Construction and Expression in Tumor Cells of a Recombinant Vaccinia Virus Encoding Human Interleukin-1β

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Background: Human interleukin-1β (hIL-1β) injected intratumorally has demonstrated growth inhibition of transplanted subcutaneous tumors in mice, regression of metastatic lesions, resistance to tumor rechallenge, and increased survival. Vaccinia virus (VV) can be genetically engineered to produce cytokines and may be an effective vector for gene therapy of cancer. This study was designed to (a) construct a VV expressing hIL-1β, (b) assess tumor cell infection in vitro with this construct, (c) measure hIL-1β production, and (d) assess the bioactivity of the secreted cytokine.

Methods: The hIL-1β gene was amplified from a plasmid clone using polymerase chain reaction (PCR) and then cloned into a homologous recombination (HR) and expression vector, which was used to insert the hIL-1β gene into the VV genome. Selection of the recombinant VV (vMJ601hIL-1β) was based on inactivation of viral TK and expression of β-galactosidase. vMJ601hIL-1β infectivity and cytokine production was assessed by infecting tumor cell lines and analyzing culture supernatants for hIL-1β. Bioactivity of the hIL-1β produced was demonstrated using an IL-1 dependent T helper cell line.

Results: The hIL-1β gene was successfully cloned into the VV genome by HR, which was confirmed by PCR. vMJ601hIL-1β efficiently infected tumor cells, as shown by increased hIL-1β secretion (0 to >500 ng/ml) and morphologic evidence of viral cytopathic effect. vMJ601hIL-1β-infected cells secreted large amounts of hIL-1β (mean 772 ng/10⁶ cells/24 h). The secreted hIL-1β was bioactive (mean bioactivity 6.8 × 10⁸ U/mg of hIL-1β).

Conclusions: (a) hIL-1β can be cloned into VV, (b) vMJ601hIL-1β retains its infectivity, (c) a large amount of hIL-1β is secreted, and (d) the secreted hIL-1β is bioactive. Recombinant VV may allow in situ cytokine gene delivery and expression in established tumors.

Key Words: Interleukin-1β—Neoplasm—Vaccinia—Gene therapy.

Biological therapy based on gene transfer is a new and promising strategy in treating cancer (1–4). One approach of gene therapy is to stimulate the host immune system to eradicate cancer cells. Molecular biology technology now permits the cloning and transfer of almost any known gene into eukaryotic cells. Cytokine gene insertion into tumor cells directly may result in the selective expression of a specific cytokine at the tumor site and reduce undesirable side effects associated with systemic administration (5). Results in animals have shown failure of cytokine-transfected tumors to grow in vivo and stimulation of systemic antitumor immunity (5–8). Retroviral vector–mediated gene transfer into tumor cells recently has been implemented in human trials (2,4,9,10). However, the feasibility and practicality of this type of therapy depends to a great degree on the gene transfer vector used. Retroviral-based vectors are not practical for routine
clinical use in human patients (11). Cells must be cultured, multiple therapeutic genes cannot be transferred easily, genes are transferred at low efficiency, retroviral vector size constraints limit the number of genes transduced, a large amount of time is required to grow tumor cells and expand gene transduced cells, and difficulty exists in transferring and expressing genes in vivo. Using vaccinia virus as a gene transfer vector is a unique system that has not been investigated thoroughly and may be a practical system of gene therapy.

Vaccinia virus (VV) was introduced as a vector for the transient expression of genes in mammalian cells in 1982 (12,13). The biology of VV has been extensively studied (14,15). VV is a member of the orthopoxvirus genus of the Poxviridae family and consists of a linear, double-stranded DNA genome of ~200 kb. The viral genome encodes most of the proteins needed for replication and transcription of viral genes. VV replicates in the cytoplasm of infected cells as opposed to the nucleus. The virus can infect most mammalian cell lines, resulting in lytic cell death (16). A number of plasmid vectors have been constructed that allow the insertion of foreign genes downstream from VV promoters and between flanking sequences homologous to VV genes. This permits homologous recombination with foreign gene insertion into the VV genome at targeted sites (16).

When recombinant human interleukin-1β (hIL-1β) is injected intratumorally into transplanted subcutaneous tumors in mice, dramatic antitumor effects are observed (17-22). However, the therapeutic efficacy of biological therapy after transfection of the hIL-1β gene into tumor cells has been unsuccessful because transfected cells fail to secrete bioactive hIL-1β (23,24). This is explained by a unique mechanism of processing and secretion involving pre–IL-1β convertase activity, which is absent in most cells other than monocytes (25). Recently, Pecceu et al. fused a synthetic gene encoding the 153 amino acid C-terminal part of hIL-1β with a synthetic sequence encoding the human growth hormone signal peptide, resulting in a novel hybrid gene construct. Transfection of an expression plasmid containing this construct into cultured Chinese hamster ovary cells resulted in secretion of bioactive hIL-1β into the culture supernatant (26). To date, there have been no reports of hIL-1β secretion from human or murine tumor cells after transfer of this synthetic gene.

Given the potent antitumor effects observed with recombinant hIL-1β and the potential advantages of using VV as a tumor cell–targeted gene delivery and expression vector, the present study was conducted to assess the feasibility of constructing a genetically engineered vaccinia virus that encodes hIL-1β. In order for such a construct to be used as a vector for gene transfer in cancer immunotherapy, the recombinant virus should retain its infectivity of tumor cells and should cause infected cells to secrete sufficient quantities of hIL-1β, which is biologically active.

**MATERIALS AND METHODS**

**Cells, media, and reagents**

D10.G4.1 cells (ATCC TIB-224) were maintained in 24-well plates in Click's (EHAA) complete medium (GIBCO BRL, cat. no. 11128-014) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 50 mM 2-mercaptoethanol, and gentamicin at 37°C without CO₂. Cell proliferation was stimulated by adding 3 μg/ml concanavalin A (Con A; Sigma, cat. no. C-5275), 3 U/ml recombinant murine IL-1β (GIBCO BRL, cat. no. 3321SS), 40 U/ml recombinant human IL-2 (DuPont, #A10-A2215), and irradiated (5,000 rad) splenocytes isolated from C57Bl/6 female 8-week-old mice. Cells were maintained in Click's medium without stimulation for at least 7 days before use for the bioassay.

CV-1 African green monkey kidney (ATCC CCL-70), BS-C-1 African green monkey kidney (ATCC CCL-26), human 143B thymidine kinase-deficient (ATCC CRL-8303), L929 mouse fibroblast tumor (ATCC CCL-1), SKBR3 human breast adenocarcinoma (ATCC HTB-30), and BT-20 human breast carcinoma (ATCC HTB-19) cell lines were obtained from ATCC and maintained according to ATCC instructions. C3HBA mouse breast carcinoma (NCIG00758), PAN02 mouse pancreatic carcinoma (NCIG002502), and B16 mouse melanoma (NCIG0062) cell lines were obtained from the National Cancer Institute and maintained according to supplied instructions. MCA205 is a mouse fibrosarcoma cell line of C57Bl/6 origin (27). The cell line was cultured in RPMI-1640 medium supplemented with 10% FBS.

For homologous recombination, lipofectin reagent (cat. no. 18292-011) and Opti-MEM I (cat. no. 320-1985AJ) were obtained from GIBCO BRL; Opti-MEM I was supplemented with gentamicin. For the bioassay, recombinant murine IL-1β was