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刚地弓形虫感染对大鼠胸腺细胞的影响

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Effect of Toxoplasma gondii Infection on Thymus Cells in Rats

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摘要

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摘要 目的 研究刚地弓形虫感染对大鼠胸腺的病理损伤和胸腺细胞凋亡的影响。方法 将7~8周龄Wistar雄性大鼠50只随机分为感染组(40只)和对照组(10只)。感染组腹腔注射弓形虫速殖子 5×10^4 /只,对照组注射等量PBS,分别于感染后第3、6、9和12天取感染组10只和对照组2只,麻醉后摘取胸腺。部分胸腺用于常规制片和苏木素-伊红(HE)染色;制备胸腺单细胞样品,流式细胞仪分析细胞周期,计算细胞增殖指数;制备胸腺冰冻切片,经Hoechst 33258细胞核染色,荧光显微镜下观察胸腺细胞的凋亡情况;免疫组化检测胸腺细胞凋亡相关蛋白Bcl-2和Bax的表达水平。结果 HE染色结果发现,感染组于大鼠感染后第3天即出现病理学改变,胸腺被膜间隙增宽,皮质和髓质细胞稀疏,可见较多吞噬细胞和淤血;至感染后第6天损伤加重;第12天未见明显改善。感染后第3、6、9和12天胸腺细胞增殖指数分别为 $(11.15 \pm 0.99)\%$ 、 $(6.17 \pm 1.02)\%$ 、 $(5.45 \pm 0.96)\%$ 和 $(6.63 \pm 1.52)\%$,均显著低于对照组 [$(13.81 \pm 1.18)\%$] (均 $P < 0.01$)。细胞核染色结果显示,感染后第3天凋亡细胞增多,第6天凋亡细胞明显增多,第6~12天凋亡细胞数量差异不大。免疫组化结果显示,感染后第3、6、9和12天胸腺组织Bax蛋白阳性细胞灰度值分别为 88.21 ± 4.74 、 64.69 ± 6.82 、 83.62 ± 5.79 和 101.09 ± 6.72 ,均显著低于对照组 (128.69 ± 8.95) (均 $P < 0.01$),表明感染后大鼠胸腺的Bax蛋白水平均显著增加。大鼠感染弓形虫后胸腺细胞Bcl-2表达无明显变化 ($P > 0.05$)。结论 弓形虫感染可导致中枢免疫器官胸腺的病理损伤、细胞增殖水平下降、凋亡细胞增多,并伴有凋亡蛋白Bax的高表达。

关键词: 刚地弓形虫 胸腺 细胞凋亡

Abstract: Objective To study the pathological damage of thymus and thymus cell apoptosis of male rats infected with *Toxoplasma gondii*. Methods Fifty Wistar male rats (7-8-week-old) were randomly divided into infection group (40) and control group (10). Rats in infection group were infected with 5×10^4 tachyzoites by intraperitoneal injection, while those in control group received same volume of PBS. On the 3rd, 6th, 9th and 12th day post infection, ten rats from infection group and two from control group were sacrificed, the thymus glands were removed. The thymus tissue sections were stained with hematoxylin and eosin (HE) for observation on histopathological changes. Single thymus cell suspensions were prepared. Cell cycle analysis was performed by flow cytometry, and proliferation index was calculated. Thymus frozen sections were stained with Hoechst 33258, and morphologic changes in apoptotic nuclei were observed under fluorescence microscope. Expression of Bcl-2 and Bax proteins were determined by using immunohistochemistry. Results Microscopic examination showed that pathological changes occurred in thymus gland on the 3rd day after infection. The space between connective tissue capsules was widened, cells in cortex and medulla cells were sparse, and more phagocytes and extravasated blood were found in thymus. On the 6th day post infection the thymus damage was aggravated, and no significant improvement was seen on day 12. On the 3rd, 6th, 9th and 12th day after infection, thymocyte proliferation index was $(11.15 \pm 0.99)\%$, $(6.17 \pm 1.02)\%$, $(5.45 \pm 0.96)\%$ and $(6.63 \pm 1.52)\%$, respectively, and each of them was significantly lower than that of the control [$(13.81 \pm 1.18)\%$] ($P < 0.01$). On the 3rd day after infection, the number of apoptotic cells increased, significantly increased on day 6, and there was no much difference in the number of apoptotic cells between day 6 and day 12. The immunohistochemistry results showed that on the 3rd, 6th, 9th and 12th day post-infection, the gray scale value of Bax positive cells was 88.21 ± 4.74 , 64.69 ± 6.82 , 83.62 ± 5.79 , and 101.09 ± 6.72 , respectively, and each of them was significantly lower than that of the control (128.69 ± 8.95) ($P < 0.01$), while there was no significant change in the Bcl-2 protein level ($P > 0.05$). Conclusion *T. gondii* causes severe pathological damage in host thymus tissue with a decrease in the proliferation index, an increase in the number of apoptotic cells, and high expression of Bax protein.

Keywords: *Toxoplasma gondii* Thymus Apoptosis

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