0483

Defective proliferative capacity and cell cycle of hematopoietic progenitor cells in rheumatoid arthritis

JI CHEN, LO QIAN, MM QIN, GS WANG, XP LI

Anhui Provincial Hospital, Hefei,Anhui, China

Purpose: In rheumatoid arthritis (RA), telomeres of hematopoietic progenitor cells are age-inappropriately shortened, suggesting excessive apoptosis of hematopoietic precursor-cells (HPCs). The purpose of this study was to examine the functional competence (proliferative capacity, cell cycle dynamics) of CD34 HPCs in RA.

Methods: Frequencies of peripheral blood CD34+ HPCs from 17 RA patients and nine controls matched for age, sex, and ethnicity were measured by flow cytometry. Carboxyfluorescein diacetate succinimidyl ester (CFSE)staining was used to assess the proliferative capacity in purified CD34 HPCs after stimulation with early hematopoietins (IL-6 20 ng/mL, IL-3 20 ng/mL,SCF 100 ng/mL, Flt-3L 100 ng/mL) by flow cytometry . Propidium iodide (PI) was used to assess cycle dynamics in the fresh purified CD34 HPCs and after stimulation with early hematopoietins were measured by flow cytometry. HPC functions and frequencies were correlated with the duration, activity, and severity of RA as well as its treatment.

Results: In healthy donors, the frequencies of CD34 HPCs in lymphoid cells was significantly higher than in RA patients (0.37% $\pm$ 0.18% versus 0.82% $\pm$ 0.10% respectively, $P < 0.05$). After growth factor stimulation, control HPCs passed through nine replication cycles over 4 days. In contrast, RA-derived HPCs completed only four generations. For the cell cycle status of fresh purified CD34 HPCs, a larger percentage of normal CD34+cells were in (S+G2/M) stage of cell cycle than these from RA patients(6.15% $\pm$ 3.34% versus 1.90% $\pm$ 0.69% respectively, $P < 0.05$), and after stimulation with early hematopoietins a larger percentage of normal CD34+cells were in (S+G2/M) stage of cell cycle than these from RA patients(33.79% $\pm$ 9.03%versus 25.40% $\pm$ 6.25% respectively, $P < 0.05$). All HPC defects were independent of disease duration, disease activity, and were present to the same degree in treated patients.

Conclusion: Circulating bone marrow derived progenitor cells In RA maybe were diminished. HPCs from RA patients displayed growth factor nonresponsiveness and sluggish cell cycle progression. Defective HPC function independent of disease activity markers suggests bone marrow failure as a potential pathogenic factor in RA.

0546

Morphologic and functional characterization of bone marrow mesenchymal stem cells from rheumatoid arthritic patients

Chee-Yin WONG, Sze-Piaw CHIN, Esha Das GUPTA, Naveed WAZIR NAYYER, Mei-Theng NG, Soon-Keng CHEONG

International Medical University, Seremban, Malaysia

Background: Rheumatoid arthritis (RA) is an autoimmune T-cell-mediated systemic disease and usually accompanied by articular cartilage damage. Joint destruction renders RA a candidate disease for cartilage repair using mesenchymal stem cells (MSC). However, the issues of whether MSC from RA patients were functionally altered, especially when patients are also on immunosuppressive agents, must be addressed before proceeding to clinical application. The aim of this study is to investigate bone marrow MSC from patients with active RA.

Methods: Bone marrow aspirate was obtained from active RA patients on methotrexate. MSC were isolated from bone marrow mononuclear cells based on plastic adherent properties and expanded in vitro in culture medium. RA-MSC were characterized using microscopy, differentiation potential and immunosuppression ability.

Results: Isolated RA-MSC showed typical morphology, were able to differentiate into adipocytes, chondrocytes and osteocytes when incubated with appropriate differentiation media. When RA-MSC were co-cultured with autologous lymphocyte, RA-MSC were shown to suppress the proliferation of autologous T-cell in a dose-dependent manner.

Conclusion: RA-MSC showed typical morphology, ability to differentiate into adipocytes, chondrocytes and osteocytes, and anti-T proliferative activity. Autologous MSC may be a feasible source of treatment for active RA.

0548

Clinical safety and efficacy of autologous bone marrow mesenchymal stem cell injection for the treatment of severe osteoarthritis

Esha Das GUPTA[1], Naveed NAYYER[1,2], Sze Piaw CHIN[1,2], Chee Yew CHEOK[3], Chee Yin WONG[1], Soon Keng CHEONG[3]

[1]*International Medical University, Seremban, Malaysia,*[2]*Mawar Medical Centre, Seremban, Malaysia,*[3]*Universiti Tunku Abdul Rahman., KL, Malaysia,*[4]*3Penang Adventist Hospital, Penang, Malaysia*

Background: Bone marrow derived mesenchymal stem cells (BM-MSC) are precursors of hyaline cartilage. Autologous BM-MSC injection is safe and may be beneficial for patients with mild to moderate osteoarthritis (OA). Whether the treatment is also feasible and efficacious for patients with severe OA is not known; yet may be valuable when knee replacement is not desirable or contraindicated.

Objective: We aim to investigate if sufficient viable BM-MSC can be obtained from patients with severe OA for proliferation and chondrogenic differentiation; and its efficacy on pain and disease progression up to 1 year.

Methods: Five patients (4 women, mean age 63 $\pm$ 4 years) were recruited sequentially. All patients had bilateral severe symptomatic OA and significant varus deformity. All patients were refractory to multiple hyaluronic acid (HA) injections and ineligible for or refused surgery due to comorbidities. 40 mLs of bone marrow aspirate was obtained from the posterior iliac crest. BM-MSC was isolated and proliferated using animal free medium. Tridifferentiation into cartilage, bone and fat were performed using commercially available induction media. For intra-articular injection, each knee received 1.0–2.0 $\times$ 10^6 MSC per kg body weight, suspended in 2 mLs of 20 g HA as a single injection. We used visual analog score (VAS, 0 ''least painful''-50 ''most painful'') to assess pain and magnetic resonance imaging (MRI) for radiologic progression of disease of each knee.

Results: BM-MSC were successfully obtained from all patients and demonstrated adequate proliferation and tridifferentiation potential. The mean $\pm$ 1 SD VAS at baseline was significantly higher compared to assessments at 6 weeks, 3 months, 6 months and 12 months (25.7 $\pm$ 7.9, 20.5 $\pm$ 7.3, 18.1 $\pm$ 9.0, 16.6 $\pm$ 10.8, 15.9 $\pm$ 10.1; paired t-test $P < 0.01$). Mean 12-month VAS reduction = 40.7%. There were no adverse events. Serial MRI demonstrated increased cartilage thickness from 1.0 mm to 1.4 mm in one patient and complete resolution of subchondral cysts in another. Two patients showed no significant changes. One had increased effusion. All patients noted significant pain and functional improvement. Four patients were very satisfied with outcome.

Conclusion: Treatment of severe OA by a single intra-articular injection of autologous BM-MSC is feasible, safe and may provide sustained pain relief. Improvement may be due to articular cartilage repair or regeneration while radiologic progression of disease appear to be halted. This novel therapy could benefit patients who are ineligible for knee replacements or where deferment is desirable. Larger randomized studies are warranted.

0579

BMSCs suppressed the responses of CII-reactive B cells from RA patients in vitro

Zhaohui ZHENG, Jin DING, Ping ZHU

Department of Clinical Immunology, Xi-jing hospital, Fourth Military Medical University, Xi'an, China

Objective: The immunosuppressive nature of bone mesenchymal stem cells (BMSCs) suggest that allogenic BMSCs may be promising candidates for immunotherapy in the treatment of rheumatoid arthritis (RA). As B cells were confirmed play an important role in the pathogenesis of RA, in the present study, we investigated the suppressive effects of MSCs on CII-reactive B cell in vitro.

Methods: Bone marrow samples (3~5 mL) were obtained from healthy adult human donors, and BMSCs were separated by centrifugation in percoll solution followed by adherence to the plastics cultured in vitro. Paired peripheral blood mononuclear cells and synovial fluids mononuclear cell were isolated from patients with RA; then the effects of MSCs on proliferation, anti-CII antibody production, B-cell subsets in RA patients were investigated with the stimulation of CII or not by BrdU-incorporate detection, flow cytometry and ELISA, respectively; CD19/Annexin V staining was used to evaluate B-cell apoptosis in the inhibition by FCM; the role of TGF-β1 underlying the inhibition was also investigated.

Results: Allogenic BMSCs also failed to elicit positive responses of CII-reactive B cells, whereas significantly suppressed CII-stimulated B-cell proliferation and anti-CII antibody prduction without inducing B-cell apoptosis; in addition, BMSCs were also found significantly inhibited the up-regulation of CD27+IgG+non-swiched memory B cells with the stimulation of CII and down-regulation of CD27-IgG+ naïve B cells ($P < 0.05$); as for CD27+IgD- memory B cells, its elevation in the presence of CII was still suppressed by BMSCs, but insignificantly ($P > 0.05$); TGF-β1 also played a critical role in the inhibition.

Conclusion: Allogenic BMSCs could significantly suppressed the responses of CII-reactive B cells in vitro, which suggested that BMSCs could be a potential candidate for RA therapy.

0810

The expression of Wnt5a and Beta-catenin genes in hematopoietic stem cells in patients with rheumatoid arthritis

MM QIN, LO QIAN, JI CHEN, GS WANG, XP LI

Anhui Provincial Hospital, Hefei, China

Objective: The proliferative ability of hematopoietic stem cell (HSC) proliferation in rheumatoid arthritis(RA) patients is impaired and regulation of HSC cell cycle or proliferation signaling pathway may be abnormal. Wnt signaling pathway plays an important role in regulation of HSC proliferation signaling pathway and may be involved. To observe whether the expression of Wnt5a and Beta-catenin genes are impaired in HSC derived from RA.

Methods: The clinical date of 24 RA patients and nine healthy control were collected. Peripheral blood mononuclear cells (PBMCs) derived from 50 mL of whole blood were separated by gradient centrifugation and $CD34^+$ cells were purified using an MACS magnetic separation device. Total RNA of $CD34^+$ cells was extracted and transcripted into cDNA. SYBR Green I dye based real-time quantitative PCR method was used to compare the expression of Wnt5a and Beta-catenin in patients and those in the control.

Results: The levels of Wnt5a and Beta-catenin mRNA were 14.389 (0.014–25.206) and 8.87 $\pm$ 4.81 respectively in normal controls. In RA patients, Wnt5a mRNA expression was 0.115 (0.009–7.152), lower than that in normal control ($P = 0.004$).Beta-catenin mRNA expression was 4.96 $\pm$ 4.52,lower than that in normal control ($P = 0.037$).