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间质干细胞培养上清对日本血吸虫SEA诱导活化的巨噬细胞株RAW264.7的抑制作用

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Inhibition of Culture Supernatant of Mesenchymal Stem Cells on Macrophages RAW264.7 Activated by Soluble Egg Antigen of *Schistosoma japonicum*

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摘要 | 参考文献 | 相关文章

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摘要 目的 观察大鼠骨髓间质干细胞（mesenchymal stem cell，MSC）培养上清对日本血吸虫可溶性虫卵抗原（soluble egg antigen，SEA）诱导活化的巨噬细胞的抑制作用。 方法 用5、10、20和40 μg/ml SEA分别诱导巨噬细胞株RAW264.7 12 h，或用20 μg/ml SEA分别诱导小鼠巨噬细胞株（RAW264.7）4、8、12和24 h后，用实时荧光定量PCR检测α肿瘤坏死因子（TNF-α）的mRNA水平，选择SEA的最佳作用浓度和作用时间。将巨噬细胞分成5组，分别为阴性对照组、SEA组、SEA+MSC上清组（MSC组）、SEA+大鼠肾小管上皮细胞株（NRK-52E）上清组（NRK-52E组）和SEA+DMEM细胞培养基组（DMEM组）。除阴性对照组外，其他各组给予20 μg/ml SEA诱导巨噬细胞活化12 h后，MSC组、NRK-52E组和DMEM组换液撤SEA，分别给予MSC培养上清、NRK-52E细胞培养上清和DMEM培养液，继续培养。显微镜观察细胞上清培养12 h后各组细胞形态。实时荧光定量PCR检测细胞上清培养12 h和24 h后TNF-α mRNA水平。蛋白质印迹（Western blotting）分析检测细胞上清培养12 h后转化生长因子β1（TGF-β1）的蛋白表达水平。噻唑蓝比色法（MTT法）测定细胞上清培养24 h和48 h后巨噬细胞增殖情况。 结果 SEA活化巨噬细胞的最佳浓度和时间分别为20 μg/ml和12 h。镜下观察显示，MSC上清培养12 h后，MSC组与SEA组、NRK?鄄52E组和DMEM组相比，细胞变圆，体积明显较小，伪足较少。MSC上清作用12 h和24 h后，MSC组TNF-α mRNA水平分别为阴性对照组的（1.0±0.4）和（1.0±0.5）倍，显著低于NRK-52E组［分别为（10.4±3.9）和（16.5±5.0）倍（12 h：P<0.05；24 h：*P*<0.01）］和DMEM组［分别为（6.0±2.1）和（2.4±0.7）倍（均*P*<0.05）］。MSC上清作用12 h后，MSC组蛋白TGF?鄄β1/GAPDH为0.31±0.10，显著低于NRK-52E组（0.88±0.10，*P*<0.01）和DMEM组（0.58±0.06，*P*<0.05）。MSC上清作用48 h后，MSC组吸光度（A490值）为0.22±0.05，与NRK-52E组（0.53±0.02）和DMEM组（0.31±0.03）比较差异均有统计学意义（均*P*<0.05）。 结论 MSC培养上清能抑制SEA诱导的巨噬细胞株RAW264.7活化。

关键词： 间质干细胞 日本血吸虫 巨噬细胞 可溶性虫卵抗原

Abstract： Objective To observe the inhibitive effect of rat mesenchymal stem cells (MSC) culture supernatant on macrophages activated by soluble egg antigen （SEA） of *Schistosoma japonicum*. Methods To select optimal SEA effecting concentration and time，macrophages RAW264.7 were induced by 5，10，20 or 40 μg/ml SEA for 12 h，or by 20 μg/ml SEA for 4，8，12 or 24 h before examination of TNF-α mRNA by RT-PCR. Macrophages were divided into five groups，i.e. negative control group，SEA group，SEA+MSC supernatant group （MSC group），SEA+NRK-52E supernatant group （NRK-52E group） and SEA+DMEM group （DMEM group）. Except negative control group，macrophages in other four groups were induced by 20 μg/ml SEA for 12 h. SEA was then removed from MSC group，NRK-52E group and DMEM group and replaced with MSC supernatant，NRK-52E supernatant and DMEM，respectively. Morphology of macrophages in each group was observed by microscope after cultured with supernatant for 12 h. TNF-α mRNA in macrophages was detected by real-time quantitative PCR after cultured with supernatant for 12 h and 24 h. TGF-β1 in macrophages was observed by Western blotting analysis after cultured with supernatant for 12 h. Macrophage proliferation was tested by MTT method after cultured with supernatant for 24 h and 48 h. Results The optimal SEA concentration and time for macrophage activation was 20 μg/ml and 12 h，respectively. Compared with SEA group，NRK-52E group，and DMEM group，macrophages in MSC group were round and small with less pseudopodia after cultured with supernatant for 12 h. TNF-α mRNA after cultured with MSC supernatant for 12 h and 24 h was （1.0±0.4） and （1.0±0.5） fold of negative control group，respectively，significantly less than NRK-52E group ［（10.4±3.9） and （16.5±5.0） fold］ （12 h: P<0.05；24 h：*P*<0.01） and DMEM group ［（6.0±2.1） and （2.4±0.7） fold］ （*P*<0.05）. The grey density image analysis of TGF-β1/GAPDH was 0.31±0.10 in MSC group，much lower than 0.88±0.10 in NRK-52E group （P<0.01） and 0.58±0.06 in DMEM group （*P*<0.05） after cultured with supernatant for 12 h. After 48 h culture，A490 of macrophages in MSC group was 0.22±0.05，much lower than 0.53±0.02 in NRK-52E group and 0.31±0.03 in DMEM group （*P*<0.05）. Conclusion MSC supernatant can inhibit activation and proliferation of macrophages which were induced by SEA of *S. japonicum*.

Keywords： Mesenchymal stem cell *Schistosoma japonicum* Macrophage Soluble egg antigen

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