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Systemic Delivery of IL-10 by Bone Marrow Derived Stromal Cells Has Therapeutic Benefits in Sepsis Therapy*

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Abstract Sepsis is one of the most serious problems of modern medicine nowadays and improvements in treatments are urgently needed. Bone marrow derived stromal cells (BMSCs) have a potent immunosuppressive effect in humans *in vivo*. IL-10, a cytokine synthesis inhibitory factor, plays an important role in anti-inflammatory response. Here BMSCs were infected with recombinant adenovirus encoding murine IL-10 (mIL-10) and a GFP marker to obtain GFP positive BMSC-mIL-10 cells. The BMSC-mIL-10 cells were injected into mice with cecal ligation and puncture (CLP)-induced sepsis, and the enhanced expression of IL-10 in blood was confirmed by ELISA. Indeed, in CLP mice mode, the systematic delivery of IL-10 *via* BMSCs effectively suppressed the production of proinflammatory cytokines, including TNF-α, IL-6, IL-1α and IL-1β, compared to BMSCs alone. The therapeutic benefits were further demonstrated by an increased prevention from body loss, increased survival rate, and the suppression of inflammatory response in lung and kidney. Furthermore, these effects may be mediated by inhibiting the activation of NF-κB in macrophages and neutrophils. Collectively, these results suggest that systemic delivery of IL-10 by BMSCs may serve as a potential treatment in sepsis therapy.

Key words sepsis, IL-10, inflammation, bone marrow derived stromal cells (BMSCs), gene therapy
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Sepsis, a disease with a mortality of 18% to 33%, is one of the top ten causes of death worldwide[1]. It is a generalized inflammatory response elicited by microbial infections. Patients with sepsis show the systemic inflammatory response syndrome (SIRS) accompanied by the inability to regulate the inflammatory response that leads to organ dysfunction[2-3]. It has been a big challenge to modern medicine to treat patients with severe sepsis and septic shock, and sepsis continues to be a substantial burden on healthcare, therefore, a new treatment regimen is desperately on demand.

Bone marrow derived stromal cells (BMSCs) were first reported to have a potent immunosuppressive effect in humans *in vivo* in 2004[4]. Studies have shown that BMSCs are potent immunomodulators in both humans and animals[5-6]. Recently, BMSCs have been shown to work as therapeutic vectors to treat a wide variety of diseases, such as myocardial infarction[7], lung injury[8-10], kidney disease[11], diabetes[12], graft versus host disease[13] and various neurological disorders[14]. Similarly, BMSCs may provide a novel cellular vector for treating sepsis.

Interleukin-10 (IL-10) was initially characterized as a cytokine synthesis inhibitory factor (CSIF)[15]. It has been shown to attenuate inflammatory responses by decreasing the proinflammatory cytokines including IL-1, IL-12 and TNF-α and by downregulating IL-2 production in T cells[16]. Since sepsis is caused by the

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inability to regulate the inflammatory response, we hypothesize that systemic delivery of IL-10 by BMSCs may have therapeutic benefits in sepsis therapy.

1 Materials and methods

1.1 Cell culture

Primary cultures of bone marrow stromal cells (BMSCs) were performed as previously described [17]. Peritoneal neutrophils and macrophages were elicited by 4% (*w*/*v*) thioglycolate (Sigma) *via* intraperitoneal injection. At 6 hours and 4 days after injection, peritoneal lavage was collected to obtain peritoneal neutrophils and macrophages, which were then maintained in complete RPMI 1640 medium with 10% (*v*/*v*) fetal calf serum plus 1% penicillin-streptomycin.

1.2 Construction and production of recombinant adenovirus over-expressing IL-10 (Ad-IL-10)

Mouse IL-10 was amplified from cDNA derived from mouse splenocytes by PCR using the following primers: 5′ CCCAAGCTTATGCCTGGCTCAGCAC-TGCTAT 3′ and 5′ ACGCGTCGACACGCCGGGT-GGTTCAAT 3′. The PCR product was cloned into pMD18-T by using a TA cloning kit (Invitrogen). The gene of IL-10 was subcloned into a shuttle vector pAdTrack-CMV between *Hin*dⅢ and *Sal* Ⅰ sites. The resulting pAdTrack-CMV-IL-10 was linearized with *Pme* Ⅰ (Invitrogen), and subsequently cotransformed into *E. coli*. BJ5183 cells with an adenoviral backbone plasmid pAdEasy-1. The recombinant adenovirus encoding murine IL-10 (Ad-IL-10) was confirmed by restriction endonuclease analysis. Finally, Ad-IL-10 was linearized and transfected into HEK293 cells. Recombinant adenoviruses (Ad-IL-10) were typically harvested within 7 to 12 days.

1.3 Mice and model of sepsis

All C57BL/6 (B6) mice were from animal center of Sun Yat-sen University. All the animal experiments were performed in compliance with the institutional guidelines and according to the protocol approved by Institutional Animal Use and Care Committee of Sun Yat-sen University. The surgical procedure to generate CLP-induced sepsis was carried out as previously described [18]. Mice were randomly allocated to four groups (10 mice/group): (1) Sham group (Sham-operated controls were subjected to the same surgical laparotomy, but the cecum was neither ligated nor punctured); (2) CLP group (Mice underwent CLP and received intravenous administration of 1 ml PBS for each mouse at 1 hour after CLP); (3) BMSCs treated group (Mice underwent CLP and received intravenous administration of 1×10^6 BMSCs in 1 ml PBS for each mouse at 1 hour after CLP); (4) IL-10BMSCs treated group (Mice underwent CLP and received intravenous administration of 1×10^6 BMSC-mIL-10 in 1 ml PBS for each mouse at 1 hour after CLP). For the CLP model, mice were lightly anesthetized with gaseous diethyl ether and a middle abdominal incision was made. The cecum was mobilized, ligated, and punctured twice with a 21-gauge needle. The bowel was repositioned and the abdomen was closed.

1.4 Flow cytometry analysis

Flow cytometry was performed on FACSCalibur with CellQuest Pro software (BD Biosciences) using directly conjugated mAbs against the corresponding markers. All reagents were purchased from BD Biosciences. The FACS buffer used for incubating cells with antibodies and for washing consisted of 1× PBS, 0.5% BSA and 0.05% Azide. The sample preparation for FACS analysis was performed following standard FACS staining protocols.

1.5 ELISA

IL-10, TNF-α, IL-6, IL-1α and IL-1β levels in the culture supernatant or serum were assayed by using ELISA kits from BD Biosciences according to the manufacturer's instructions.

1.6 Total RNA extraction, RT-PCR and quantitative RT-PCR

Total RNA was extracted by the TRIzol/ chloroform (Invitrogen) from tissues or cells. The mRNA level of IL-10 in BMSCs was detected by RT-PCR using following primers: 5′ CCCAAGCT-TATGCCTGGCTCAGCACTGCTAT 3′ and 5′ ACG-CGTCACACGCCGGGTGGTTCAAT 3′. The mRNA levels of TNF-α, IL-6 and GAPDH in kidney and lung tissues were measured using a real-time PCR machine (MJ Research, USA) with following primers. TNF-α: Forward, 5′ GGTCCCCAAAGGGATGAGA 3′, Reverse, 5′ TCTGGGCCATAGAACTGATGAGA 3′; IL-6: Forward, 5′ CCACGGCCTTCCCTACTTC 3′, Reverse: 5′ TTGGGAGTGGTATCCTCTGTGA 3′; GAPDH: Forward, 5′ AGGCCGGTGCTGAGTATG-TC 3′, Reverse, 5′ TGCCTGCTTCACCACCTTCT 3′. The real-time PCR was performed as the following protocol: 50℃ for 2 min, 95℃ for 10 min, followed by 40 cycles of 95℃ for 15 s and 60℃ for 1 min. Results were generated as the ratio of target mRNA over GAPDH mRNA.

1.7 Histological analysis

Mice were injected with PBS, BMSCs or BMSC-mIL-10 at 1 hour after surgery. 24 hours later, mice were sacrificed. Kidney and lung tissues were fixed in 10% buffered-formalin, embedded in paraffin, cut to 4 mm thickness. The sections were then stained with hematoxylin and eosin, and observed with a bright-field microscope. Histological evaluations were made by two independent expert observers without prior knowledge of the experimental design.

1.8 Western blot and immunoprecipitation

1.5×10^6 peritoneal macrophages were serum starved for overnight. The cells were pretreated with indicated conditioned medium for 1 h, then stimulated with LPS (50 μg/L) for 20 min, and lysed in 100 μl of lysis buffer (150 mmol/L NaCl, 50 mmol/L HEPES at pH 7.4, 1 mmol/L EDTA, 1% NP-40, protease inhibitors). Of the resulting lysates, 12～15 μl were subjected to SDS-PAGE and probed for p-IκBα, IκBα or actin antibody (Cell Signal Technology). For immunoprecipitation, the culture supernatants were collected after centrifugation at various time points. Each sample was incubated with anti-IL-10 antibody (Cell Signal Technology) and protein A-agarose beads (Roche Applied Science) at 4℃ overnight. The agarose beads were washed three times in cold lysis buffer and the levels of IL-10 were detected by Western blot with anti-IL-10 antibody.

1.9 Immunofluoresent staining

The peritoneal neutrophils were stimulated with LPS (50 μg/L) in normal medium or conditioned medium. At 1 hour post stimulation, cells were fixed in acetone at –30℃ for 5 min. For the staining of p65, fixed cells were permeablized with 0.1% Triton X-100 for 10 min at room temperature and blocked with 5% goat serum for 1 hour. Then the cells were incubated with rabbit anti-p65 antibody (Cell Signal Technology) for 1 hour. After 3 times wash in PBS, the cells were incubated with FITC labeled goat anti-rabbit antibody (Molecular Probes) for 1 hour at room temperature. The cells were washed 3 times with PBS, stained with propidium iodide (PI), and mounted for fluorescence microscopy. Cells were viewed with an oil objective len and the staining was photographed with a Nikon Eclipse 400 microscope.

1.10 Statistical analysis

Data are expressed as $\bar{x} \pm s$. Statistical analysis was performed using a one-way ANOVA to compare animals at the same time point receiving different treatments. And a Student's *t*-test was used for comparison between two groups. Survival rate and weight loss were assessed with Fisher's exact probability test or the log-rank test. Statistical differences were considered to be significant at $P < 0.05$.

2 Results

2.1 Construction of BMSCs over-expressing IL-10 (BMSC-mIL-10) and the expression of IL-10 *in vitro* and *in vivo*

To construct recombinant adenoviruses over-expressing IL-10 (Ad-IL-10), we used a simplified recombinant adenoviruses system, AdEasy System (Figure S1, See Supplement online, http://www.pibb.ac.cn/cn/ch/common/view_abstract.aspx?file_no=20100107&flag=1).

Freshly isolated BMSCs showed adherence and expansion in culture and displayed a fibroblast-like morphology when observed under a light microscope. To test the stability of BMSCs, we analyzed the BMSC cell surface markers by FACS. After the fifth passage, the cells displayed high levels of surface CD29, CD105 and CD166, but not CD45 (Figure 1a), which is consistent with the previous reports[17]. BMSCs were cultured for three passages and then infected with Ad-IL-10 at different MOIs and GFP expression was examined two days after infection (Figure 1b). The best infection efficiency (75%) was observed at an MOI of 150 (Figure 1c). Therefore, an MOI of 150 was chosen for the following studies.

To test the expression of IL-10, we first performed RT-PCR to detect the mRNA level of IL-10 in BMSCs. As expected, the IL-10 mRNA level was much higher in the BMSC-mIL-10 (Figure S2a, lane*2*, See Supplement online, http://www.pibb.ac.cn/cn/ch/common/view_abstract.aspx?file_no=20100107&flag=1), compared to the BMSCs infected with vector alone (Figure S2a , lane*1*, *3* served as a positive control. See Supplement online, http://www.pibb.ac.cn/cn/ch/common/view_abstract.aspx?file_no=20100107&flag=1). Furthermore, as shown in Figure 1d, IL-10 concentration in the supernatant was much higher when infected with Ad-IL-10, compared with that vector alone, as determined by ELISA at day 2 after infection. To further confirm these results, we immunoprecipitated IL-10 in the supernatant with anti-IL-10, then immunoblotted with anti-IL-10. As shown in Figure S2b (See Supplement online, http:

//www.pibb.ac.cn/cn/ch/common/view_abstract.aspx?file_no=20100107&flag=1), higher amount of IL-10 in the medium derived from BMSC-mIL-10 was observed. To test the expression of IL-10 *in vivo*, we injected PBS, BMSCs and BMSC-mIL-10 into three groups of mice through the tail vein (5 mice/group) and the IL-10 level in the blood were measured at 24 h and 48 h after injection. We found that the IL-10 level was much higher in BMSC-mIL-10 group than that in BMSCs group (Figure 1e). These results indicated that IL-10 was secreted to the blood by injected BMSC-mIL-10.

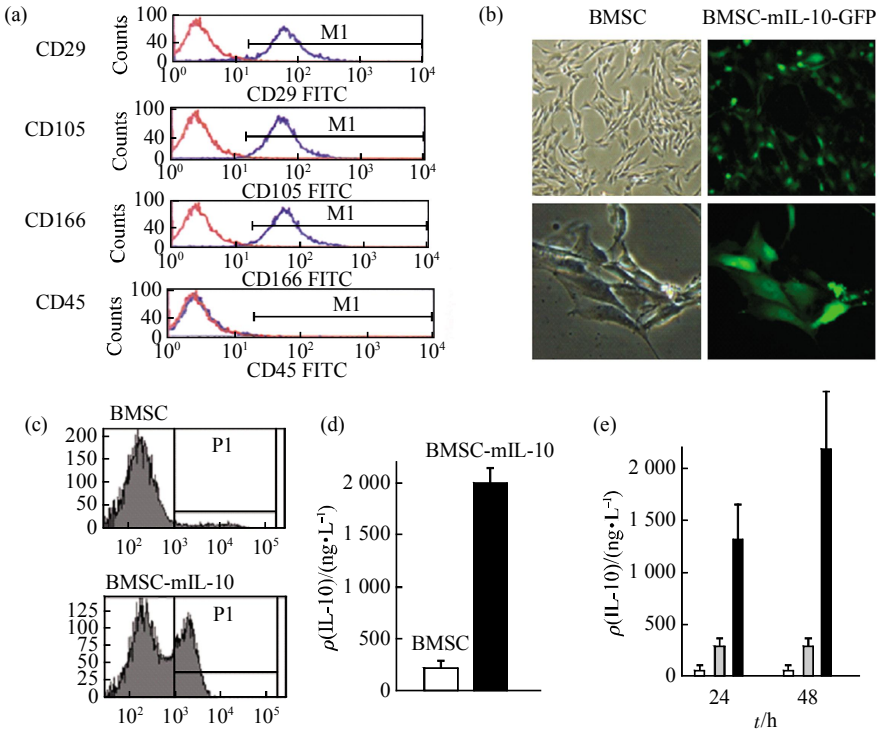

Fig. 1 Generation and characterization of BMSC-mIL-10 *in vitro* and *in vivo*

(a) Phenotypes of mouse BMSCs. BMSCs at the 5th passage were analyzed with flow cytometry. Blue lines represent the specific fluorescence-labeled antibodies, and red lines represent isotype controls. (b) Determination of recombinant adenovirus infection. GFP expression in the infected cells was observed under light (left) or fluorescence microscopy (right) after infection with Ad-IL-10 at a MOI of 150. (c) Determination of adenovirus infection efficiency. Infection efficiency was estimated by flow cytometry 2～3 days after infection with Ad-IL-10 at a MOI of 150. (d) *In vitro* expression of IL-10 in BMSCs. The concentration of IL-10 in the medium from BMSCs and BMSC-mIL-10 group was detected by ELISA at 48 h after injection. (e) *In vivo* over expressing of IL-10. BMSCs or BMSC-mIL-10 were injected into mice intravenously; the serum was collected at 24 and 48 h after injection and IL-10 levels in the serum were measured by ELISA (N: Normal mice group). Results are representative of three independent experiments. □: N; ▨: BMSC; ■: BMSC-mIL-10.

2.2 Systematic delivery of IL-10 by BMSCs improved mice survival rate, prevented body weight loss, and decreased inflammatory cytokine production in the CLP induced sepsis model

Both BMSCs and IL-10 play an important role in anti-inflammatory response. In order to demonstrate the potential therapeutic effect of BMSCs and IL-10 in sepsis, we first established a sepsis model by cecal ligation and puncture (CLP). Then we monitored survival rates in four groups: sham, CLP with PBS injection alone (CLP: Untreated), CLP treated with BMSCs (CLP-BMSC), and CLP treated with BMSC-mIL-10 groups (CLP-BMSC-mIL-10). As shown in Figure 2a, the survival rate in CLP-BMSC-mIL-10 group is much higher than CLP and CLP-BMSC group at the end of day 4 after surgery. We also monitored the weight loss in the surviving mice, and we found that the CLP-BMSC-mIL-10 mice lost less than 20% of the weight, which is significantly less than that of CLP-BMSC mice (30%) or CLP mice (50%) at the end of day 4 (Figure 2b). The beneficial effect on survival rate and the weight loss by BMSC-mIL-10 is significantly better than BMSCs alone ($P < 0.05$). These results suggested that combination of BMSCs and IL-10 can dramatically improve survival rate and prevent body weight loss in sepsis.

CLP induced sepsis leads to the production of large quantities of proinflammatory cytokines, including IL-6, tumor necrosis factor (TNF), IL-1α and IL-1β 24 hours after surgery [18]. We found that treatment with BMSCs alone could slightly reduce the production of proinflammatory cytokines (Figure 2c) and more significantly, treatment with BMSC-mIL-10 remarkably reduced the levels of proinflammatory cytokines (Figure 2c). These results demonstrated that combination of BMSCs and IL-10 can dramatically reduce the production of proinflammatory cytokines in sepsis. Therefore, systematic delivery of IL-10 by BMSCs appears to effectively inhibit the inflammatory response in CLP induced sepsis model.

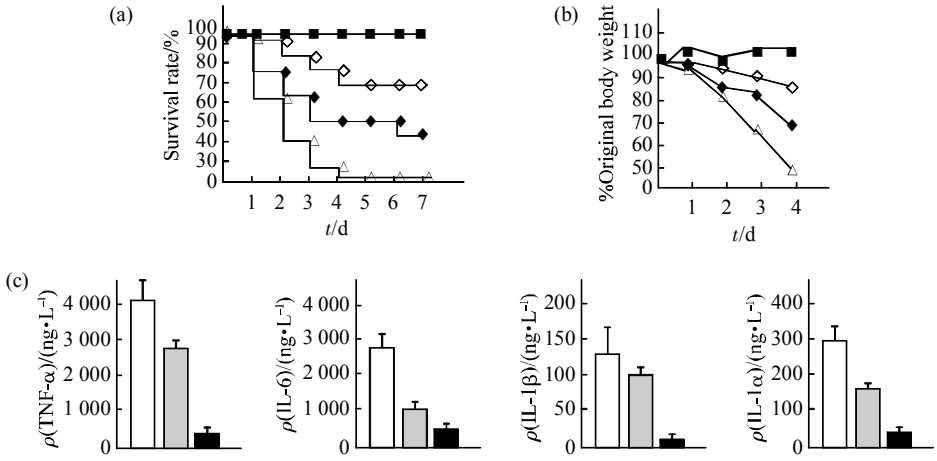

Fig. 2　Systematic delivery of IL-10 by BMSCs improved survival rate, prevented weight loss, and inhibited the production of proinflammatory cytokines in a sepsis model

BMSCs or BMSC-mIL-10 were injected into mice intravenously 1 h after CLP. The survival rate (a) and body weight (b) of each group was monitored over the indicated time (Un: CLP injected with PBS alone). Results are representative of three independent experiments. ■—■: Sham mice; △—△: Un; ◆—◆: BMSC; ◇—◇: BMSC-mIL-10. (c) PBS, BMSCs or BMSC-mIL-10 were injected into mice intravenously 1 h after CLP, and TNF-α, IL-6, IL-1α and IL-1β levels in the serum were measured by ELISA at 24 h($\bar{x} \pm s$, $n = 3$). □: Un; ▨: MSC; ■: MSC-mIL-10.

2.3　Treatment of BMSC-mIL-10 inhibited the inflammation in organs in sepsis model

Generally, the lethality in sepsis is associated with organ failure. Therefore, we examined the pathology of major organs often injured in sepsis. As shown in Figure 3a, the treatment of BMSCs can suppress the expression of TNF-α and IL-6 in lung and kidney in CLP model, and this suppression became more efficient when treated with BMSC-mIL-10, suggesting that the combination of BMSCs and IL-10 plays a better role in anti-inflammatory response.

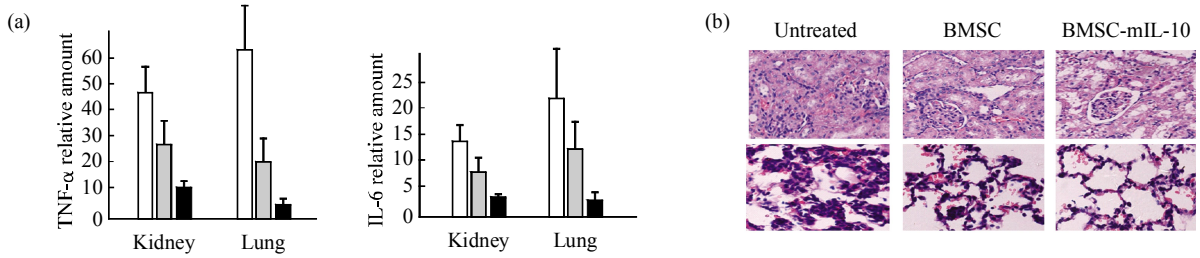

Fig. 3　Treatment of BMSC-mIL-10 inhibited the inflammation in the organs in sepsis model

Mice were injected with PBS, BMSCs or BMSC-mIL-10 at 1 hour after surgery. 24 hours later, lungs and kidneys were collected from each group of mice. (a) The mRNA levels of TNF-α and IL-6 in lungs and kidneys were detected by real-time PCR. Results shown are $\bar{x} \pm s$ of three independent experiments. □: Un; ▨: BMSC; ■: BMSC-mIL-10. (b) Morphological changes in the lungs and kidneys were evaluated by hematoxylin and eosin staining. Results are representative of three independent experiments.

To further demonstrate the efficacy of systematic delivery of IL-10 by BMSCs in suppressing inflammation in this sepsis model, we performed tissue histological evaluation. As shown in Figure 3b, both lung and kidney from CLP group mice showed classical features of inflammation, including thickening of luminal wall, loss of normal architecture and cellular infiltration. These inflammatory features were a little attenuated in the organs from the CLP-BMSC group mice. In contrast, sepsis-bearing mice treated with BMSC-mIL-10 showed a dramatic decrease in inflammation in the lung and kidney. These results suggested that the treatment of BMSC-mIL-10 can significantly reduce the inflammation in organs in sepsis model.

2.4 Treatment of BMSC-mIL-10 may inhibit the inflammatory response through the inactivation of NF-kappa B in macrophages and neutrophils

NF-kappa B(NF-κB) is one of the key transcription factors controlling the expression of proinflammatory gene in immune cells, such as macrophages and neutrophils[19]. Since IL-10 has been shown to inhibit the activation of NF-κB, we hypothesized that the treatment of BMSC-mIL-10 may inhibit the inflammatory response in the sepsis through the inactivation of NF-κB in macrophages and neutrophils. To test our hypothesis, we grew the BMSCs and BMSC-mIL-10 *in vitro* for 24 h to generate condition medium, CM and CM-mIL-10, respectively. Then the peritoneal macrophages were pre-incubated in these mediums for 1 hour before stimulated for 20 min with endotoxin lipopolysaccharide (LPS), which is the cell wall of gram-negative bacteria and plays the most important role in the pathogenesis of sepsis. CM-mIL-10 conditioned medium significantly decreased the phosphorylation and degradation of IκBα in macrophages when stimulated with LPS, compared to that of the CM conditioned medium. These results suggested that the conditioned medium from BMSC-mIL-10 could effectively inhibit the LPS induced NF-κB activation, which is likely due to the IL-10 secreted by BMSC-mIL-10.

To further demonstrate whether the treatment of BMSC-mIL-10 can also affect the activation of NF-κB in neutrophils, we performed immunofluorescent staining to observe the nuclei translocation of NF-κB. As shown in Figure 4b, the p65 protein, a key member of the NF-κB transcription activators, translocated from cytoplasm to nucleus after LPS stimulation in peritoneal neutrophils. However, when peritoneal neutrophils were pretreated with the BMSC-mIL-10 conditioned medium, the translocation of p65 was reduced dramatically. Collectively, these results showed that the treatment of BMSC-mIL-10 may effectively inhibit the activation of NF-κB in both macrophages and neutrophils, and thus inhibit the inflammatory response in the sepsis model.

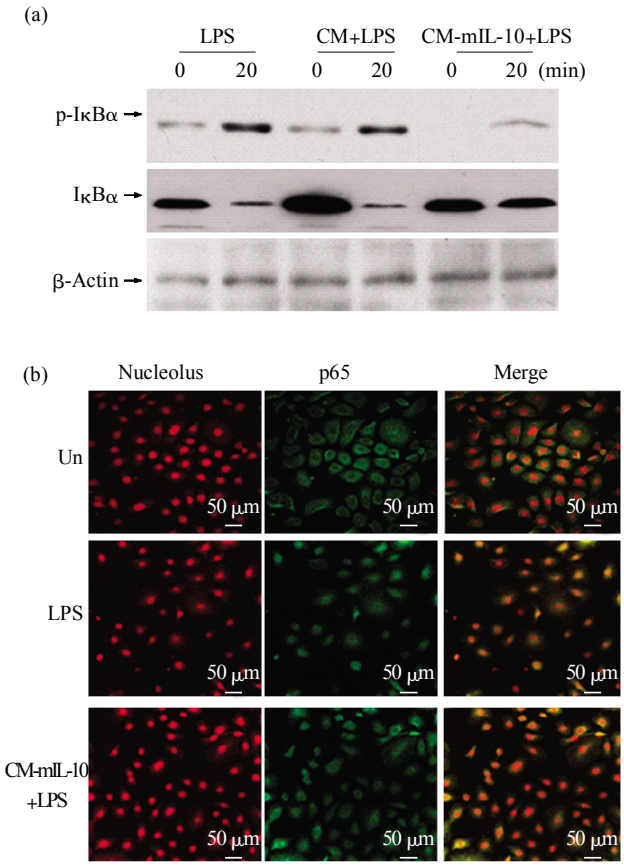

Fig. 4 Treatment of BMSC-mIL-10 reduced the inflammatory response by inhibiting the activation of NF-kappa B in macrophages and neutrophils

(a) Immunoblot of IκBα phosphorylation and degradation in cell lysates. Peritoneal macrophages were pretreated with conditioned medium of BMSCs (CM) or BMSC-mIL-10 (CM-mIL-10) for 1 h, then stimulated with LPS (50 μg/L) for 20 min. The cell lysates were collected and analyzed by immunoblotting with anti-phosphorylated IκBα and IκBα. Actin served as a loading control. (b) Immunofluoresent staining to detect the activation of NF-κB in neutrophils. Peritoneal neutrophils were pretreated with conditioned medium of BMSCs (CM) or BMSC-mIL-10 (CM-mIL-10) for 1 hour, and then stimulated with LPS (50 μg/L) for 1 hour. The cells were fixed for immunofluorescent staining with anti-p65 antibody. Propidium iodide (PI) was used for nuclear counterstaining. Results are representative of three independent experiments.

3 Discussion

Here we demonstrated a new BMSCs-based IL-10 gene therapy in CLP induced sepsis model. The administration of BMSC-mIL-10 effectively suppressed the levels of proinflammatory cytokines, including TNF-α, IL-6, IL-1α and IL-1β, compared to BMSCs alone in CLP induced sepsis model. The therapeutic benefits were further demonstrated by an increased protection from body weight loss and enhanced survival rate, the suppression of inflammatory response and damage in lung and kidney. Collectively, our results demonstrated that an intravenous injection of BMSC-mIL-10 can beneficially modulate the inflammation response of the host in sepsis. And the suppression of inflammatory response is likely achieved by inhibiting the activation of NF-κB and thus the expression of proinflammatory cytokines. Overall, the systematic delivery of IL-10 by BMSCs improved survival rate and body loss and may serve as a potential treatment in sepsis therapy.

The serum half-life of recombinant IL-10 is between 2.3 and 3.7 h[20]. If we use recombinant IL-10, it may require daily administration due to the short half-life and swift clearance of IL-10 in the circulation. Gene therapy provides a promising treatment to overcome this problem by sustained expression of target genes. Since viral vectors may induce host immune responses and tumorigenesis by coding viral proteins, we choosed cell-based gene therapy by using BMSCs in this study. A recent report showed that BMSCs could improve survival rate in CLP induced sepsis, it showed that monocytes and/or macrophages from septic lungs produced more IL-10 from BMSCs treated mice versus untreated mice[21]. Importantly, the beneficial effect of BMSCs was eliminated by macrophage depletion or pretreatment with interleukin-10 (IL-10) or IL-10 receptor antibodies[21], suggesting that IL-10 play an important role in anti-sepsis. Consistently, here we also showed that BMSCs have a therapeutic effect on CLP induced sepsis, and more interesting, over expression of IL-10 in BMSCs can significantly enhance the therapeutic effect. In addition to the secretion of IL-10, BMSCs may also release additional mediators that could be beneficial in sepsis, such as prostaglandin E2. Therefore, it will be interesting to determine whether other mediators are involved in this process in the future.

Signaling *via* NF-κB is a key process during inflammation and thus is an attractive target for anti-inflammatory therapeutic interventions. The signaling cascade to activate NF-κB depends on the activation of a multisubunit IκBα kinase (IKK) complex. The activated IKK complex phosphorylates IκBα proteins on conserved serine residues to target them for ubiquitin-dependent degradation, which frees NF-κB and allows its translocation into the nucleus for the transcriptional activation of its target genes[19]. It is well known that NF-κB is up regulated in sepsis[22] and inhibition of the activation of NF-κB can prevent circulatory failure in CLP-induced sepsis [23]. In this study, we showed that the conditioned medium from BMSC-mIL-10 inhibited the LPS induced activation of NF-κB in both macrophages and neutrophils, but not the conditioned medium from BMSCs alone. These results indicate that IL-10 may majorly function through inhibiting the activation of NF-κB and BMSCs may function through a NF-κB-independent pathway. Therefore, the combination of IL-10 and BMSCs may synergically inhibit both NF-κB and non-NF-κB pathways to reduce inflammation in sepsis.

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骨髓间充质干细胞过表达 IL-10 基因对脓毒症的治疗作用 *

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摘要 脓毒症是近年来的医学难题之一，迫切需要探讨新的治疗手段．以往研究发现，骨髓间充质干细胞具有免疫调节作用，免疫细胞因子白介素 10(IL-10)是细胞因子分泌的抑制因子，具有很好的抗炎作用．因此构建了携带小鼠 IL-10(mIL-10)的腺病毒载体，用它来感染骨髓间充质干细胞(BMSC)，并通过载体上的绿色荧光蛋白来筛选过表达 mIL-10 基因的骨髓间充质干细胞(BMSC-mIL-10)．随后，运用盲肠结扎穿孔法在小鼠体内建立脓毒症模型，并观察 BMSC-mIL-10 在脓毒症中的治疗作用．研究发现，和单纯 BMSC 治疗组相比，BMSC-mIL-10 可以明显降低脓毒症老鼠体内炎症因子的产生，这包括 TNF-α、IL-6、IL-1α 和 IL-1β 等，同时，BMSC-mIL-10 治疗组小鼠的肺部和肾脏炎症反应减轻，体重丢失下降，死亡率明显降低．为进一步探讨 BMSC-mIL-10 治疗机制，运用 Western blot 和免疫荧光染色方法发现 BMSC-mIL-10 组上清液可以抑制巨噬细胞和中性粒细胞中 LPS 引起的 NF-κB 的激活．上述结果表明，骨髓间充质干细胞过表达 IL-10 有望成为脓毒症治疗的潜在手段之一．

关键词 脓毒症，白介素 10，炎症，骨髓间充质干细胞，基因治疗
学科分类号 R63，R619 **DOI:** 10.3724/SP.J.1206.2010.00107

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Supplement

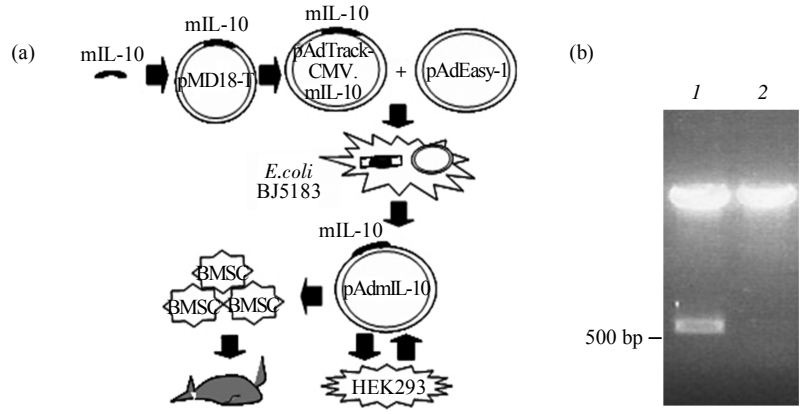

Fig. S1　Construction of recombinant adenovirus and schematic illustration of experimental studies

(a) Schematic illustration of experimental studies. (b) Digestion of the resultant plasmid pAdTrack-CMVIL-10 (left) and pAdTrack-CMV (right) with *Hin*dⅢ and *Sal* Ⅰ. *1*: pAdTrack-CMV.mIL-10; *2*: pAdTrack-CMV.

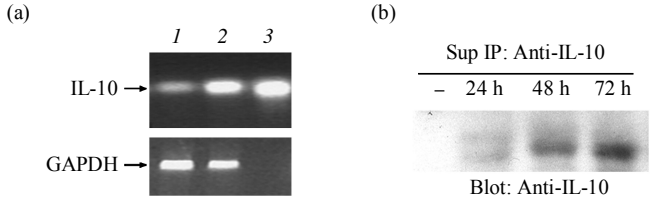

Fig. S2　The expression of IL-10 *in vitro* in BMSCs

(a) *In vitro* expression of IL-10 in BMSCs detected by RT-PCR. The expression of IL-10 in BMSCs or BMSC-mIL-10, pAd- IL-10 served as a positive control. *1*: BMSC; *2*: BMSC-mIL-10; *3*: pAd-mIL-10. (b) *In vitro* expression of IL-10 in BMSCs detected by Immunoblot. Immunoassay of fresh medium (-) or supernatants from BMSC-mIL-10 for 24, 48 and 72 h immunoprecipitated with anti-IL-10 and analyzed by immunoblotting with anti-IL-10.