TRANSLATIONAL SCIENCE

Combination of human umbilical cord mesenchymal stem (stromal) cell transplantation with IFN-γ treatment synergistically improves the clinical outcomes of patients with rheumatoid arthritis

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ABSTRACT

Objectives To clarify the key role of circulating interferon-γ (IFN-γ) and to improve the clinical efficacy of mesenchymal stem cell (MSC) transplantation (MSCT) in patients with rheumatoid arthritis (RA).

Methods Study of wild-type or IFN-γR$^{-/-}$ MSCT was first evaluated in a murine model of collagen-induced arthritis (CIA) following which a phase 1/2 randomised controlled study was conducted in 63 patients with RA who responded poorly to regular clinical treatments. Subjects were randomly assigned to an MSCT monotherapy group (n=32) or an MSCT plus recombinant human IFN-γ treatment group (n=31), with 1 year of follow-up. The primary end points consisted of efficacy as assessed as good or moderate EULAR response rates and the proportion of patients at 3 months attaining American College of Rheumatology 20 (ACR20) response rates.

Results In the murine studies, wild-type MSCT significantly improved the clinical severity of CIA, while IFN-γR$^{-/-}$ MSCT aggravated synovitis, and joint and cartilage damage. Transitioning from the murine to the clinical study, the 3-month follow-up results showed that the efficacy and ACR20 response rates were attained in 53.3% patients with MSCT monotherapy and in 93.3% patients with MSCT combined with IFN-γ treatment ($p<0.05$). No new or unexpected safety issues were encountered in 1-year follow-up for either treatment group.

Conclusions The results of this study show that IFN-γ is a key factor in determining the efficacy of MSCT in the treatment of RA, and that an MSC plus IFN-γ combination therapeutic strategy can greatly improve the clinical efficacy of MSC-based therapy in RA patients.

Key messages

What is already known about this subject?

- Despite the success of biological agents in rheumatoid arthritis (RA), there is still a significant number of patients who do not respond to these drugs, showing the need of new therapies.
- The extraordinary immunomodulatory properties of mesenchymal stem cell (MSC) transplantation (MSCT) have provided a promising treatment strategy for autoimmune diseases but their clinical efficacy remains unpredictable.

What does this study add?

- Absence of interferon-γ (IFN-γ) stimulating by specific deletion of IFN-γR on MSC invalidate the efficacy of MSCT or even aggravate collagen-induced arthritis.
- A phase 1/2 randomised controlled trial (RCT) study demonstrated that MSCT+IFN-γ combination therapy significantly increased the efficacy (good or moderate EULAR response) and American College of Rheumatology 20 response rates (93.3%) at third month of follow-up.

How might this impact on clinical practice or future developments?

- MSCT+IFN-γ combination therapy may provide a promising therapeutic strategy in patients with RA refractory to either conventional synthetic disease-modifying antirheumatic drugs (DMARDs) and/or biological DMARDs (DMARDs).
- However, limited by the small sample size, additional research including a multicentre RCT is needed.

INTRODUCTION

Rheumatoid arthritis (RA) is the most common inflammatory rheumatic disease characterised by progressive synovitis, destructive arthropathy and systemic complications.[1] Globally, the age-standardised reported prevalence and annual incidence rates of RA in 2017 were 246.6 and 14.9 per 100 000 individuals, respectively.[2] In keeping with the known critical pathogenetic role that the inflammatrory response plays in the occurrence and development of RA,[3 4] conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), biological DMARDs (bDMARDs) including anti-CD20, antitumour necrosis factor α (TNF-α) and anti-interleukin-6 (IL-6) monoclonal antibody and targeted synthetic DMARDs such as janus kinase inhibitors have achieved significant clinical efficacy in patients with RA[5–8] while only a

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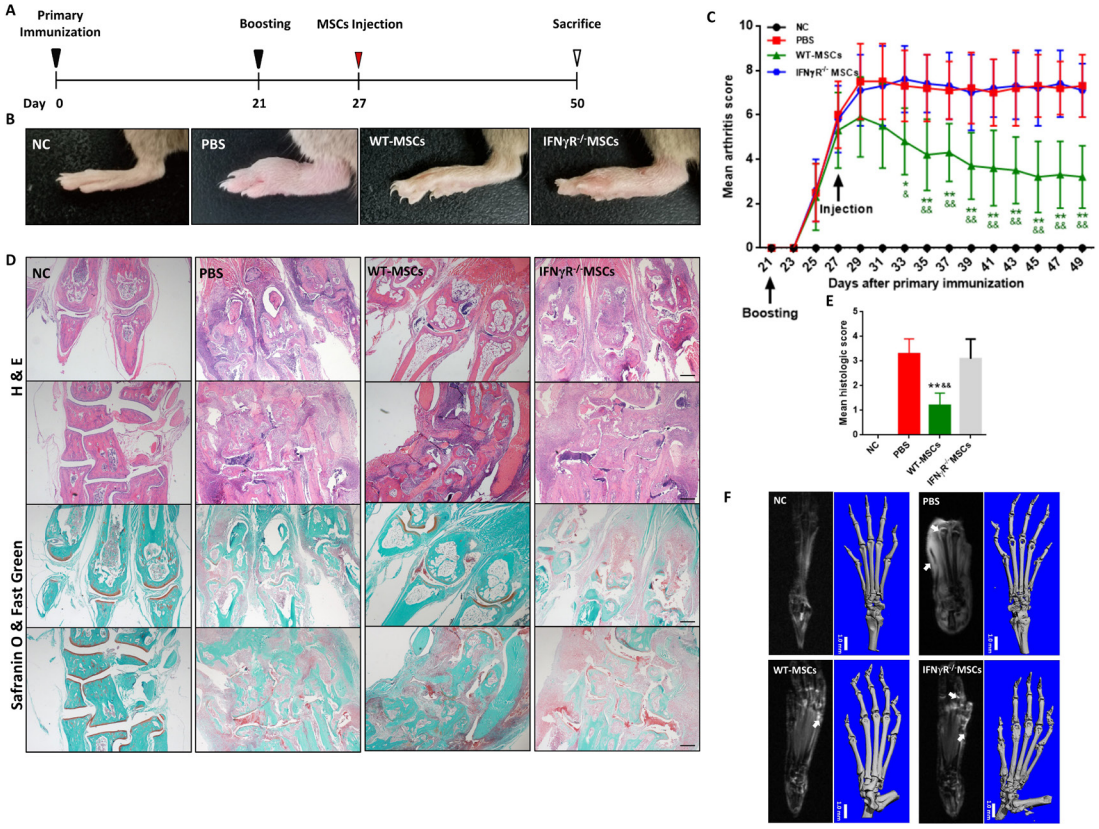

Figure 1 Intravenous injection of IFN-γR$^{-/-}$ MSCs has no therapeutic effect on CIA. (A) The schematic illustrates the protocol for CIA induction and MscS treatment. (B) Photographs of representative gross lesions in the hind limbs of CIA mice for clinical assessment are shown. (C) Clinical severity was continuously monitored, and the arthritis score was calculated until sacrifice. The mice were analysed by three independent, blinded examiners every other day and evaluated according to a scoring system for subjective evaluation of arthritis severity: 1, erythema and mild swelling confined to the tarsals or ankle joint; 2, erythema and mild swelling extending from the ankle to the tarsals; 3, erythema and moderate swelling extending from the ankle to the metatarsal joints; and 4, erythema and severe swelling encompassing the ankle, foot and digits or ankylosis of the limb. Each limb was scored, and the scores were added together, with a maximum possible score of 12 points per animal. *p<0.05, **p<0.01 versus the PBS group (two-way ANOVA for comparisons at each time point). (D and E) All mice were sacrificed on D 50 for histopathological evaluation according to the following scale: 0, normal synovial tissue; 1, synovial hypertrophy and cell infiltrates; 2, pannus and cartilage erosion; 3, major erosion of the cartilage and subchondral bone; and 4, loss of joint integrity and ankylosis. The assessment was performed by two examiners, and the average score was used as the final value. Paraffin-embedded sections of both the interphalangeal and metacarpophalangeal joints were stained with H&E, safranin O & fast green. Representative microscopic images of both joints are shown (D); histopathological integrity was calculated based on these images (E), scale bar=200 µm. *p<0.05, **p<0.01 vs PBS; $^{\&}$p<0.05, $^{\&\&}$p<0.01 versus the WT-MSCs (two-way ANOVA for the comparison of each time point). (F) Before sacrifice, the hind limbs of CIA mice were imaged with small animal MRI and CT for radiological evaluation according to the method reported previously on the following scale: 0, no bone damage; 1, tissue swelling and oedema; 2, joint erosion and 3, bone erosion and osteophyte formation. Representative images are shown with arrows indicating tissue inflammation and joint destruction. At least five mice per group were used. NC=negative control (black; n=5 mice), PBS=positive control CIA mouse PBS treatment group (red; n=10 mice), WT-MSCs=CIA mouse WT-MSCs treatment group (green; n=10 mice), IFN-γR$^{-/-}$ MSCs=CIA mouse IFN-γR$^{-/-}$ MSC treatment group (blue; n=10 mice). All results are shown as the mean±SD.

minority of patients can achieve disease remission and persistent remission off therapy.[9–12] However, targeting single proinflammatory pathways may not be sufficient, as suggested by variable results obtained with TNF-α blockade therapy.[13 14] Therefore, there is an urgent need to develop safer and more effective treatment strategies for refractory RA patients.

The ideal ultimate goal in RA would be to restore immune tolerance, thereby allowing the complete discontinuation of immunosuppressant therapy. Thus, treatment with mesenchymal stem cells (MSCs) has attracted widespread attention due to their strong immunosuppressive and anti-inflammatory effects.[15–17] Current therapeutic clinical trials have shown that MSC-mediated immunosuppression is independent of major histocompatibility complex, non-antigen-specific mechanisms,[15] but rely almost entirely on responses carried out by immune cell populations that inhibit cell cycle progression.[18 19] It is currently believed that the immunomodulatory capacity of MSCs not only depends on soluble factors secreted by the MSCs,[20 21] but also involve immunoregulatory mechanisms carried out by various types of innate and adaptive immune cells.[22–25] Since MSCs display such strong systemic immunomodulatory effects through multiple mechanisms, MSC-based cell therapy is currently considered a promising treatment strategy for the treatment of autoimmune diseases, such as graft-versus-host disease (GvHD), inflammatory bowel disease, multiple sclerosis and atopic dermatitis.[17 26–30]

However, the current clinical efficacy of MSC transplantation (MSCT) still displays huge individual differences even in the same autoimmune disease.[31–36] The immunosuppressive function of MSCs is known to be activated by interferon-γ (IFN-γ) and TNF-α and that this effect can be further amplified by cytokines such as

Table 1 Baseline clinical and demographic characteristics of the patients

Variable	MSCs (n=30)*	MSCs+IFN-γ (n=30)*
Female no (%)	26 (86.7)	25 (83.3)
Mean age (year)	47.8	48.3
Mean duration of disease (year)	3.93±2.7	4.21±2.5
DAS28-ESR	5.70±0.62	5.74±0.49
HAQ-DI	1.64±0.22	1.61±0.20
Erythrocyte sedimentation rate (mm/hr)	47.69±8.58	46.24±7.70
C reactive protein level (mg/L)	23.04±5.97	23.78±6.12
Positive for rheumatoid factor (%)	97.0	96.9
Positive for anti-CCP antibodies (%)	93.9	93.8
Medication history no (%)		
DMARDs	30(100)	30 (100)
Biologics	15 (50)	12 (40)
NSAIDs	30(100)	30 (100)
Prednisone acetate	25(83)	27 (90)

*Only data from patients who completed 48 weeks of follow-up were included here.
CCP, cyclic citrullinated peptide; DAS28-ESR, the Disease Activity Score for 28-joint counts based on the erythrocyte sedimentation rate; DMARDs, disease-modifying anti-rheumatic drugs; HAQ-DI, the Health Assessment Questionnaire-Disability Index; IFN-γ, interferon-γ ; MSC, mesenchymal stem cells; NSAIDs, nonsteroidal anti-inflammatory drugs.

IL-17.[37 38] Considering the substantial individual variations in the cytokine-mediated immunological microenvironment at different stages of disease development in patients with RA, the individual differences in clinical efficacy of MSCT can be explained. Actually, our previous study demonstrated that patients with RA with high levels of circulating IFN-γ had improved therapeutic efficacy after MSCT.[31] In the present study, we demonstrate that IFN-γ-mediated immunological microenvironment is a key factor in determining the efficacy of MSCT in the RA mouse model, and that an MSCT plus IFN-γ combination therapy strategy greatly improves efficacy in patients with RA in the clinical study.

METHODS

Induction and assessment of collagen-induced arthritis

The collagen-induced arthritis (CIA) mouse model was used according to a previous protocol.[39] To evaluate therapeutic efficacy, treatment was started after the onset of disease when the arthritis score reached three or more (approximately 27 days after the primary immunisation). Mice with established CIA received an intravenous injection of 1×10^6 wild-type mBM-MSCs (WT-MSC group), 1×10^6 *Ifngr1*^{tm1Agt}/J mBM-MSCs (IFN-γR$^{-/-}$ MSC group) or PBS (PBS group) (figure 1A). Animals were sacrificed on day 50, and the blood and limb joints were taken for flow analysis, cytokine detection, radiological evaluation (CT and MRI) and histopathological examination (H&E and safranin O & fast green). Detailed methods were listed in online supplementary materials.

Patient eligibility

All patients met the American College of Rheumatology 1987 (ACR1987) criteria for RA classification and had no other autoimmune or systemic diseases.[40] The study was registered at Chictr.org (identifier: ChiCTR-INR-17012462).Written informed consent was obtained from each subject in accordance with the Declaration of Helsinki. The enrolled RA patients responded poorly to regular clinical treatments, including csDMARDs, non-steroidal anti-inflammatory drugs (NSAIDs), biologics and steroids, or could not tolerate the serious side effects of these drugs; thus, the patients maintained an active disease state (table 1). csDMARDs treatment remained unchanged before patients were enrolled in the study, but the patients who used bDMARDs were required to have discontinued drug use for more than 6 months. Corticosteroids therapy was discontinued in all patients within 3 days before and after MSCT. A total of 63 patients with RA were enrolled in the study from August 2017 to September 2018.

Treatment protocol

Since recombinant human IFN-γ monotherapy is known to be safe but ineffective in treating RA,[41 42] subjects were randomly divided into human umbilical cord MSCs (hUC-MSCs) combined with recombinant human IFN-γ (MSCT +IFN-γ) and hUC-MSCs monotherapy (MSCT) treatment groups. The source and preparation of hUC-MSCs were established previously and there was a single hUC-MSC donor for all participating subjects.[31] Subjects received 1×10^6 cells/kg of body weight in 50 mL of 1% albumin in physiological saline via intravenous infusion with/without a single intramuscular infusion of 1 million IU of IFN-γ. If the status of a subject continued to improve, a withdrawal schedule was used to taper off the conventional drug treatment regimen in the following order: prednisone acetate, NSAIDs then DMARDs. All treatment modifications were approved by the rheumatologist in charge.

Assessment of disease status

All subjects returned for scheduled follow-up visits at 1, 2, 3, 4, 8, 12, 24 and 48 weeks. At these time points, the adverse events, general physical status, serological indicators and regulatory T cell (Treg) to T helper 17 (Th17) cell ratios of the patients were examined and recorded. The primary efficacy end points were the efficacy (good or moderate EULAR response) rate[43] and the proportion of patients with an ACR20 response at month 3. Secondary efficacy end points included the Disease Activity Score for 28-joint counts based on the erythrocyte sedimentation rate (DAS28-ESR) and Health Assessment Questionnaire-Disability Index (HAQ-DI) scores. Other key secondary end points included ACR20, ACR50 and ACR70 responses (at time points including month 1, 3 and 12).

Statistical analysis

Statistical analyses were conducted using a t-test for parametric data and the Mann-Whitney U test for non-parametric data. One-way analysis of variance (ANOVA) followed by the Bonferroni test or two-way ANOVA was used when there were more than two groups. The normal-approximation test for the difference in binomial proportions was used to determine the superiority of MSCT plus IFN-γ over MSCT monotherapy with respect to two of the primary end points: the efficacy rate and the percentage of patients with an ACR20 response. Changes from the baseline scores for the DAS28-ESR and HAQ-DI and other continuous end points were analysed with the use of a mixed-effect longitudinal model. All statistical tests were two sided, and the significance level was set at $p<0.05$. All analyses were conducted with SPSS V.17.0 (SPSS). The data are shown as the mean±SD.

RESULTS

Intravenous infusion of IFN-γR$^{-/-}$ MSCs has no therapeutic effect on CIA

In addition to its immunological, histological and clinical similarities with RA, the CIA model is also characterised by high levels of endogenous IFN-γ.[44] Therefore, to verify the hypothesis that the clinical efficacy of MSCT in the treatment of RA depends on the

IFN-γ, we used IFN-γR$^{-/-}$ MSCs for treatment in the CIA model mice. Compared with an intravenous injection of WT-MSCs, treatment with IFN-γR$^{-/-}$ MSCs not only did not significantly ameliorate the clinical severity of CIA in mice, but also led to the development of severe arthritis symptoms (figure 1B,C). Furthermore, compared with the WT-MSCT group, the IFN-γR$^{-/-}$ MSCT group did not show a decrease in the IFN-γ levels in the serum of CIA mice (online supplementary figure S2). Histological evaluation revealed aggravated synovitis and articular and cartilage destruction in IFN-γR$^{-/-}$ MSC-treated CIA mice compared with WT-MSC-treated mice (figure 1D,E). In addition, IFN-γR$^{-/-}$ MSC-treated CIA mice exhibited a significantly greater degree of tissue inflammation and joint destruction as assessed by radiological evaluation (figure 1F). Finally, mice treated with WT-MSCs did not show any side effects or death before the end of the experiment. Collectively, these findings demonstrate that the systemic administration of WT-MSCs can exert a significant therapeutic effect on CIA without any significant side effects, while treatment with IFN-γR$^{-/-}$ MSCs had almost no therapeutic effect on CIA suggesting that the therapeutic efficacy of MSCT on RA is dependent on the IFN-γ in vivo.

Immunosuppressive activity by MSCs is induced by IFN-γ in vitro

To further confirm that the therapeutic efficacy of MSCT on RA is dependent on the IFN-γ in vitro, we analysed the immunosuppressive capacities of IFN-γR$^{-/-}$ MSCs or IFN-γ-primed MSCs on T cell proliferation and function. Our results demonstrate that both mouse and human MSCs efficiently inhibit T cell proliferation. However, IFN-γR$^{-/-}$ MSCs had no such suppressive effect on T cell proliferation (figure 2A,B). Intracellular cytokine staining assays demonstrated that only IFN-γ-primed MSCs, not the non-primed MSCs, significantly inhibited the production of IFN-γ by T cells (figure 2C,D). These results demonstrate that IFN-γ is a key factor in determining the immunosuppressive capacity of MSCs in vitro.

Clinical outcomes

On the basis of these findings, we inferred that the clinical efficacy of MSCT in RA depends on IFN-γ and that combination with IFN-γ treatment may synergistically enhances the clinical efficacy of MSCT on RA. A cohort of 63 enrolled RA patients were randomised into either the MSCT+IFN-γ group or the MSCT group (online supplementary figure S1). No severe acute adverse events occurred during or after MSCT or MSCT+IFN-γ treatment. Eight patients developed chills or fever (≤39°C) after treatment but recovered within 2–3 hours without any further intervention. No patient developed GvHD, and no serious infections occurred (online supplementary table S1). Notably, although most patients had a transient decrease in the absolute number of lymphocytes within 1 month after MSCT or MSCT+IFN-γ treatment, these returned to normal by the eighth week or 12th week on follow-up examination (online supplementary table S2). In addition, no significant abnormalities were found in routine blood tests, liver and kidney function analysis, chest radiography, urine analysis, or electrocardiography.

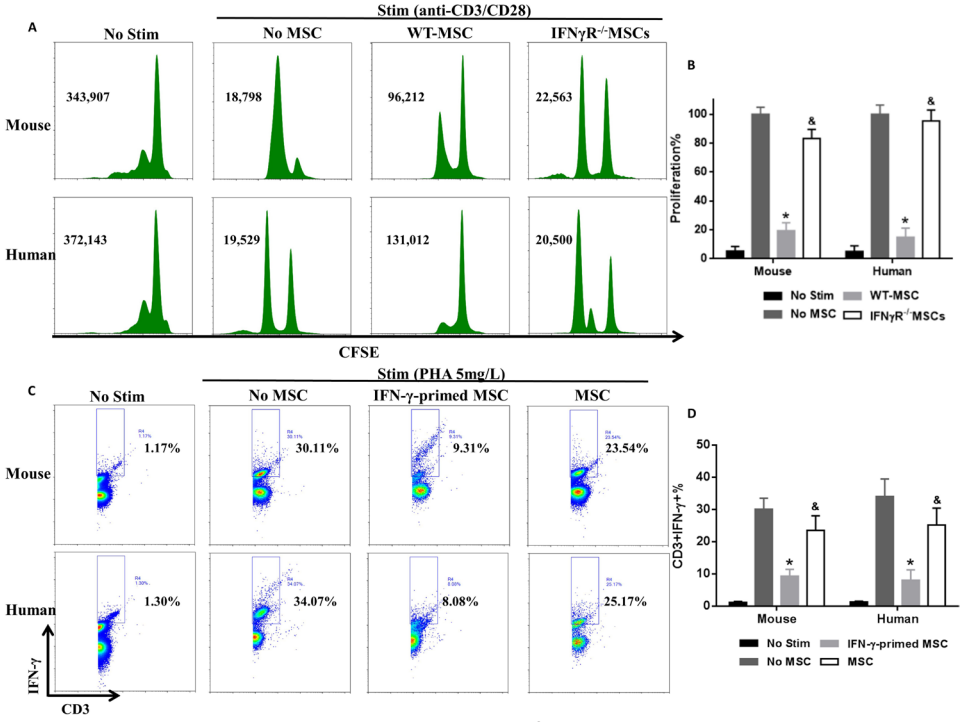

Figure 2 Immunosuppression by MSCs is induced by IFN-γ in vitro. Cells (1×10^6 cells /mL) were activated by plastic-bound anti-CD3 and soluble anti-CD28 antibodies for 48 hours and then cultured with IL-2 (200 U/mL) alone for 48 hours. (A, B) Peripheralblood mononuclear cells (PBMCs) were cocultured in the presence or absence of either MSCs or IFN-γR$^{-/-}$ MSCs and were stimulated with anti-CD3/CD28. For the carboxyfluoresceinsuccinimidyl ester (CFSE) proliferation assay, cells were incubated for three dimensions in the absence of brefeldin A and subjected to anti-CD3 antibody staining. *P<0.05 vs the no MSC group; &p<0.05 vs the MSC group by one-way ANOVA. (C, D) PBMCs were cocultured in the presence or absence of either MSCs or IFN-γ-primed MSCs and stimulated with phytohemagglutinin (PHA). for the intracellular IFN-γ cytokine staining assay, cells were incubated with brefeldin A for 12–14 hours and subsequently stained with antibodies specific for CD3 and IFN-γ for flow cytometry analysis. *P<0.05 versus the no MSC group; & p<0.05 versus the IFN-γ MSC group by one-way ANOVA. ANOVA, analysis of variance; IFN-γ, interferon-γ; MSCs, mesenchymal stem cells; WT, wild-type.

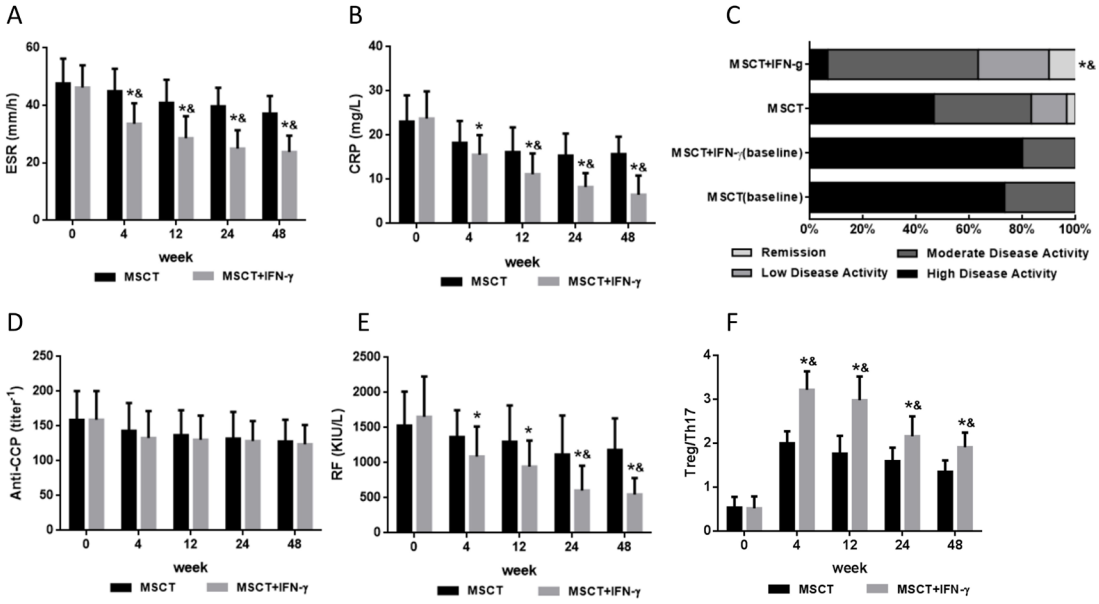

Figure 3 Assessment of disease activity. The ESR, CRP, anti-CCP and RF levels and the ratio of CD4 +Foxp3+Tregs to CD4 +IL-17A+Th17 cells in the CD4 +T cell population were measured before and after treatment for each group. (A) Serum ESR level; (B) serum CRP level; (C) cluster assessment of disease activity in the MSCT+IFN-γ and MSCT groups before and after 12 weeks of treatment; (D) serum anti-CCP level; (E) serum rf level; (F) ratio of CD4 +CD25+Foxp3+Tregs to CD4 +IL-17A+Th17 cells in the CD4 +T cell population. *P<0.05 versus the MSCT+IFN-γ-responsive group before treatment; &P<0.05 versus the MSCT group by one-way ANOVA; the error bars indicate the SD. CCP, cyclic citrullinated peptide; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; IFN-γ, interferon-γ; IL-17, interleukin-17; RF, rheumatoid factor; Th17, T helper 17.

Taken together with the previous study, MSCT monotherapy was effective in only some of the patients with RA, with an efficacy rate of 53.3% (16/30). Surprisingly, most of the patients in the MSCT+IFN-γ treatment group had a rapid improvement in clinical symptoms, with an efficacy rate of 93.3% (28/30). Meanwhile, the mean (±SD) proportion of patients who had an ACR20 response at month 3 was 40.0%±49.0% (12 of 30 patients) in the MSCT group and 93.3%±24.9% (28 of 30 patients) in the MSCT+IFN-γ group (p<0.05) (table 2). Additional data, including those for ACR20, ACR50 and ACR70, are provided in table 2 and online supplementary table S2. Compared with MSCT monotherapy, MSCT+IFN-γ treatment produced a rapid improvement in disease activity, which was reflected not only by the C reactive protein (CRP) and ESR levels (figure 3A,B) but also by a decrease of HAQ-DI and DAS28-ESR scores (table 2). In addition, the general condition of patients, including mental state, physical strength, diet and sleep activity, had also improved significantly were maintained for 12 months after single treatment (table 2). However, excluding infection and other disease factors, it is worth mentioning that in the MSCT group, two patients (6.7%) relapsed at the 24th week of follow-up, as shown by increases in the ESR, CRP levels, joint swelling and pain. In contrast, no relapse events occurred in the MSCT+IFN-γ group. The disease activity clusters of the two groups were also significantly changed after treatment. Compared with that in the MSCT group, the improvement in disease activity in the MSCT+IFN-γ group was more obvious. Only a few patients had high disease activity, while most patients had moderate or low disease activity, and a few patients showed remission (figure 3C).

Further investigations found that the RF level of the MSCT+IFN-γ treated group declined faster than in the MSCT group, but changes in anti-CCP levels were not significantly different (figure 3D,E). Prior to treatment, the cell ratios of Tregs/Th17 at baseline were less than one in all patients with RA,

Table 2 Coprimary and secondary efficacy end points

Variable	Month 1		Month 3		Month 12	
	MSCs (n=30)*	MSCs+IFN-γ (n=30)*	MSCs (n=30)	MSCs+IFN-γ (n=30)	MSCs (n=30)	MSCs+IFN-γ (n=30)
Efficacy (Good or Moderate EULAR response)—% of patients (SD)	23.3 (42.3)	86.7 (34.0)†	53.3 (49.9)	93.3 (24.9)†	50.0 (50.0)	93.3 (24.9)†
DAS28-ESR <2.6—% of patients (SD)	0.0 (0.0)	3.3 (18.0)	3.3 (18.0)	10.0 (30.0)	3.3 (18.0)	13.3 (34.0)
DAS28-ESR ≤3.2—% of patients (SD)	0.0 (0.0)	33.3 (47.1)†	16.7 (37.3)	40.0 (49.0)	10.0 (30.0)	36.7 (48.2)†
ACR 20 response—% of patients (SD)	23.3 (42.3)	86.7 (34.0)†	53.3 (49.9)	93.3 (24.9)†	50.0 (50.0)	93.3 (24.9)†
ACR 50 response—% of patients (SD)	6.7 (24.9)	33.3 (47.1)†	20.0 (40.0)	36.7 (48.2)	20.0 (40.0)	36.7 (48.2)
ACR 70 response—% of patients (SD)	3.3 (18.0)	10.0 (30.0)	3.3 (18.0)	13.3 (34.0)	3.3 (18.0)	13.3 (34.0)
△DAS28-ESR (SD)	−0.69 (0.64)	−2.14 (0.60)†	−1.05 (0.95)	−2.51 (0.58)†	−1.28 (1.03)	−2.54 (0.60)†
△HAQ-DI (SD)	−0.17 (0.29)	−0.59 (0.28)†	−0.37 (0.24)	−0.73 (0.24)†	−0.40 (0.26)	−0.87 (0.27)†

*Only data from patients who completed 48 weeks of follow-up were included here.
†P<0.05 for the comparison with MSCs.
ACR, American College of Rheumatology; DAS28-ESR, Disease Activity Score for 28-joint counts based on the erythrocyte sedimentation rate; EULAR, European League against Rheumatism; HAQ-DI, Health Assessment Questionnaire-Disability Index; IFN-γ, interferon-γ; MSCs, mesenchymal stem cell; SEM, SE of mean.

indicating imbalanced immune homoeostasis and excessively inflammatory microenvironment (figure 3F). As expected, the Treg/Th17 cell ratio increased more rapidly in the MSCT+IFN-γ than in the MSCT group, suggesting that combination therapy with IFN-γ significantly improved immunomodulatory function and therapeutic efficacy of MSCT. Most enrolled patients in this study completed a 48 week follow-up, except one subject in the MSCT+IFN-γ group and two in the MSCT group.

DISCUSSION

Several studies have confirmed that MSCT treatment significantly improve clinical symptoms of patients with autoimmune diseases via the immunomodulatory functions of MSCs.[45 46] However, such immunomodulatory functions of MSCs are not innate and immutable, but plasticity-dependent on the induction of the inflammatory microenvironment.[37] The immunosuppressive functions of MSCs are induced by IFN-γ together with the concomitant presence of any of three other proinflammatory cytokines, TNF-α, IL-1α, or IL-1β *via* expressing high levels of several chemokines and inducible nitric oxide synthase (iNOS).[33] Consistent with the above results, our previous clinical study found that patients with high IFN-γ levels responded well to MSCT, while patients with low IFN-γ levels responded poorly.[31] However, there are substantial variations in the immune microenvironment among different stages of disease or different patients, which may affect the therapeutic efficacies of MSCT. Therefore, we hypothesise that the combined use of recombinant IFN-γ to reconstruct immune microenvironment would improve the clinical efficacy of MSCT in patients ith RA. Although many clinical trials have attempted to treat RA with IFN-γ, the final results have demonstrated that the treatment of RA with IFN-γ alone is safe but ineffective.[41 42] To date, it is still unclear whether MSCT combined with IFN-γ treatment is safe and synergistically improves the clinical outcome in patients with RA.

In this study, we first demonstrated that WT-MSCT significantly ameliorated the severity of CIA in a mouse model, including that of joint synovitis and articular and cartilage destruction, with a high level of endogenous IFN-γ. However, these therapeutic effects were not observed with IFN-γR$^{-/-}$ MSCT treatment. The in vitro studies also confirmed that IFN-γ was a key factor in determining the immunosuppressive capacity of mouse and human MSCs. These results indicate that the therapeutic efficacy of MSCT on RA is dependent on IFN-γ and could significantly improve disease severity of CIA without any side effects.

Furthermore, our clinical study in patients with RA showed that at the third month of follow-up, the efficacy and ACR20 response rate of MSCT +IFN-γ group was 93.3% (28/30), while that of MSCT group was 53.3% (16/30). Along with a better improvement in disease activity clusters, MSCT +IFN-γ significantly improved clinical symptoms and disease activities, including DAS28-ESR, HAQ-DI, ESR and CRP values, RF level, than MSCT monotherapy. As reported in other studies, we found a low cell ratio of Treg/Th17 in patient with RA which orchestrate the imbalanced immune homoeostasis and excessively inflammatory microenvironment.[47 48] The rapidly increase of Treg/Th17 cell ratio in the patients with RA treated with MSCT +IFN-γ indicated an enhancing activity of Treg cells which were beneficial for the treatment of RA. These results also demonstrate for the first time that combination IFN-γ treatment synergistically enhances the therapeutic efficacy of MSCT on RA patients without any adverse side effects.

Besides the patient's immune microenvironment, the immunomodulatory plasticity of MSCs was also affected by immunosuppressive agent. In fact, MSC-based cell therapy was successfully applied in the transplantation of a third-party, semimatched grade IV acute GvHD patient with cyclosporine and steroid resistance.[49] However, prochymal, an MSC-based agent for GvHD, achieved no therapeutic efficacy in GvHD patients when combined with steroids.[45] In this study, we also found that a non-responsive patient of MSCT+IFN-γ group took steroids beginning on the day after MSCT until the fourth week of follow-up without the consent of the researchers. It has been confirmed that dexamethasone affects the immunoregulatory capacity of MSCs by inhibiting the expression of iNOS and Indoleamine 2,3-dioxygenase (IDO).[50] These studies suggest that combination administration of immunosuppressive agents and MSCs should be avoided. In addition, therapeutic effects of MSCT may also be achieved by enhancing the sensitivity of patients to DMARDs, as the types and doses of DMARDs used in all responsive patients were gradually decreased after MSCT or MSCT combined with IFN-γ treatment.

In summary, our study combined preclinical and clinical studies to demonstrate for the first time that IFN-γ is a key factor in determining the efficacy of MSCT in the treatment of RA. We proposed a novel clinical strategy for the treatment of RA with MSCT+IFN-γ and confirmed that MSCT+IFN-γ combination therapy synergistically enhances the efficacy of MSCT in patients with RA without any side effects during a 1-year observation period. However, to further confirm the safety and efficacy of this new strategy, further research, including performing a multicentre randomised controlled trial to expand the number of patients, extending the follow-up time to evaluate the long-term relapse rate, resolving conflict between MSCs combined with IFN-γ treatment and conventional antirheumatic treatment, standardising the frequency of treatment, evaluating long-term side effects, and exploring the variability of MSCs in surviving and how this can affect the clinical outcomes, is still needed.

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