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不同棘球蚴抗原诱导树突状细胞表达吲哚胺 2，3-双加氧酶的实验研究

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Different Echinococcus granulosus Antigens Induced Indoleamine 2，3-dioxygenase Expression in Dendritic Cells

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摘要 目的 检测不同的棘球蚴抗原体外诱导树突状细胞（DCs）表达吲哚胺2，3-双加氧酶（IDO）的情况。 方法 从C57BL/6小鼠股骨中分离出骨髓细胞，用小鼠重组巨噬细胞集落刺激因子（rmGM-CSF）诱导后，获得小鼠骨髓源树突状细胞（BMDCs）。分别用重组抗原B（rAgB，15 μg/ml）、小鼠细粒棘球蚴囊液（MHF，5 mg/ml）、γ干扰素（IFN-γ，1 000 U/ml，为阳性对照）和RPMI 1640完全培养液（阴性对照）刺激DCs，于18、24和48 h后收集细胞和细胞上清。采用流式细胞术检测各组DCs的表面标志物CD40、CD80、CD86和I-A/I-E的阳性表达情况，并利用实时荧光定量PCR（FQ-RT-PCR）检测各组的IDO mRNA相对转录水平。利用高效液相色谱法（HPLC）检测各组细胞上清中的色氨酸（Try）浓度。 结果 流式细胞术检测结果显示，DCs受rAgB和MHF刺激后，其表面标志物CD40、CD80、CD86和I-A/I-E的阳性表达率均降低。刺激24 h后，rAgB组的CD40、CD86和I-A/I-E 阳性表达率分别为（22.60±2.69）%、（35.50±4.38）%和（57.30±4.38）%，与MHF组［（38.00±3.54）%、（53.00±3.39）%和（77.10±1.70）%］和阴性对照［（37.95±3.61）%、（19.55±1.06）%和（85.45±1.63）%］的差异均有统计学意义（P<0.05）。FQ-RT-PCR结果显示，刺激18、24和48 h后，rAgB组的IDO mRNA水平［（9.20±0.01）、（29.44±0.02）和（16.48±0.04）］和MHF组的［（9.67±0.02）、（17.52±0.01）和（16.81±0.01）］均高于阴性对照组［（2.46±0.01）、（7.77±0.01）和（10.56±0.01）］（P<0.01），rAgB组和MHF组IDO mRNA水平的差异均有统计学意义（P<0.05）。HPLC结果显示，刺激18、24和48 h后，rAgB组DCs上清中的色氨酸浓度［（23.65±0.64）、（13.95±1.06）和（19.05±0.64）μmol/L］均较其他3组低，刺激24 h后的色氨酸浓度与阴性对照组（22.90±0.14）和MHF组（20.65±0.35）的差异均有统计学意义（P<0.05）。 结论 在体外实验条件下，rAgB、MHF均可上调DCs表面IDO的表达，作用24 h后，rAgB上调IDO的能力强于MHF。

关键词： 细粒棘球蚴 抗原 树突状细胞 吲哚胺2 3-双加氧酶 免疫逃避

Abstract: Objective To observe the expression of indoleamine 2, 3-dioxygenase （IDO） in dendritic cells （DCs） via different Echinococcus granulosus antigens in vitro. Methods Bone Marrow DCs generated from bone marrow precursor cells of C57BL/6 mice and cultured in the presence of recombinant mouse GM-CSF（rmGM-CSF）. Then, DCs were induced with 15 μg/ml recombinant antigen B （rAgB）, 5 mg/ml mouse hydatid fluid （MHF）, 1 000 U/ml IFN-γ （as positive control）, and RPMI 1640 complete medium（as negative control）, respectively. Meanwhile, the treated DCs and cell supernatants were collected at 18, 24 and 48 h after induction. The positive expressions of D40, CD80, CD86 and I-A/I-E on DCs were determined by flow cytometry. By real-time fluorescent quantitative reverse-transcription polymerase chain reaction（FQ-RT-PCR）, the expression level of IDO mRNA in DCs was measured. Concentrations of tryptophan （Try） were tested by high-performance liquid chromatography （HPLC） assay in cell supernatant. Results The data from flow cytometry showed that the positive expressions of CD40, CD80, CD86, I-A/I-E were decreased after stimulated by rAgB and MHF. At 24 h after induction, there was significant difference in the level of CD40, CD86 and I-A/I-E among rAgB-treated group［（22.60±2.69）%,（35.50±4.38）%,（57.30±4.38）%］, MHF-treated group［（38.00±3.54）%,（53.00±3.39）%,（77.10±1.70）%］ and negative control［（37.95±3.61）%,（19.55±1.06）% and（85.45±1.63）%］（P<0.05）. At 18, 24 and 48 h after induction, the levels of IDO mRNA in rAgB-treated group［（9.20±0.01）,（29.44±0.02）,（16.48±0.04）］ and MHF-treated group［（9.67±0.02）,（17.52±0.01）,（16.81±0.01）］ was higher than that of negative control group［（2.46±0.01）,（7.77±0.01）, and（10.56±0.01）］（P<0.01）. And significant difference was found between rAgB-treated group and MHF-treated group（P<0.05）. At 18, 24 and 48 h after induction, the concentrations of Try were lowest in rAgB-treated group［（23.65±0.64）,（13.95±1.06）,（19.05±0.64） μmol/L］. At 24 h after induction, Try concentration in negative control group（22.90±0.14） was higher than that of MHF-treated group （20.65±0.34）（P<0.05）. Conclusion Under in vitro condition, rAgB and MHF can up-regulate IDO expression. The ability of rAgB to up-regulate IDO activity was stronger

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