCs for different days. (d) Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis of RAR-related orphan receptor C (RORC) transcripts in anti-CD3/CD28 Dynabeads-stimulated CD4<sup>+</sup> T cells in the presence or absence of hUC-MSCs for different days. The data are expressed as the mean  $\pm$  s.e.m. from three different experiments, each performed in triplicate. \* $P < 0.05$ , \*\* $P < 0.01$ . (e) Flow cytometry analysis of percentages of T helper type 17 (Th17) cells in the presence or absence of hUC-MSCs for 3 days of culture. All data were excluded from the autofluorescence and were analysed based on four repeat experiments. \*\* $P < 0.01$ .

hUC-MSCs). The hUC-MSCs showed promotive effects on hPBMCs from different donors (Fig. 1b). Furthermore, these promotive effects appeared to be a consistent and stable feature of hUC-MSCs, because similar effects were observed with hUC-MSCs prepared from eight different donors and with different numbers of cell passages (data not shown).

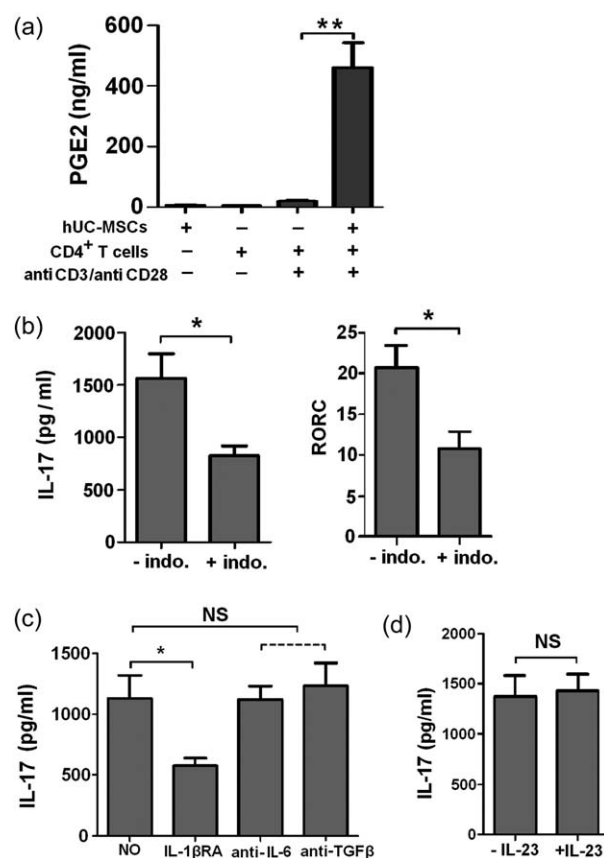
To assess whether hUC-MSCs acted directly on T cells or indirectly through accessory cells, we activated freshly sorted CD4<sup>+</sup> T cells by anti-CD3/CD28 Dynabeads at different culture times in the absence of accessory. IL-17 levels were almost tripled when CD4<sup>+</sup> T cells were co-cultured with hUC-MSCs for 7 days, while these levels doubled in other co-culture systems (Fig. 1c).

Recently, retinoid-related orphan receptor gamma t (ROR $\gamma$ t) has been reported as a crucial transcription factor that regulates IL-17 expression and Th17 differentiation in mouse and human [15]. We further evaluated mRNA levels for retinoic acid receptor-related orphan receptor C (RORC). As shown in Fig. 1d, RORC mRNA levels were slightly higher in isolated CD4<sup>+</sup> T cells in response to anti-CD3/CD28 Dynabeads than in an unstimulated culture at day 7 ( $2.10 \pm 0.72$  versus  $8.53 \pm 1.02$ ;  $P < 0.01$ ). However, it was increased significantly by the addition of hUC-MSCs at day 5 ( $7.77 \pm 0.79$  versus  $19.07 \pm 1.91$ ;  $P < 0.01$ ) or at day 7 ( $8.53 \pm 1.02$  versus  $22.10 \pm 1.86$ ;  $P < 0.01$ ).

We further used flow cytometry to analyse the percentages of Th17 cells when CD4<sup>+</sup> T cells were co-cultured with or without hUC-MSCs. After 3 days of culture, CD4<sup>+</sup> T cells were further restimulated for PMA and ionomycin, and were labelled with anti-IFN- $\gamma$  and anti-IL-17 fluorescence. Meanwhile, in another experiment, the cells were labelled with CD4CD25<sup>high</sup> without restimulation. We found that the percentage of Th17 cells in hUC-MSCs/CD4<sup>+</sup> T cells activated by anti-CD3/CD28 Dynabeads was significantly higher than that in activated-CD4<sup>+</sup> T cell cultures ( $13.67 \pm 3.79\%$  versus  $5.82 \pm 1.53\%$ ,  $P < 0.01$ ). The flow cytometry data suggest that hUC-MSCs promote the expansion of Th17 cells (Fig. 1e).

#### PGE<sub>2</sub> and IL-1 $\beta$ might promote the augmentation of Th17 cells mediated by hUC-MSCs

PGE<sub>2</sub> is an important cytokine involved in promotion of Th17 cell differentiation [27]. Cell co-culture experiments were performed to investigate effects of some important soluble factors on Th17 cell augmentation mediated by hUC-MSCs. As in Fig. 2a, very low levels of PGE<sub>2</sub> were produced by hUC-MSCs or CD4<sup>+</sup> T cells when they were cultured separately. In co-cultures of hUC-MSCs and CD4<sup>+</sup> T cells stimulated with anti-CD3/CD28 Dynabeads, PGE<sub>2</sub> was increased dramatically. In the presence of the COX inhibitor indomethacin (indo), the IL-17 production was decreased ( $1564.83 \pm 235.3$  versus  $829.0 \pm 91.43$ ;  $P < 0.05$ ), as was RORC expression ( $20.67 \pm 2.74$  versus  $10.47 \pm 2.01$ ;  $P < 0.05$ ) in CD4<sup>+</sup> T cells (Fig. 2b). When CD4<sup>+</sup> T cells stimulated with anti-CD3/CD28 Dynabeads were co-cultured with hUC-MSCs in the presence of anti-IL-6 antibody or anti-TGF- $\beta$  antibody, no effects were observed on IL-17 production. In contrast, IL-1 $\beta$ RA decreased the production of IL-17 (Fig. 2c). Furthermore, exogenous PGE<sub>2</sub> and IL-23 have been reported to synergize in inducing IL-17 [27,28], while we did not detect the difference of IL-23 in the supernatant of co-culture system (data not shown), and no significant difference ( $P > 0.05$ ) was found in IL-17 production between activated CD4<sup>+</sup> T cells/hUC-MSCs in the presence of IL-23 ( $1429 \pm 166.6$ ) and absence of IL-23 ( $1374 \pm 208.3$ ) (Fig. 2d). Thus, PGE<sub>2</sub> and IL-1 $\beta$  appeared to be involved in the expansion of

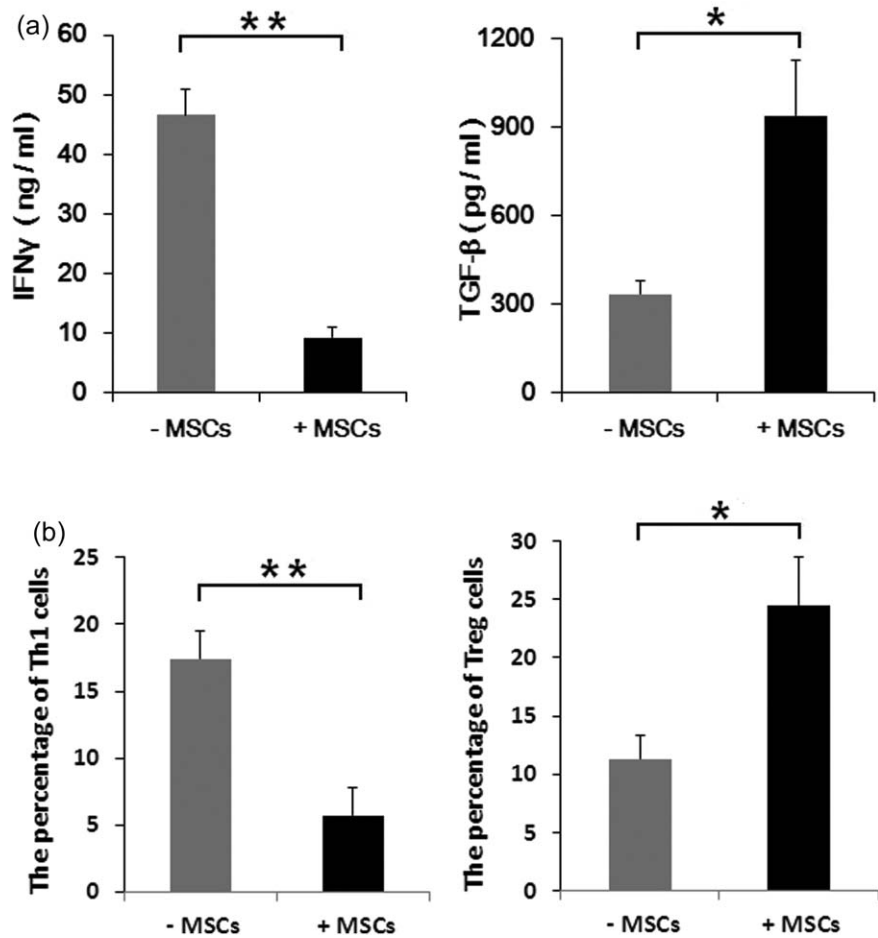


**Fig. 2.** Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) promote interleukin (IL)-17 release by CD4<sup>+</sup> T cells partly through prostaglandin E<sub>2</sub> (PGE<sub>2</sub>). (a) Expression levels of PGE<sub>2</sub> compared with CD4<sup>+</sup> T cells and CD4<sup>+</sup> T cells/hUC-MSCs. (b) Activated CD4<sup>+</sup> T cells were co-cultured with hUC-MSCs in the presence or absence of 10  $\mu$ M indomethacin (indo). Cytokines were measured in the supernatant and transcripts for RAR-related orphan receptor C (RORC) were determined by real-time polymerase chain reaction (PCR) in culture. (c) Activated CD4<sup>+</sup> T cells were co-cultured with hUC-MSCs in the presence or absence of indicating neutralizing antibodies. (d) Detection of IL-17 secretion in hUC-MSCs/CD4<sup>+</sup> co-cultures in the presence or absence of IL-23 by enzyme-linked immunosorbent assay (ELISA). All the data are expressed as the mean  $\pm$  standard error of the mean (s.e.m.) from three different experiments, each performed in triplicate. \* $P < 0.05$ ; \*\* $P < 0.01$ .

Th17 cells mediated by hUC-MSCs, but this response did not require IL-23.

#### hUC-MSCs might decrease the production of Th1 and increase the production of T<sub>reg</sub>

To investigate the effect of hUC-MSCs on the production of Th1 and T<sub>reg</sub>, ELISA was performed to detect the corresponding cytokines from the CD4<sup>+</sup> T cells/hUC-MSCs co-culture supernatant. We observed that addition of hUC-MSCs to cultures of CD4<sup>+</sup> T cells activated with anti-CD3/CD28 Dynabeads resulted in decreased IFN- $\gamma$  ( $46.63 \pm 4.27$  versus



**Fig. 3.** The different effect of human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) on CD4<sup>+</sup> T cells. CD4<sup>+</sup> T cells were stimulated with anti-CD3/CD28 beads in the presence or absence of hUC-MSCs. (a) Interferon (IFN)- $\gamma$  and transforming growth factor (TGF)- $\beta$  were measured in the supernatant. (b) Flow cytometry analysis of T helper type 1 (Th1) cells and regulatory T cells (T<sub>reg</sub>) in CD4<sup>+</sup> T cell cultures and hUC-MSC/CD4<sup>+</sup> co-cultures. All the data are expressed as the mean  $\pm$  standard error of the mean (s.e.m.) from three different experiments, each performed in triplicate. \* $P$  < 0.05; \*\* $P$  < 0.01.

$9.23 \pm 1.70$ ;  $P < 0.01$ ) and enhanced TGF- $\beta$  production ( $333.3 \pm 43.72$  versus  $938.3 \pm 187.5$ ;  $P < 0.05$ ) (Fig. 3a). Furthermore, we detected the percentages of Th1 and T<sub>reg</sub> separately using flow cytometry. These data were consistent with the data from ELISA analysis (Th1:  $17.40 \pm 2.07$  versus  $5.71 \pm 2.92\%$ ,  $P < 0.01$ ; T<sub>reg</sub>:  $11.35 \pm 2.05$  versus  $24.49 \pm 4.17\%$ ,  $P < 0.05$ ) (Fig. 3b).

#### hUC-MSCs promote IL-17 production but not IL-23 production in hPBMCs of SLE patients

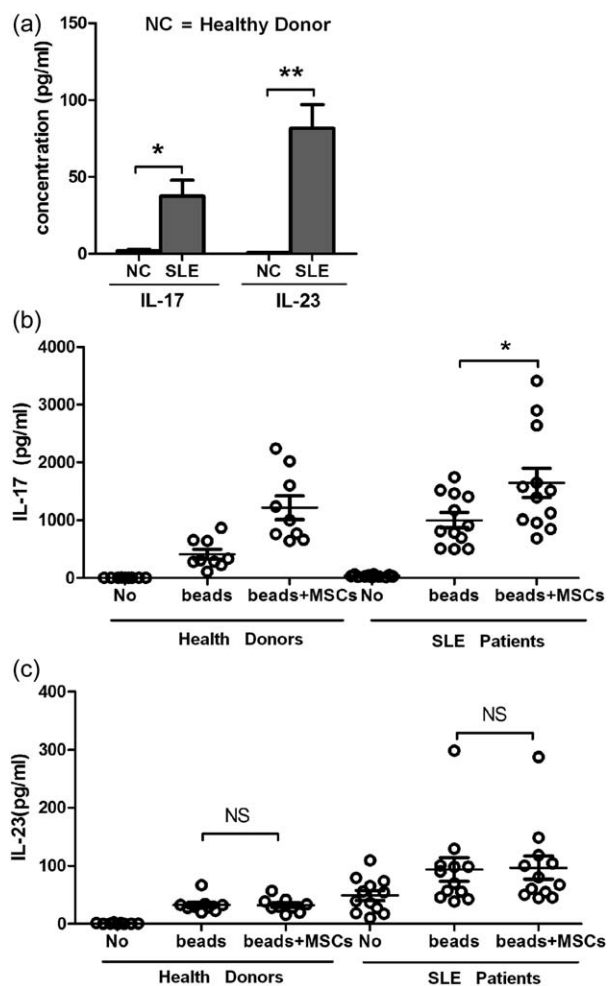
Plasma IL-23 and IL-17 concentrations were reported to be significantly higher in SLE patients than in healthy individuals. Using ELISA, we also detected concentrations of IL-23 and IL-17 from healthy donors and SLE patients. As shown in Fig. 4a, the level of IL-17 secretion in all SLE patients ( $37.4 \pm 18.1$ ) was higher than that of the control group ( $1.99 \pm 0.85$ ) ( $P < 0.05$ ). Plasma IL-23 levels were significantly different between patients and healthy donors ( $81.70 \pm 15.37$  versus  $0.56 \pm 0.11$ ;  $P < 0.01$ ). Furthermore, we investigated the *ex-vivo* production of IL-17 and IL-23 from CD4<sup>+</sup> T cells co-cultured with hUC-MSCs activated with anti-CD3/CD28 Dynabeads in the patient cohort. hUC-MSCs induced the release of IL-17 ( $P < 0.05$ ) (Fig.

4b) but not IL-23 ( $P > 0.05$ ) (Fig. 4c) in activated CD4<sup>+</sup> T cells in SLE patients than the healthy donors.

#### Discussion

For years, Th1 cells have been considered to be the major mediators of inflammation in most autoimmune syndromes. However, with the discovery of the Th17 subtype of CD4<sup>+</sup> T cells, it is now evident that Th17 cells are the primary drivers of these autoimmune disease responses [18]. In the case of lupus, many studies have examined the potential for a role for T cell-derived cytokines in its pathogenesis. IL-17 levels have been reported as significantly elevated in the serum of lupus patients compared with healthy donors [29], and have been implicated in at least some lupus mouse models [30].

More recently, fetal bone marrow-derived mesenchymal stem cells (FBM-MSCs) have been reported to promote the expansion of Th17 cells from CD4<sup>+</sup> T cells [9]. As a promising candidate for many potential clinical applications, hUC-MSCs appear to directly suppress T cell activation [31,32], but the effect of hUC-MSCs on the differentiation of human CD4<sup>+</sup> T cells is still unknown. In the current study, hUC-MSCs also promoted Th17 production from CD4<sup>+</sup> T cells.



**Fig. 4.** Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) promote interleukin (IL)-17 release by CD4<sup>+</sup> T cells from systemic lupus erythematosus (SLE) patients. (a) The concentration of IL-17 and IL-23 in the plasma of SLE patients and the NC (healthy donors) group. *Ex-vivo* production of IL-17 (b) and IL-23 (c) from anti-CD3/CD28-activated CD4<sup>+</sup> T cells of all SLE ( $n = 12$ ) and NC healthy donors ( $n = 9$ ) groups in the presence or absence of hUC-MSCs.

Because recent evidence indicates that transplantation of human MSCs can inhibit significantly autoimmune progression in Murphy Roths Large/lymphoproliferation (MRL/lpr) mice, it was of interest to determine if a similar effect would be observed on the function of Th17 cells from SLE patients.

Previous studies have reported elevated plasma levels of IL-17 in SLE patients [33]. In contrast, Kurawata *et al.* reported no elevation of IL-17 in the serum of a cohort of 24 Japanese lupus patients [34]. Our current results revealed a clearly higher level of IL-17 secretion in SLE patients than that in healthy donors. Our data also showed that hUC-MSCs promoted IL-17 production in activated CD4<sup>+</sup> T cells from SLE patients.

A recent study reported that IL-23 was essential for Th17 differentiation and expressing IL-17 in the process

mediated by BM-MSCs. IL-23 is also required for disease pathogenesis in many models of autoimmunity, and appears to play a role in the exacerbation of SLE symptoms [35]. Roles for IL-23 are thought to possibly involve a Th17 effector function as well as recruitment of Th17 to sites of inflammation. We observed no significant increases in *ex-vivo* production of IL-23 in activated CD4<sup>+</sup> T cells from either SLE patients or healthy donors cultured in the presence of hUC-MSCs. Our results suggested that the IL-23 signal might not be involved in the production of IL-17 mediated by hUC-MSCs.

*In-vitro* PGE<sub>2</sub> specifically increases the frequency of CD4<sup>+</sup> T cells producing IL-17 [28]. The proinflammatory effect of PGE<sub>2</sub> in experimental inflammatory bowel disease/collagen-induced arthritis in mice is also mediated through the IL-23/IL-17 axis [36,37]. In the current study, the level of PGE<sub>2</sub> was increased by approximately 30-fold when hUC-MSCs were co-cultured with CD4<sup>+</sup> T cells stimulated with anti-CD3/CD28 Dynabeads. The possibility therefore exists that hUC-MSCs induce IL-17 production and ROR $\gamma$ t expression in activated CD4<sup>+</sup> T cells through the secretion of PGE<sub>2</sub>. Moreover, in a reciprocal manner, the production of PGE<sub>2</sub> by MSCs was observed to be increased strongly by soluble factors produced by Th17 cells [38]. Guo and co-workers have suggested that FBM-MSCs might promote the expansion of Th17 cells partly through increasing expression of IL-6 and IL-1 [9]. The role of TGF- $\beta$  in the induction of IL-17 in human CD4<sup>+</sup> T cells is controversial [39,40]. Our blocking studies showed that IL-1 $\beta$ , but not IL-6/TGF- $\beta$ , was essential for the production of IL-17 mediated by hUC-MSCs. These controversial effects may due to the diverse types of MSCs and the complicated inflammatory environment *in vivo*.

General points are that the immunomodulatory capacity of MSCs such as inducing Th17 differentiating from naive cells and further secreting IL-17 is not intrinsic. These effects of MSCs on the inflammatory cytokine-production profile were dependent in part upon their secretion of soluble proinflammatory factors. Combined with the activity of TGF- $\beta$ , IL-1 $\beta$  and IL-21 have been reportedly involved in the MSC-mediated process of Th17 differentiation and IL-17 production. These cytokines modulated IL-17 production by inducing the expression of a key lineage-specific transcription factor, ROR $\gamma$ t [39,41].

Naive CD4<sup>+</sup> T cells are believed to differentiate into Th17 cells, but also two other possible lineages, Th1 and T<sub>reg</sub>. In our study, hUC-MSCs suppressed significantly the expansion of Th1 cells and promoted the expansion of T<sub>reg</sub> cells, which is consistent with a previous report [6].

MSC effects might be highly dependent upon the timing of MSC administration [42]. We assumed that, during the early phase, hUC-MSCs promoted Th17 cell production, followed by conversion of the Th17 into T<sub>reg</sub> cells. However, the extent of how many Th17 could be converted into T<sub>reg</sub> cells might be impacted by the co-culture period time, such

as the long-term MSC survival with T cells *in vivo*, or some additional soluble factors from the complicated *in vivo* environment, or some additives used, such as mycophenolate mofetil [43]. As discussed above, the key to success of using MSCs might be the timing of the MSC administration and the additives used in combination with MSCs.

Various studies have demonstrated that MSCs have the ability to mediate immunomodulatory function *in vitro* and *in vivo*, with beneficial effects observed in animal models and clinical studies in disease such as graft-*versus*-host disease (GVHD), autoimmune diseases, bone, cartilage and cardiovascular diseases [44,45]. Some other studies have also reported that MSCs alone accelerated allograft rejection [43,46], which prompts us to explore further how to optimize the use of MSCs.

In conclusion, this study reports for the first time that hUC-MSCs can promote the production of IL-17 from hPBMCs of SLE patients. This finding provides some controversial effects of BM-MSCs reported recently, and gives a new insight into the potential immunomodulatory function of MSC-based clinical studies. This is the first report, to our knowledge, demonstrating that PGE<sub>2</sub> is also critical for IL-17 production by hUC-MSCs. Given that serum abundance of IL-17 has been correlated with SLE disease activity, clinical treatments using hUC-MSCs in SLE may require more careful research.

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## Disclosure

None declared.

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