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弓形虫ESA诱导小鼠产生的CD8+ T细胞对早期黑色素瘤生长的影响

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Role of CD8+ T cells in the Tumor Growth Delay Induced by Toxoplasma gondii Excreted-secreted Antigen in B16F10 Mouse Melanoma Model

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摘要 目的 观察弓形虫排泄分泌抗原（TgESA）对B16F10黑色素瘤接种小鼠CD8+ T细胞的影响，以及CD8+ T细胞对B16F10黑色素瘤的作用。 方法 取RH株弓形虫速殖子体外培养12 h后，收集培养液，离心取上清，获得弓形虫ESA。15只C57BL/6小鼠随机分为A、B和C三组（每组5只）。B组和C组每鼠右腋窝皮下接种B16F10黑色素瘤细胞2×105个，A组注射等量无菌PBS。接种后第7天，C组每只小鼠腹腔注射TgESA 100 μl。接种后第13天，处死小鼠，无菌取脾。流式细胞仪检测各组小鼠CD3+CD8+ T细胞所占脾细胞比例；采用免疫磁珠分选B组和C组脾细胞中的CD8+ T细胞，乳酸脱氢酶（LDH）释放法检测两组CD8+ T细胞对B16F10细胞的杀伤作用，分别设效靶细胞比为2.5 ： 1、5 ： 1和10 ： 1。另取30只C57BL/6小鼠随机分为E、F和G三组（每组10只），每鼠接种B16F10细胞2×105个。接种同时，F组和G组小鼠分别尾静脉注射分离自B组和C组的CD8+ T细胞(2×105个/鼠)。观察小鼠瘤体生长情况，记录各组小鼠死亡数和死亡时间，共观察35 d。 结果 流式细胞仪检测结果显示，A、B和C组脾脏CD3+CD8+ T细胞占脾细胞的比例分别为（13.86±0.13）%、（14.18±0.27）%和（15.74±0.28）%，C组显著高于其他两组（P<0.05）。LDH释放试验结果显示，不同效靶细胞比时，C组的CD8+ T细胞杀伤活性均显著高于B组（均P<0.05）。G组的平均出瘤时间［（14.9±1.2）d]晚于F组［（11.9±0.7）d]和E组［（9.4±1.2）d］（P<0.05）。3组小鼠自出瘤后瘤体均不断增长，但增长速度无明显差异，G组的瘤体面积始终小于其他两组（均P<0.05）。E、F和G组小鼠分别于B16F10细胞接种后第26、29和30天开始出现死亡，至观察终点(第35天)，3组小鼠存活的数量分别为3、5和7只。 结论 TgESA可上调B16F10黑色素瘤小鼠CD8+ T细胞的数量和杀伤功能，上调的CD8+ T细胞具有早期延缓肿瘤生长的作用。

关键词： 刚地弓形虫 排泄分泌抗原 CD8+ T细胞 黑色素瘤

Abstract： Objective To observe the role of CD8+ T cells in the tumor growth delay induced by Toxoplasma gondii excreted-secreted antigens （TgESA） in B16F10 mouse melanoma model in the early stage. Methods TgESA were prepared by incubating T. gondii tachyzoites for 12 h in vitro. 15 C57BL/6 mice were randomly assigned to group A, B, and C （5 mice per group）. Each mouse in group B and C was subcutaneously injected in right flank with 2×105 B16F10 cells. Mice in group C were intraperitoneally injected with TgESA （100 μl per mouse） at 7 d after B16F10 cells injection. Mice of group A were only injected with PBS. On the 13th day after melanoma cell injection, the mice were sacrified and spleen was removed. The percentage of CD8+ T cells in the spleen was analyzed by flow cytometry. CD8+ T cells were isolated from spleen cells by using immunomagnetic beads. The activity of CD8+ T cells against B16F10 melanoma cells was determined by LDH release assay at different effect-to-target cell ratios （2.5 ： 1, 5 ： 1, and 10 ： 1）. Other 30 C57BL/6 mice were randomly divided into group E, F, and G. Each mice were injected with 2×105 B16F10 cells. At the same time, mice in group F and G were simultaneously injected via the tail vein with CD8+ T cells isolated from mice in group B and C. Tumor growth, mortality and survival time of mice were observed and recorded during 35-d observation period. Results The percentage of CD3+CD8+ T cells in the spleen cells of group C［（15.74±0.28）%］ was significantly higher than that of group B［（14.18±0.27）%］ and A ［（13.86±0.13）%］（P<0.05）. At different effect-to-target cell ratios, the activity of CD8+ T cells against B16F10 cells in group C was significantly higher than that of group B （P<0.05）. The average time of tumor formation in group G ［（14.9±1.2） d］ was longer than that in group F ［（11.9±0.7） d］ and E ［（9.4±1.2） d］（P<0.05）. The tumor size in these groups increased, but there was no obvious difference in the tumor growth rate among the three groups. The tumor size of group G was significantly smaller than the other two groups （P<0.05）. In group E, F and G, mice began to die on the 26th day, the 29th day and the 30th day after tumor inoculation, and the number of survival mice was 3, 5 and 7, respectively, at the 35th day after injection. Conclusions TgESA may up-regulate the quantity and function of CD8+ T cell in B16F10 melanoma mouse model, which plays a role of delaying tumor growth in early stage.

Keywords： Toxoplasma gondii Excreted-secreted antigens CD8+ T cell Melanoma

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