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腺病毒介导MHC Ⅱ类分子反式激活因子突变体基因治疗小鼠实验性自身免疫性甲状腺炎 点此下载全文

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摘要：

目的：观察腺病毒介导MHC Ⅱ类分子(major histocompatibility complex class Ⅱ molecules)反式激活因子突变体(CⅡTAm)基因治疗小鼠实验性自身免疫性甲状腺炎(experimental autoimmune thyroiditis, EAT)的效果，并探讨其可能的作用机制。方法：31只健康雌性CBA/J小鼠随机分成EAT模型组(n=8)、CⅡTAm治疗组(n=9)、GFP对照组(n=9)和正常对照组(n=5)。除正常对照组不作特殊处理外，其余3组均以猪甲状腺球蛋白(porcine thyroglobulin, pTg)+弗氏佐剂(complete or incomplete Freund adjuvant, CFA/IFA)建立EAT小鼠模型，CⅡTAm治疗组和GFP对照组分别静脉注射重组腺病毒Ad-CMV-CⅡTAm及Ad-GFP进行治疗，EAT模型组注射等量生理盐水。首次免疫后第29日处死小鼠，进行H-E染色观察甲状腺病理形态；免疫组织化学染色测定甲状腺MHC Ⅱ类分子表达；分析pTg刺激下脾脏淋巴细胞的增殖及其上清液中IFN-γ的分泌水平；ELISA法检测血浆中抗-pTg自身抗体滴度；流式细胞术分析外周血和脾脏淋巴细胞中CD4+ T淋巴细胞上可诱导共刺激分子(inducible costimulator,ICOS)的表达水平。结果：H-E染色结果表明，CⅡTAm治疗组甲状腺淋巴细胞浸润指数(0.3±0.5)低于EAT模型组(1.4±0.4)和GFP对照组(1.5±0.2，P<0.01)。免疫组化结果显示，EAT模型组和GFP对照组甲状腺组织有弥漫性MHC Ⅱ类分子表达，而CⅡTAm治疗组未见明显表达，正常对照组表达呈阴性。80 μg/ml pTg刺激下，CⅡTAm治疗组小鼠淋巴细胞刺激指数(SI)明显低于EAT模型组或GFP对照组(P<0.05)；培养上清各组IFN-γ分泌水平与SI结果类似(P<0.01)。CⅡTAm治疗组血浆抗-pTg自身抗体滴度显著低于EAT模型组或GFP对照组(P<0.01)；CⅡTAm治疗组外周血和脾脏CD4+ T细胞ICOS分子阳性表达率亦显著低于EAT模型组或GFP对照组(P<0.01)。结论：重组腺病毒Ad-CMV-CⅡTAm能抑制EAT小鼠甲状腺组织MHC Ⅱ类分子表达，抑制自身反应性T细胞增殖，减轻甲状腺炎性细胞浸润，降低自身抗体滴度，对EAT有一定的治疗作用。

关键词：MHC Ⅱ类分子反式激活因子突变体 腺病毒科 自身免疫性甲状腺炎 基因疗法

Adenovirus-mediated MHC class Ⅱ transactivator mutant in gene therapy of mouse experimental autoimmune thyroiditis Download Fulltext

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Abstract:

Objective: To observe the therapeutic effect of major histocompatibility complex (MHC) class Ⅱ transactivator mutant (CⅡTAm) for gene therapy of mouse experimental autoimmune thyroiditis (EAT) and explore the possible mechanisms. Methods: Thirty-one healthy female CBA/J mice were randomly divided into 4 groups, namely EAT model group (n=8), CⅡTAm therapy group (n=9), GFP control group (n=9), and normal control group (n=5). Animals in the first 3 groups were immunized with porcine thyroglobulin (pTg) and complete or incomplete Freud's adjuvant (CFA/IFA) to establish EAT model; mice in the CⅡTAm therapy group and GFP control group were also treated by intravenous recombinant adenovirus Ad-CMV-CⅡTAm and Ad-GFP, respectively, while those in the EAT model group were injected with equal volume of normal saline. Mice in the normal control group received no special treatment. All mice were sacrificed on the 29th day after the first immunization. The thyroid pathological changes were examined using H-E staining; the expression of MHC Ⅱ molecules in the thyroid was examined using immunohistochemical staining; the spleen lymphocyte proliferation and IFN-γ secretion stimulated by pTg were examined in their culture supernatant; the titer of plasma anti-pTg autoantibody was assayed by ELISA; and the CD4+ T cells in both peripheral blood and spleen was analyzed by flow cytometry. Results: H-E staining showed that the infiltration index of thyroid lymphocyte in the CⅡTAm therapy group (0.3±0.5) was significantly lower than that in the EAT model group (1.4±0.4) and the GFP control group (1.5±0.2, both P<0.01). Immunohistochemical staining showed diffused expression of MHC Ⅱ molecules in the thyroid of the EAT model group and GFP control group, compared to very weak expression in the CⅡTAm therapy group and the negative expression in the normal control group. The lymphocyte stimulation index (SI) against 80 μg/ml pTg in the CⅡTAm therapy group was significantly lower than that in the EAT model group and the GFP control group (P<0.05). The IFN-γ secretion in the culture supernatants showed a similar difference as SI in all the groups (P<0.01). The titer of plasma anti-pTg autoantibody in the CⅡTAm therapy group was significantly lower than those in the EAT model group and the GFP control group (both P<0.01). The positive rate of ICOS on CD4+ T cells in the CⅡTAm therapy group was significantly lower than that in the EAT model group and the GFP control group (both P<0.01). Conclusion: Ad-CMV-CⅡTAm recombinant adenovirus can inhibit the MHC Ⅱ molecule expression in the thyroid of EAT mouse and the proliferation of self-reactive T cells, attenuate the inflammatory cells infiltration in the thyroid, and decrease the titer of plasma anti-pTg autoantibody, indicating that CⅡTA mutants might have therapeutic effect for EAT.

Keywords:MHC class Ⅱ transactivator mutant adenoviridae autoimmune thyroiditis gene therapy

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