·论 著·

间充质干细胞通过调控巨噬细胞极化减轻1型糖尿病模型小鼠炎症反应

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摘要:目的 探讨间充质干细胞(MSC)减轻1型糖尿病(T1DM)小鼠炎症反应的作用机制。**方法** 从7日龄小鼠股骨和胫骨分离并培养MSC,应用流式细胞术检测MSC表面标志物Sca-1,CD29,CD105,CD31,CD34,CD45,Ia和CD11b,并采用油红O染色检测体外成脂诱导分化,碱性磷酸酶染色检测成骨诱导分化,实时荧光定量PCR(RT-PCR)检测成脂和成骨关键转录因子,以鉴定分离培养的MSC。6周龄C57BL/6J雄性小鼠ip给予链脲佐菌素50 mg·kg^{-1},每天1次,连续5 d,制备T1DM小鼠模型,于制备成功后第1和第15天分别尾静脉注射MSC(每只5×10^5 MSC)。第1次MSC治疗后,每周检测小鼠随机血糖和体质量,治疗4周后取胰腺组织和腹腔巨噬细胞,采用HE染色法检测胰腺组织病理变化,免疫组化法检测胰岛素分泌和巨噬细胞数量的变化,流式细胞术检测M1和M2型巨噬细胞比例,RT-PCR检测M1和M2巨噬细胞标志分子诱导型一氧化氮合酶(iNOS),精氨酸酶1(Arg-1)及炎症因子白细胞介素1β(IL-1β),IL-4,IL-6,IL-10,IL-12,干扰素γ(IFN-γ)、肿瘤坏死因子α(TNF-α)和转化生长因子β(TGF-β)mRNA表达水平。**结果** 分离培养的MSC高表达Sca-1,CD29和CD105,不表达或低表达CD31,CD34,CD45,Ia和CD11b;成脂诱导分化后,出现大量脂滴,成脂关键转录因子CEBPα和PPARγ表达显著增加(P<0.01);成骨诱导后,碱性磷酸酶活性增加,成骨关键转录因子骨钙素和 Runx2 mRNA表达亦显著增加(P<0.01)。与T1DM模型组相比,MSC治疗组小鼠随机血糖浓度低(25.0±0.1)%,体质量增加(12.0±0.4)%(P<0.05),胰岛素分泌和巨噬细胞增加,巨噬细胞炎症因子IL-1β,IL-6,IL-12和TNF-α mRNA水平显著降低,而IL-10,IL-4和TGF-β mRNA水平显著升高(P<0.01);M2巨噬细胞百分比从(22.5±4.0)%增至(72.5±3.4)%,Arg-1 mRNA表达上调(P<0.05);M1巨噬细胞百分比从(50.1±1.2)%降至(15.6±1.2)%,iNOS mRNA表达降低(P<0.05)。**结论** MSC移植可减轻T1DM模型小鼠的炎症反应,其机制可能与调控M1和M2巨噬细胞极化有关。

关键词: 间充质干细胞; 巨噬细胞; 1型糖尿病; 炎症因子
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随着现代生活水平的不断提高,1型糖尿病(type 1 diabetes mellitus,T1DM)患者呈不断上升趋势,已成为威胁青少年和儿童健康的主要代谢疾病之一。T1DM是一种自身免疫性疾病,多由T淋巴细胞介导,造成胰岛β细胞破坏,使其丧失合成和分泌胰岛素的功能,进而引起糖代谢紊乱[1]。目前,T1DM仍以胰岛素治疗为主,如果治疗不及时则可能出现严重并发症,影响患者的生活质量并危及生命[2]。因此,寻找最佳的治疗方法是当今研究的热点。近年来,间充质干细胞(mesenchymal stem cells,MSC)治疗T1DM受到广泛关注[3-4]。MSC是一种来源于中胚层的具有自我更新和多向分化潜能的成体干细胞[5-6],具有低免疫原性和免疫调节等生物学功能[7-8],可作用于多种免疫细胞,如T和B淋巴细胞为主的适应性免疫细胞及巨噬细胞、自然杀伤细胞和树突状细胞等固有免疫细胞[9-10]。目前已有研究表明,MSC通过改善T1DM的免疫紊乱状态及保护残存的β细胞,进而有效改善血糖状态[11]。但具体机制仍不清楚。

巨噬细胞作为人体天然免疫的主要成员之一,

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可参与多种炎症性疾病的发生发展[12]。在不同微环境下,可分化为作用相反的2个亚群,即经典活化M1型和选择性活化M2型。M1型巨噬细胞激活后可分泌促炎症反应因子,如诱导型一氧化氮合酶(inducible nitric oxide synthase,iNOS),肿瘤坏死因子α(tumor necrosis factor-α,TNF-α),白细胞介素1β(interleukin-1β,IL-1β)和IL-12等;M2型巨噬细胞活化后可促进抗炎因子IL-10和IL-4及转化生长因子β(transforming growth factor β,TGF-β)分泌而抑制炎症发生,精氨酸酶1(arginase-1,Arg-1)是其表达的主要分子之一[13-14]。现有研究已表明,MSC可调控巨噬细胞表型,促进其由促炎M1型向抗炎M2型极化,从而抑制炎症反应,维持微环境的稳态[15-16];且移植MSC可通过募集M2型巨噬细胞使胰岛β细胞增殖和再生,进而影响T1DM的病理生理过程[17-18]。但MSC是否通过调节巨噬细胞极化,从而减轻T1DM模型小鼠的炎症反应仍不清楚。为此,本研究通过对T1DM模型小鼠进行MSC治疗,进一步探讨MSC调控巨噬细胞极化、减轻T1DM小鼠炎症反应的作用机制。

1 材料与方法

1.1 实验动物

30只6周龄雄性C57BL/6J小鼠,体质量为18~21 g,购自北京维通利华实验动物有限公司,动物合格证号:SCXK-(京)2016-0006。在12 h昼夜交替下,饲养于军事医学研究院实验动物中心;环境温度保持在22~25℃,相对湿度为35%~50%。另5只1周龄雄性C57BL/6J小鼠(购自北京维通利华)用于MSC的分离培养。所有的动物实验过程经院实验动物伦理委员会批准。

1.2 试剂和主要仪器

链脲佐菌素(streptozotocin,STZ)、Trizol、油红O、地塞米松、3-异丁基-1-甲基黄嘌呤(3-isobutyl-1-methylxanthine,IBMX)、胰岛素、β-磷酸甘油、维生素C磷酸盐、丙酮酸和碱性磷酸酶购自美国Sigma公司;柠檬酸、柠檬酸钠和10%中性甲醛购自国药集团化学试剂有限公司;牛血清白蛋白购自北京Solarbio科技有限公司;兔抗小鼠胰岛素抗体(一抗)购自美国Abcam公司;HRP-山羊抗兔IgG抗体(通用型,二抗)及DAB显色剂购自丹麦DAKO公司;α-MEM、0.25%胰酶、PBS及胎牛血清购自美国Gibco公司;流式抗体(抗小鼠Ia,CD11b,Sca-1,CD105,CD34,CD45,CD31和CD29抗体)购自美国ThermoFisher公司,APC-F4/80,PE-CD16/32和FITC-CD206抗体购自美国eBioscience公司;RTase M-MLV、RNA酶抑制剂及5×RTase M-MLV缓冲液购自日本TaKaRa公司;DTT、dNTP、无RNA酶水和2×Mltra Master Mix购自中国康为世纪有限公司;所用引物由安徽通用生物系统有限公司合成,引物序列见表1。

Tab.1 PCR primer sequence

Gene	Primer Sequence
CEBPα	F: 5′ACGAGACGTCTATAGACATCAG3′
	R: 5′AGGAACTCGTCGTTGAAGG3′
PPARγ	F: 5′GATGTCTCACAATGCCATCAG3′
	R: 5′ATATCACTGGAGATCTCCGC3′
Osteocalin	F: 5′GGAGTCTGTTCACTACCTTATTG3′
	R: 5′CTCTGTCTCTCTGACCTCAC3′
Runx2	F: 5′TGATGAGAACTACTCCGCC3′
	R: 5′GTGAAACTCTTGCCTCGTC3′
Arg-1	F: 5′GAACTGAAAGGAAAGTTCCCA 3′
	R: 5′AATGTACACGATGTCTTTGGC 3′
IL-10	F: 5′TTAATAAGCTCCAAGACCAAGG 3′
	R: 5′CATCATGTATGCTTCTATGCAG 3′
IL-1β	F: 5′CCTCAAAGGAAAGAATCTATACCTG 3′
	R: 5′CTTGGGATCCACACTCTCC 3′
IL-6	F: 5′TACCACTTCACAAGTCGGA 3′
	R: 5′AATTGCCATTGCACAACTC 3′
iNOS	F: 5′CCAACAATACAAGATGACCCT 3′
	R: 5′TTCTGGAACATTCTGTGCTG 3′
IFN-γ	F: 5′CACCTGATTACTACCTTCTTCAG3′
	R: 5′GTTGTTGACCTCAAACTTGG3′
TNF-α	F: 5′GGCAGGTCTACTTTFFAGTCATTGC 3′
	R: 5′ACATTCGAGGCTCCAGTGAATTCGG3′
TGF-β	F: 5′ACCAAGGAGACGGAATACAG3′
	R: 5′CGTTGATTTCCACGTGGAG3′
IL-4	F: 5′GATTCATCGATAAGCTGCACC3′
	R: 5′CATGATGCTCTTTAGGCTTTCC 3′
IL-12	F: 5′TCCCTCAAGTTCTTTGTTCG3′
	R: 5′CGCACCTTTCTGGTTACAC 3′
GAPDH	F: 5′ACTCTTCCACCTTCGATGC 3′
	R: 5′CCGTATTCATTGTCATACCAGG3′

CEBPα: CCAAT enhancer binding protein α; *PPARγ*: peroxisome proliferator activated receptor γ; *Runx2*: runt related transcription factor 2; *Arg-1*: arginase-1; *IL-10*: interleukin-10; *iNOS*: inducible nitric oxide synthase; *IFN-γ*: interferon-γ; *TNF-α*: tumor necrosis factor-α; *TGF-β*: transforming growth factor-β.

稳择易型血糖试纸和血糖仪购自美国强生公司;Qubit® 2.0荧光定量仪、台式低温冷冻离心机、7500 Real Time System、常温台式离心机及细胞培养箱购自美国Thermo Fisher公司;流式细胞仪

Facscalibur购自美国BD公司；光学显微镜购自日本Olympus公司。

1.3 骨实质来源MSC的分离培养和鉴定

在无菌条件下分离7日龄小鼠股骨及胫骨，用眼科镊将骨碎片转移至盛有1 mL Ⅱ型胶原酶的EP管中，37℃恒温消化45 min后，将骨碎片转移至含10%胎牛血清的α-MEM完全培养基中，采用骨片贴壁法培养小鼠骨片来源的MSC。传至第3代（P3），采用流式细胞术、体外成脂及成骨诱导分化等方法鉴定分离培养MSC[8]。流式细胞仪检测所用抗体为FITC-抗小鼠Ia，FITC-抗小鼠CD11b，FITC-抗小鼠Sca-1，PE-抗小鼠CD105，FITC-抗小鼠CD34，FITC-抗小鼠CD45，PE-抗小鼠CD31及PE-抗小鼠CD29。同时将细胞接种6孔板，用含10%胎牛血清的α-MEM培养基进行成脂（地塞米松1 μmol·L^{-1}，胰岛素10 μg·L^{-1}，IBMX 0.5 mmol·L^{-1}和吲哚美辛0.5 mmol·L^{-1}）及成骨（地塞米松0.1 μmol·L^{-1}，维生素C磷酸盐50 μmol·L^{-1}和β-磷酸甘油10 mmol·L^{-1}）诱导分化，分别培养8和10 d，用油红O染料工作液染色及实时荧光定量PCR（RT-PCR）检测成脂分化关键转录因子CCAAT增强子结合蛋白α（CCAAT enhancer binding protein alpha，CEBPα）和过氧化物酶体增殖物激活受体（peroxisome proliferator activated receptor gamma，PPARγ）mRNA表达。用碱性磷酸酶染料工作液染色及RT-PCR检测成骨分化关键转录因子骨钙素（osteocalcin）和Runt相关转录因子2（Runt related transcription factor 2，Runx2）mRNA表达。

1.4 1型糖尿病小鼠模型制备和MSC治疗

所有小鼠适应性喂养2 d后随机分为2组：正常对照组（*n*=10）和T1DM组（*n*=20）。正常对照组不做任何处理；T1DM组小鼠禁食12 h、禁水8~10 h后，ip给予含STZ 50 mg·kg^{-1}柠檬酸钠缓冲液，每日1次，连续5 d。第1次注射后，即刻恢复自由饮食；给药第12天，连续3 d通过尾静脉取血测随机血糖，血糖平均值≥16.7 mmol·L^{-1}即为建模成功。将建模成功的小鼠随机分为T1DM模型组（*n*=10）和MSC治疗组（*n*=10）。成模后第1和第15天，MSC治疗组由尾静脉注射MSC 5×10^5，正常对照组和T1DM模型组注射PBS；每周检测小鼠随机血糖和体质量。28 d后处死小鼠，用PBS反复灌洗腹腔，将灌洗液6000×*g*离心3 min，获腹腔巨噬细胞；随后取胰腺等组织待测。

1.5 胰腺组织HE染色和免疫组化检测

胰腺组织取出后，立即置10%中性甲醛溶液中固定，采用HE染色检测胰腺中胰岛组织形态是否饱满及胰岛细胞分布是否均匀，以判断病变程度[19]；采用免疫组化法检测胰岛素分泌水平及巨噬细胞（F4/80）数量[18]。

1.6 实时荧光定量PCR法检测腹腔巨噬细胞标志分子和炎症因子mRNA表达

Trizol法提取腹腔巨噬细胞总RNA，取1 μg RNA逆转录为cDNA；RT-PCR法检测腹腔巨噬细胞标志分子（iNOS和Arg-1）及炎症因子（IL-1β，IL-4，IL-6，IL-10，IL-12，IFN-γ，TNF-α和TGF-β）mRNA表达。反应体系为cDNA 1 μL，PCR上下游引物（10 μmol·L^{-1}）1 μL，q-PCR Master Mix 10 μL和无RNA酶水8 μL；反应条件为95℃ 10 min，（95℃ 15 s，60℃ 1 min）40个循环，95℃ 15 s，60℃ 1 min，95℃ 15 s。GAPDH为内参基因，采用$2^{-\triangle\triangle Ct}$表示待测基因mRNA表达水平。

1.7 流式细胞术检测腹腔巨噬细胞M1和M2表型

取腹腔巨噬细胞，加入流式抗体APC-抗小鼠F4/80，PE-抗小鼠CD16/32及FITC-抗小鼠CD206抗体，避光孵育30 min；加入PBS 1 mL洗涤，450×*g*，离心5 min，弃上清，加入4%多聚甲醛200 μL固定，上机检测。

1.8 统计学分析

实验结果数据以$\bar{x}\pm s$表示，用GraphPad Prism 6软件作图及进行数据分析。各组间差异采用方差分析（SNK进行两两比较）。P<0.05认为差异具有统计学意义。

2 结果

2.1 骨实质来源MSC的鉴定

分离培养的MSC呈均一的纤维样长梭形、旋涡状贴壁生长（图1A）。流式细胞仪检测结果表明，细胞高表达Sca-1，CD29和CD105；不表达或低表达CD31，CD34，CD45，Ia和CD11b（图1B）。油红O染色结果显示，分离培养的MSC成脂诱导后出现大量红色脂滴（图1C），且成脂关键转录因子CEBPα和PPARγ表达显著增加（P<0.01，图1D）。成骨诱导后，碱性磷酸酶活性增加（图1E），成骨关键转录因子骨钙素和Runx2表达亦显著增加（P<0.01，图1F）。上述结果提示，成功分离培养小鼠骨实质来源的MSC。

2.2 MSC治疗对T1DM模型小鼠随机血糖和体质量的影响

与正常对照组比较，T1DM模型组小鼠随机血糖显著升高（P<0.05）；与T1DM模型组相比，MSC治疗组小鼠随机血糖在治疗7 d后逐渐下降，治疗

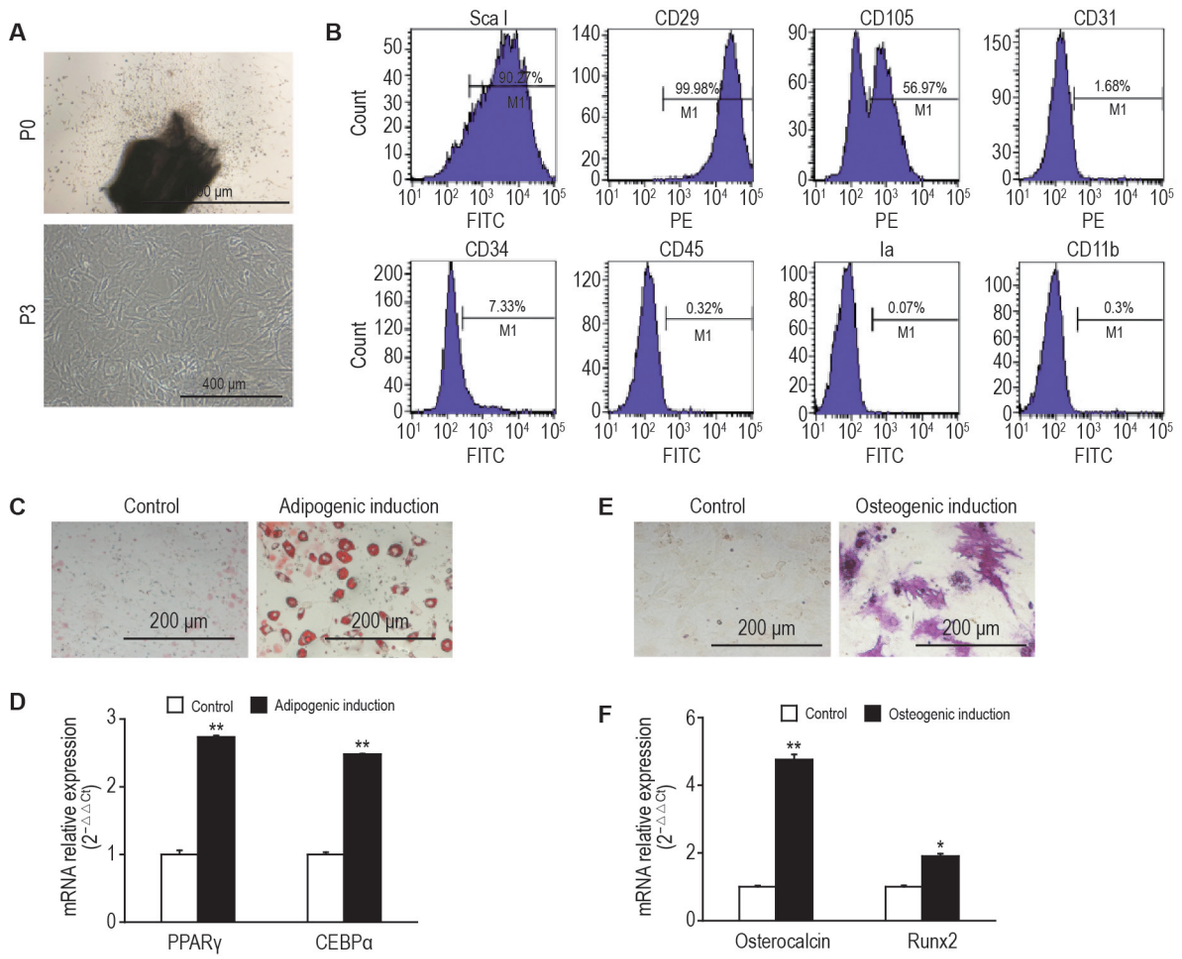

Fig.1 Pluripotency characteristics of mesenchymal stem cells (MSCs) derived from mouse compact bone. A: MSCs derived from murine compact bone were digested by collagenase and amplified to the passage 3 (P3) *in vitro*. Cell morphology was shown; B: expression status of surface molecules on MSCs was analyzed by flow cytometry; C: oil red O staining of adipocytes differentiated from MSCs; D: RT-PCR analyses of adipogenic markers in MSCs after adipogenic induction; E: alkaline phosphatase staining of osteoblasts differentiated from MSCs; F: RT-PCR analyses of osteogenic markers in MSCs after osteogenic induction. $\bar{x}\pm s$, n=3, $^{**}P$<0.01, compared with corresponding control group.

21 d后显著降低(P<0.05),28 d后降低(25.0±0.1)%(P<0.05),但未降至正常对照组水平(P<0.05)。与正常对照组相比,T1DM模型组小鼠体质量明显降低(P<0.05),MSC治疗组体质量在治疗后7 d内即开始增长,28 d后增加(12.0±0.4)(P<0.05)(图2)。

2.3 MSC治疗对T1DM模型小鼠胰岛素分泌和巨噬细胞数的影响

HE染色结果显示,T1DM模型组小鼠胰腺组织形态已完全被破坏,炎症细胞浸润明显较多;MSC治疗组胰腺组织形态较模型组改善,细胞分布大体

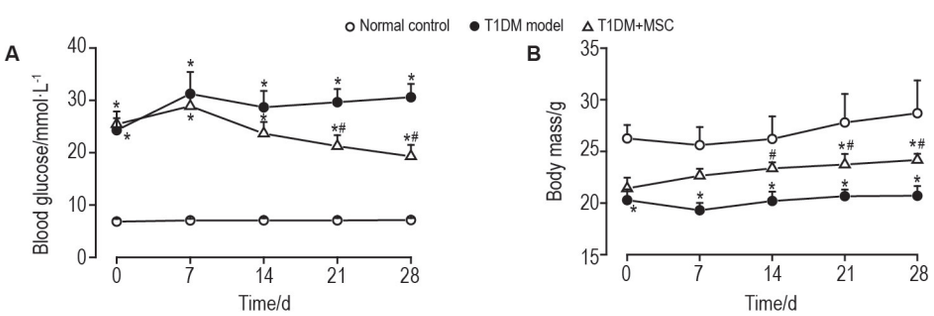

Fig.2 MSCs ameliorated non-fasting blood glucosed (A) and body mass (B) of streptozotocin (STZ)-induced type 1 diabetes mellitus (T1DM) model mice. T1DM was induced in male C57BL/6 mice using STZ 50 mg·kg^{-1} intraperitoneal injection, 5×10^5 MSCs were injected into mice via tail vein injection route on the 1st day and the 15th day after incidence of diabetes. Blood glucose concentration exceeding 16.7 mmol·L^{-1} in three consecutive daily measurements was considered diabetes. $\bar{x}\pm s$, n=10. $^*P$<0.05, compared with normal control group; $^{\#}P$<0.05, compared with T1DM model group.

均匀一致，炎症细胞浸润较少（图3A）。免疫组化结果表明，T1DM模型组小鼠与正常对照组相比胰岛素分泌显著减少；与模型组相比，MSC治疗组胰岛素分泌增多（图3B）；胰腺组织中F4/80阳性细胞数即巨噬细胞数量明显增加（图3C）。

2.4 MSC治疗对T1DM模型小鼠腹腔巨噬细胞炎症因子mRNA表达的影响

与正常对照组比较，T1DM模型组小鼠腹腔巨噬细胞促进炎症因子IL-1β，TNF-α，IL-6和IL-12 mRNA表达显著增加（P<0.05），IFN-γ无明显变化；抑制炎症因子IL-4，IL-10和TGF-β表达则显著降低（P<0.05）。与模型组相比，MSC治疗组炎症因子IL-1β，TNF-α，IL-6和IL-12 mRNA表达显著下降（P<0.05，P<0.01），抑制炎症反应IL-4，IL-10和TGF-β mRNA表达显著增加（P<0.05）（图4）。

2.5 MSC治疗促进T1DM模型小鼠M2型巨噬细胞极化

由图5所示，与正常对照组比较，T1DM模型组M1型巨噬细胞百分比升高到至（50.1±1.2）%（P<0.05），其标志分子iNOS mRNA表达升高（P<0.01）；M2型巨噬细胞百分比下降到（22.5±4.0）%，其标志分子Arg-1 mRNA表达下降（P<0.05）；M2/M1比值降低（P<0.05）。与T1DM模型组比较，MSC治疗组M1型巨噬细胞百分比降低至（15.6±1.2）%，iNOS mRNA表达降低（P<0.01）；M2型巨噬细胞百分比升高至（72.5±3.4）%，Arg-1 mRNA表达升高（P<0.01）；M2/M1比值升高（P<0.05）。

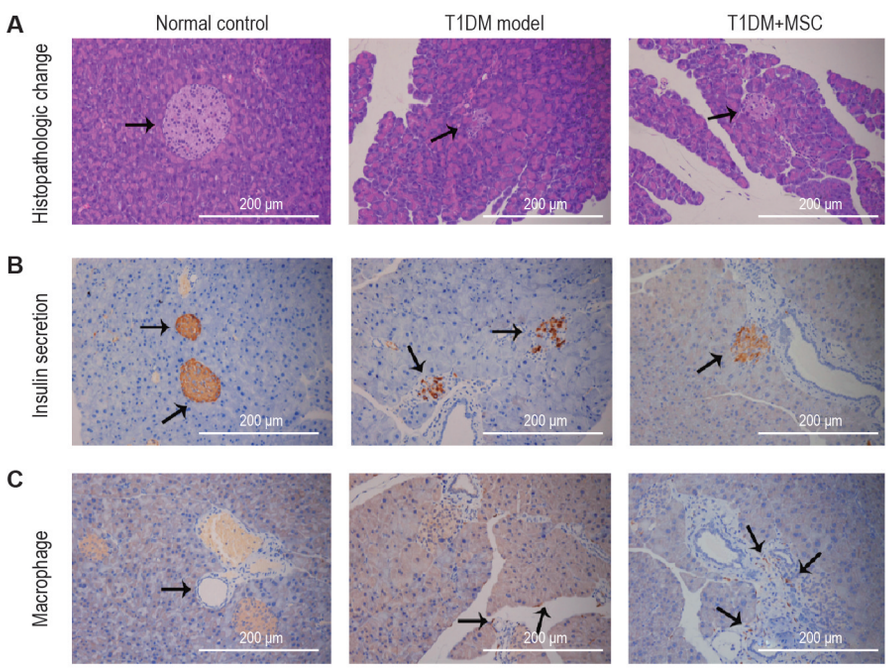

Fig.3 MSC treatment for 28 d decreased severity of insulitis and macrophage count in STZ-induced T1DM model mice. See Fig.2 for the mouse treatment. A: HE staining, the arrows show islet tissue; B: immunohistochemistry examination, the arrows show insulin secretion; C: immunohistochemistry examination, the arrows show the positive of F4/80.

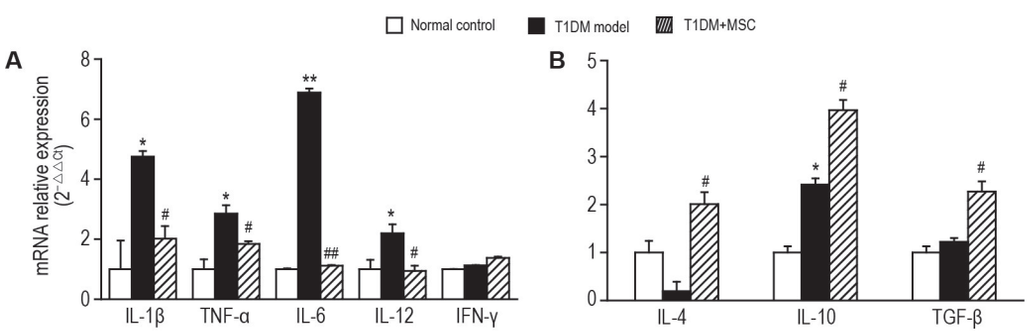

Fig.4 MSC treatment up-regulated anti-inflammatory factors and down-regulated proinflammatory factors expression on peritoneal macrophages in STZ-induced T1DM model mice detected by RT-PCR. See Fig.2 for the mouse treatment. A: proinflammatory factors; B: anti-inflammatory factor. $\bar{x}\pm s$, n=10. *P<0.05, **P<0.01, compared with normal control group; #P<0.05, ##P<0.01, compared with T1DM model group.

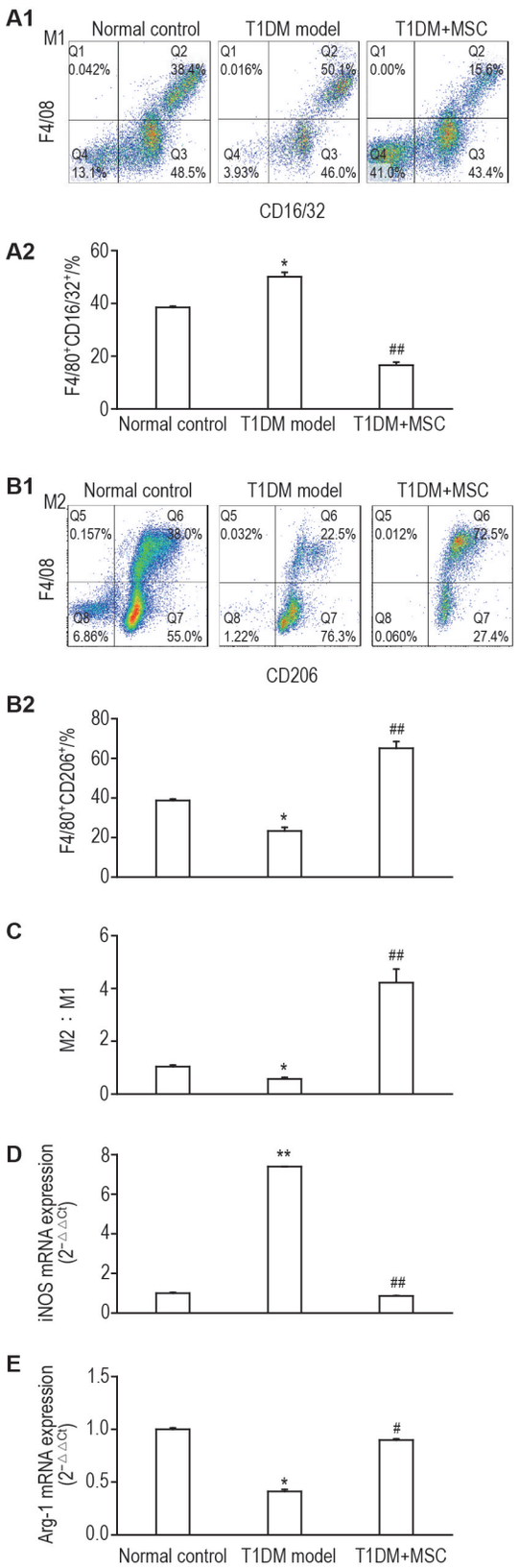

Fig.5 MSC treatment for 28 d promotes M2 macrophage polarization in STZ-induced T1DM model mice. See Fig.2 for the mouse treatment. A: population of M1 cells; B: population of M2 cells; C: ratios of M2/M1; D: mRNA expression of iNOS on M1 cells; E: mRNA expression of Arg-1 on M2 cells. $\bar{x} \pm s$, n=10. *P<0.05, **P<0.01, compared with normal control group; #P<0.05, ##P<0.01, compared with T1DM model group.

3 讨论

T1DM是青少年和儿童中常见的一种自身免疫性疾病，目前依旧是以胰岛素治疗为主，尽管随着胰岛素给药方式的改进，已能改善患者的生活质量和身心健康[19]，但尚不能有效的降低其并发症的发生率。目前研究发现，T1DM发病原因有很多，但主要病因是由于胰岛β细胞进行性破坏，进而导致胰岛素分泌不足和调节障碍。具体发病机制尚不清楚。但现有研究已证实，T1DM的发病机制涉及T细胞及多种固有免疫细胞如巨噬细胞等之间的相互调控，而这些免疫细胞数量的改变可造成胰岛β细胞的损伤或减少[19-20]。因此，机体免疫紊乱状态的纠正有望为T1DM的治疗提供新的思路和靶点[22]。

MSC作为一种多能的成体干细胞，具有多向分化潜能、低免疫原性和免疫调节能力[23]，可作用于多种免疫细胞，如T及B淋巴细胞为主的适应性免疫细胞及巨噬细胞、自然杀伤细胞及树突状细胞等固有免疫细胞[9-10]。现有实验研究已证实，MSC治疗可改善T1DM的免疫紊乱状态，但具体机制尚不清楚[24]。本研究发现，MSC治疗组小鼠腹腔巨噬细胞的炎症因子IL-1β，IL-6，IL-12和TNF-α mRNA表达下降，其中早期炎症因子IFN-γ mRNA表达无明显差异，而抑制炎症反应的IL-10，IL-4及TGF-β mRNA表达显著增加，提示MSC可通过巨噬细胞调节免疫，从而减轻糖尿病的炎症反应。IFN-γ表达无明显差异可能与检测时间等相关，需进一步研究。

巨噬细胞作为人体一种重要的固有免疫细胞，在抗击病原微生物及损伤修复等方面有重要作用，并能随微环境的变化而向不同亚群（M1和M2）进行分化。其中M1型巨噬细胞有助于宿主防御外来微生物的侵袭，但持续活化可造成机体慢性炎症的发生，此时若M2型巨噬细胞发生极化，可发挥抗炎的作用。但体内外实验表明，机体M1型巨噬细胞不易向M2型巨噬细胞进行极化。目前已有研究发现，MSC移植能调节巨噬细胞的表型，促进其从促炎的M1型向抗炎的M2型极化，从而发挥抑制炎症反应、维持微环境稳态的作用[15,26]。此外，也有动物实验结果表明，给T1DM小鼠移植MSC，可募集M2巨噬细胞，促使胰岛β细胞再生，以减轻糖尿病反应[27]。而巨噬细胞极化是否参与T1DM小鼠的炎症反应过程仍不清楚。为此本研究制备了T1DM模型小鼠，用MSC移植治疗，检测体内腹腔巨噬细胞的M2/M1比值，RT-PCR检测腹腔巨噬细胞相关炎症因子mRNA表达。结果显示，MSC治疗组小

鼠的血糖、体质量等生理指标均优于T1DM模型组。HE染色和免疫组化结果也显示，MSC治疗组胰岛损伤程度均优于模型组，说明MSC可治疗小鼠的T1DM。流式细胞术结果表明，MSC组M2/M1型巨噬细胞的比值显著高于T1DM模型组；RT-PCR比较分析两组小鼠腹腔巨噬细胞中M1和M2型巨噬细胞相关基因的表达水平。结果表明，相较于T1DM模型组，MSC治疗组M2型巨噬细胞相关基因Arg-1表达水平显著上升，M1型巨噬细胞相关基因iNOS表达水平显著下降。据报道，MSC免疫调节具有可塑性，其在T1DM模型小鼠中如何促进M2型巨噬细胞极化，以及糖尿病炎症微环境如何诱发MSC调节固有免疫细胞的炎症反应，仍需进一步探究。IL-1β作为固有免疫反应的重要因子，不仅可介导炎症反应，也可参与细胞凋亡过程；MSC治疗T1DM后IL-β分泌减少，同时可能抑制了其诱发凋亡反应，仍需深入研究，这也对MSC治疗T1DM疾病提供了新的研究方向。

综上所述，MSC移植可减轻T1DM模型小鼠的炎症反应，其机制可能与调控MI和M2巨噬细胞极化有关，MSC促进M2型巨噬细胞增多，抑制M1型巨噬细胞在小鼠T1DM模型中产生的炎症反应，从而减轻T1DM的发展。本研究为MSC治疗T1DM提供了新的理论依据，为深入了解MSC治疗T1DM的机制及临床应用提供有益参考。

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Mesenchymal stem cells alleviate inflammatory response by regulating macrophage polarization in type 1 diabetes mellitus mouse model

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Abstract: OBJECTIVE To explore the mechanism by which mesenchymal stem cells (MSCs) alleviate inflammatory response in the type 1 diabetes mellitus (T1DM) mouse model. **METHODS** To iden-

tify the MSCs isolated from 7-day-old mouse femur and tibia, the cell surface markers of Sca-1, CD29, CD105, CD31, CD34, CD45,1a and CD11b were examined by flow cytometry. Oil red O staining and alkaline phosphatase staining were performed to evaluate the adipogenic and osteogenetic differentiation activity of MSCs. The osteogenetic and adipogenic key transcription factors were analyzed by real-time quantitative PCR (RT-PCR). T1DM was induced in 6-week-old C57BL/6 male mice using ip injection of streptozotocin (STZ) 50 mg·kg^{-1}, once a day, for 5 d. To determine intervention effects, MSCs 5.0×10^5 were given through iv injection on the 1st day and the 15th day suuessful preparation. Randomized blood glucose and body mass of each group of mice were monitored weekly after the first MSC treatment. Pancreas glands and peritoneal macrophages were harvested in the T1DM model for 4 weeks. The pathological response of pancreas, insulin expression and the percentage of M2 and M1 macrophages were examined by HE staining, immunohistochemistry and flow cytometry, respectively. Moreover, the expressions of macrophage marker molecules such as inducible nitric oxide synthase (iNOS) and arginase 1 (Arg-1) and related inflammatory factors such as interleukin-1β (IL-1β), IL-4, IL-6, IL-10, IL-12, interferon gamma (IFN-γ)、tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β) were detected by RT-PCR. **RESULTS** The murine compact bone-derived MSCs had high expressions of Sca-1, CD29 and CD105, and negative expressions of CD31, CD34, CD45, 1a and CD11b. After adipogenic differentiation, a large number of lipid droplets appeared, and the key transcription factors of CCAAT enhancer binding protein-α (CEBPα) and peroxisome proliferator-activated receptor-γ (PPARγ) mRNA expression were significantly increased (P<0.01). For osteogenic induction, the alkaline phosphate enzyme activity was increased, and the osteogenic key transcription factors of osteocalcin and Runt-related transcription factor 2 (Runx2) mRNA expression were also increased significantly (P<0.01). The random blood glucose concentration of T1DM+MSC group was decreased by (25.0±0.1)% and body mass was increased by (12.0±0.4)% (P<0.05) compared with the T1DM model group. Pathologic examination at the MSC infusion for 4 weeks demonstrated that MSC infusion could promote the insulin secretion and increase the number of macrophages. Moreover, the inflammatory cytokine level (IL-1β, IL-6, IL-12 and TNF-α mRNA) was significantly down-regulated (P<0.01), while the level of anti-inflammatory cytokine (IL-10, IL-4 and TGF-β mRNA) was up-regulated in the macrophages of the T1MD+MSC group compared with that of T1MD model group (P<0.01). Flow cytometry results showed that the percentage of M2 macrophages in T1DM+MSC group was increased from (22.5±4.0)% to (72.5±3.4)% and M1 macrophages was decreased from (50.1±1.2) to (15.6±1.2)% (P<0.05). The expression of M1 macrophage marker iNOS was reduced, but M2 macrophage marker molecule Arg-1 was increased (P<0.05). **CONCLUSION** MSCs can alleviate the inflammation of T1DM model mice. The mechanism could be related to regulation by the M1/M2 macrophage polarization.

Key words: mesenchymal stem cells; macrophages; type 1 diabetes mellitus; inflammatory factors

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