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论著

细粒棘球绦虫重组Bb-Eg95-EgA31蛋白对感染小鼠脾细胞凋亡的抑制作用

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摘要
目的 探讨细粒棘球绦虫（Eg）重组蛋白Bb?鄂Eg95?鄂EgA31免疫小鼠被Eg原头节攻击感染后小鼠体内生成的棘球蚴囊重减少率及脾细胞凋亡的变化。 方法 56只雌性BALB/c小鼠随机均分7组，分别为重组蛋白皮下注射组（A组）、 肌肉注射组（B组）、 鼻腔内接种组（C组）、 灌胃组（D组）、 空载体对照组（E组）、 双歧杆菌对照组（F组）和双歧杆菌液体培养基（MRS）对照组（G组）。A、B和D组分别以5×106、5×106和5×108蛋白克隆形成单位（CFU）悬浮于100 μl MRS免疫小鼠，C组以5×105蛋白克隆形成单位（CFU）悬浮于10 μl MRS免疫小鼠，E和F组分别以空载体［Bb（pGEX-1λT）］和Bb 5×106 CFU悬浮于100 μl MRS皮下注射小鼠，G组以100 μl MRS皮下注射小鼠。8周后各组均用Eg原头节（50个/只）攻击感染，25周后剖杀小鼠，分离细粒棘球蚴包囊并称重，计算囊重减少率；取脾，分离脾细胞，伴刀豆球蛋白（ConA）刺激培养，流式细胞仪检测脾细胞的凋亡发生率。结果 A、B、C和D组小鼠的棘球蚴囊重分别为（41.0±23.0）mg、（44.0±22.0）mg、（22.0±21.0）mg和（28.0±16.0）mg，均低于G组（75.0±33.0）mg（P＜0.05，P＜0.01），A、B、C组与D组间囊重差异无统计学意义（P＞0.05）； E组（63.0±30.0）mg、 F组（69.0±22.0）mg和G组间囊重差异无统计学意义（P＞0.05）。未加ConA培养的脾细胞凋亡发生率， A（0.14±0.01）、 B（0.14±0.01）、 C（0.13±0.01）和D组（0.14±0.01）均低于G组（0.21±0.01）（P＜0.05）； C组低于A、 B和D组（P＜0.05）； E组（0.20±0.01）、 F组（0.20±0.01）和G组间差异无统计学意义（P＞0.05）；加ConA培养后的脾细胞凋亡发生率，A（0.19±0.01）、 B（0.20±0.00）、 C（0.17±0.01）和D组（0.19±0.01）均显著低于G组（0.26±0.01）（P＜0.01），C组低于A和B组（P＜0.01）， C组低于D组（P＜0.05）， D组低于B组（P＜0.05），A组与B组、D组间差异无统计学意义（P＞0.05）， E组（0.25±0.01）、 F组（0.25±0.01）和G组间差异无统计学意义（P＞0.05）。各组小鼠加ConA培养的脾细胞凋亡率显著高于相应的未加ConA培养组（P＜0.01）。 结论 Eg原头节感染可引起小鼠脾细胞凋亡，用Eg重组蛋白Bb?鄂Eg95?鄂EgA31免疫小鼠在一定程度上可抑制感染鼠脾细胞的凋亡，诱导小鼠产生一定的保护力。

关键词 细粒棘球绦虫；重组蛋白；脾细胞；凋亡

分类号

Inhibition on Apoptosis of Splenocytes in Infected Mice by Immunization with Recombinant Bb-Eg95-EgA31 Protein of *Echinococcus granulosus*

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Abstract
Objective To investigate the weight reduction of hydatid cysts and apoptosis of splenocytes in infected mice by recombinant Bifidobacteria bifidum（Bb）-Eg95-EgA31 protein of Echinococcus granulosus（Eg）and challenged with Eg protoscoleces. Methods 56 female BALB/c mice were randomly divided into 7 groups. Groups A and B were injected subcutaneously and intramuscularly respectively with 5×106 colony-forming unit （CFU） recombinant Bb-Eg95-EgA31 protein, group C was immunized intranasally by 5×105 CFU protein, group D was vaccinated transgastrically by 5×108 CFU protein, groups E and F were injected subcutaneously with 5×106 CFU blank vector［Bb（pGEX-1λT）］ and Bb respectively, and group G was injected subcutaneously with 100 μl MRS. Mice in all groups were challenged with 50 Eg protoscoleces on the 8th week after vaccination and sacrificed on the 25th week after

infection. The weight of hydatid cysts was measured and weight reduction rate was calculated. Spleens were collected to prepare splenocytes which were cultured under stimulation with concanavalin A（ConA）. The apoptotic rate was determined by flow cytometry （FCM）. Results The average weight of hydatid cysts in groups A ［（41.0±23.0）mg］, B ［（44.0±22.0）mg］, C ［（22.0±21.0）mg］, and D ［（28.0±16.0）mg］ was lower than that of group G［（75.0±33.0）mg］ （P＜0.05, P＜0.01）, and there was no significant difference among groups A, B, C and D （P＞0.05）; no significant difference was found between group G and groups E ［（63.0±30.0）mg］, F［（69.0±22.0）mg］ （P＞0.05）. The apoptotic rate of splenocytes cultured with no ConA in groups A（0.14±0.01）, B（0.14±0.01）, C （0.13±0.01）, and D（0.14±0.01）was lower than that of group G（0.21±0.01） （P＜0.05）; that of group C was lower than groups A, B, and D（P＜0.05）; there was no significant difference between groups D and A, between groups A and B, and between groups E（0.20±0.01）, F（0.20±0.01）and group G. The apoptotic rate of splenocytes cultured with ConA in group A（0.19±0.01）, B（0.20±0.00）, C（0.17±0.01）, and D （0.19±0.01）were lower than that of group G（0.26±0.01）（P＜0.01）, that of group C was lower than groups A and B(P＜0.01), group C was lower than group D(P＜0.05), group D was lower than group B(P＜0.05); there was no significant difference between groups A and B,and between groups A and D, and between groups E（0.25±0.01）, F （0.25±0.01）, and group G（P＞0.05）. The apoptotic rate of splenocytes cultured with ConA was higher than those cultured without ConA （P＜0.01）. Conclusions Apoptosis of splenocytes may be induced by infection of Echinococcus granulosus protoscoleces in mice， while the recombinant Bb-Eg95-EgA31 protein may inhibit the apoptosis of splenocytes in mice challenged with Eg， and induce certain protective immunity in the host.

Key words *Echinococcus granulosus*；Recombinant protein；Splenocyte；Apoptosis

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