Construction of vectors expressing bioactive heterodimeric and single-chain murine interleukin-12 for gene therapy

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摘要

Abstract

It has been well demonstrated that interleukin-12 (IL-12) could be useful to defend against a variety of pathogens, to suppress tumor growth and metastasis, and even to be employed as an adjuvant of vaccines to enhance beneficial type 1 T helper (Th1) cell response over detrimental type 2 T helper (Th2) cell responses. To apply IL-12 genes in gene therapy such as a DNA vaccine, a pIL-12 vector was constructed that contained two cytomegalovirus (CMV) promoters to drive the expression of p35 and p40 subunits, respectively. In addition, a pscIL-12 vector was designed with a linker to fuse p35 cDNA with p40 cDNA to produce a single-chain IL-12 protein, ensuring not only that the expression of p35 and p40 subunits was equally expressed, but also that no free p40 subunits interfered with IL-12 activity. The data suggested pIL-12 could produce a rather high level of biologically active IL-12 after transfection of COS cell lines as well as C2C12 muscle cell lines, as measured by both concanavalin A blast proliferation assay and enzyme-linked immunosorbent assay. Interestingly, the pscIL-12 vector could also express a bioactive murine IL-12 fusion protein in vitro. Furthermore, in vivo functional studies also demonstrated that mice co-immunized with a pS vector expressing the major envelope protein of hepatitis B virus (HBV) and IL-12 vectors encoding native IL-12 or single-chain IL-12 fusion protein elicited higher levels of IgG2a anti-HBs antibody and of Th1-related cytokine. Because p35 and p40 genes can be expressed in a vector by using a single promoter, pscIL-12 should be useful in future applications for nucleic acid vaccination or for gene therapy against diseases.