Effects of Combined Therapy With Interleukin 2 and Interleukin 12 Gene–Transfected Tumor Vaccine for Head and Neck Carcinoma

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Background: The biological effects of cytokines are coming to be understood. The therapeutic effects of interleukin (IL) 2, IL-12, and interferon γ (IFN-γ) in cancer treatment have been reported, but there are problems when these cytokines are systemically used as therapeutic agents.

Objective: To examine the efficacy of IL-2 and IL-12 gene–transfected tumor cell vaccines for head and neck squamous cell carcinoma (SCC).

Methods: Homozygous mice with the autosomal recessive nude gene (BALB/c nu/nu mice) were inoculated subcutaneously in the right flank with cells from a human oral floor SCC cell line (KB cells). The mice were then injected with IL-2 and IL-12 gene–transfected KB cells (KB/IL-2 and KB/IL-12 cells, respectively) irradiated with 2000 rad (20 Gy).

Results: No mice died soon after the injection of the gene immunotherapy. The treatment with either KB/human IL-2 (hIL-2) or KB/murine IL-12 (mIL-12) was not very effective. However, the treatment with both KB/hIL-2 and KB/mIL-12 cells significantly and safely inhibited the growth of established tumors (P = .04). There was no significant difference in antitumor effect between once-weekly and twice-weekly injections of both KB/hIL-2 and KB/mIL-12 cells.

Conclusion: Double gene immunotherapy is safe and effective treatment for SCC in mice.


INTERLEUKIN (IL) 2 is an important cytokine in the generation of antitumor immunity mediated by cytotoxic T lymphocytes (CTLs) and/or natural killer (NK) cells. Intravenous, intralymphatic, and intralional IL-2 administration results in clinically significant antitumor responses in several types of cancer. However, because of the short half-life of IL-2 in serum, systemic administration of IL-2 at high doses is required, resulting in severe adverse effects such as vascular leak syndrome, edema, anemia, fevers, and hypotension. To avoid these problems, local delivery of IL-2 at low doses has been investigated in animal models, with the aim of increasing survival and reducing tumor growth without the adverse effects induced by high dosing regimens. Attention has now turned to the use of gene delivery systems to facilitate the expression of IL-2 continuously within or around the tumor as a more effective method of achieving high intratumoral or peritumoral concentrations of IL-2. The induction of effective immune responses has been demonstrated in IL-2–producing tumor cells transfected with IL-2 complementary DNA in vivo. Transfection of tumor cells with retrovirus vectors containing the IL-2 gene has been shown to reduce tumorigenicity and the metastatic potential of B16F1 (B16) melanoma, CMS-5 fibrosarcoma, and murine bladder tumor (MBT-2) bladder carcinoma.

In the present study, we used herpes simplex virus (HSV) vector to transfer the human IL-2 gene into cells from a head and neck squamous cell carcinoma (SCC) cell line (KB cells). The wide host range of HSV-1 and the relative ease of its genetic manipulation have made it an attractive candidate as a tool for gene transfer. Amplicon-based HSV vector has been termed a defective virus vector owing to its inability to replicate in the absence of the parent virus as a helper.

Interleukin 12 is a heterodimeric cytokine that is produced primarily by antigen-presenting cells such as monocytes, macrophages, and dendritic cells, and it exerts immunoregulatory effects on NK cells. In addition to this effect, IL-12 activates cytotoxic T cells and differenti-
It is made up of 2 disulfide-linked subunits designated p35 and p40, both of which are required for biological activities.10,11 Interleukin 12 is most noted for its ability to induce the production of large amounts of interferon γ (IFN-γ) from resting and activated T and NK cells.12,13 Through direct action and through its ability to induce IFN-γ secretion from T and NK cells, IL-12 plays a central role in innate and adaptive immunity important to host defense against predominantly intracellular pathogens. Additionally, IL-12 has been shown to induce an antitumor immune response against several murine tumors,16,17 and clinical trials of this effect have commenced.

Thus, IL-2 and IL-12 have very strong antitumor effects. But it is difficult to use them systemically as drugs because of their adverse effects and short half-lives. However, there are some reports indicating that combination nonviral IL-2 and IL-12 gene therapy is effective in head and neck SCC and that the nonviral gene delivery system is without severe toxic effects.18,19 Indeed, the viral vector has dangerous aspects and the dose of vector must be limited because of its toxic effects, but it has been reported that the nonviral delivery system is not as effective as the viral delivery system.18

Rashleigh et al20 reported that the combination of IL-2 and IL-12 increased NK cell activity against head and neck SCC without increasing the toxic effects attendant with increasing doses of IL-2.20 There are many reports about the antitumor effects of IL-2 and IL-12. In those articles, the systemic administration of IL-2 and/or IL-12 was noted to be very toxic. To use these cytokines as systemic agents in safety, the dose must be limited, resulting in limited antitumor effects. So strategies have been explored to use viral delivery systems to transduce the genes of antitumor cytokines.

In our study, we used IL-2 and IL-12 double gene therapy with irradiated tumor vaccine made in vitro by a small quantity of viral vector without severe toxic effects. We describe herein the potential of a transfected IL-2 gene delivery system with ampiclon-based HSV vector (HSV/human IL-2 [hIL-2]) and an IL-12 gene delivery system with retrovirus vector for the treatment of human head and neck SCC.
Tokyo, Japan) (100 µL/well). The cells were then incubated at 37°C in a 5% carbon dioxide atmosphere for 72 hours. The cells were treated with trypsin and counted by microscope observation, using trypan blue to determine if the cells survived. The mIL-12 level in the supernatant of KB/mIL-12 cells was examined using a commercially available enzyme-linked immunosorbent assay kit (R & D Systems, Minneapolis, Minn).

TREATMENT WITH hIL-2 AND mIL-12 GENE–TRANSFECTED KB CELLS ON ESTABLISHED TUMORS

Mice were inoculated subcutaneously in the right flank with 5 × 10^6 KB cells. Seven days after tumor cell inoculation, as a preliminary study, mice were injected peritumorally once a week for 4 weeks (total 4 times) with 5 × 10^6 KB/hIL-2 and KB/mIL-12 cells or KB/mIL-12 cells irradiated with 2000 rad (20 Gy) or with phosphate-buffered saline as a control (5 mice were used in each group). No KB/β-galactosidase (lacZ) or KB/Neo was experimented with as a control. The location of the injection was a point of subcutaneous tissue bordering the established tumor. Tumor growth was calculated in cubic centimeters using the formula (a × b^2) × 0.4, where a is the larger and b the smaller of the dimensions. Tumors were measured every week.

Seven days after tumor cell inoculation, the rest of the mice were injected twice a week for 4 weeks (total 8 times) peritumorally with 5 × 10^6 KB/hIL-2 and/or KB/mIL-12 cells or KB/lacZ or KB/Neo cells irradiated with 2000 rad (20 Gy), or with phosphate-buffered saline as a control. Tumors were measured once a week as described above. The mice were killed 12 weeks after tumor cell inoculation for evaluation of the IFN-γ levels. Serum samples were obtained by cardiac puncture. All data were expressed as mean ±SD; the t test was used for analysis.

RESULTS

mIL-12 SECRETION OF KB/mIL-12 CELLS AND mIL-12 SECRETION OF IRRADIATED KB/mIL-12 SUPERNATANT

The concentration of KB/mIL-12 cells in the culture supernatant gradually increased every day, which suggests that KB/mIL-12 cells keep producing mIL-12 (Figure 1). The concentration of the culture supernatant of KB/mIL-12 cells irradiated with 2000 rad (20 Gy) increased in the first 24 hours but then leveled off, which suggests that KB/mIL-12 cells irradiated with 2000 rad (20 Gy) produce mIL-12 for only the first 24 hours (Figure 2).

SURVIVAL CURVE OF IRRADIATED KB/mIL-12 CELLS

To examine the survival time of KB/mIL-12 cells irradiated with 2000 rad (20 Gy) and to see whether they had an ability to proliferate, we counted the number of cells every 12 hours. The number gradually decreased, and 72 hours after irradiation, almost all of the KB/mIL-12 cells were dead. Error bars indicate SD calculated from 3 experiments.

INHIBITION OF TUMOR GROWTH BY IRRADIATED KB/hIL-2 AND/OR KB/mIL-12 CELLS IN VIVO

Tumors were measured in mice treated with KB/hIL-2 and KB/mIL-12 cells or KB/mIL-12 cells irradiated with 2000 rad (20 Gy). The KB tumor–bearing mice were treated with peritumor injections of those cells for 4 weeks (5 × 10^6 cells, once a week). The injection of 5 × 10^6 irradiated KB/hIL-2 cells and 5 × 10^6 irradiated KB/mIL-12 cells once a week significantly inhibited the growth of established tumors (P = .05) (Figure 4). Based
kbil-2 and kb/mil-12 were injected peritumorally once a week for 4 weeks. The treatment with both kb/hil-2 and kb/mil-12 significantly inhibited the growth of established tumors. lacZ indicates β-galactosidase; Neo, neomycin phosphotransferase gene; PBS, phosphate-buffered saline; asterisk, P<.05; dagger, P<.01; and double dagger, not significant. Error bars indicate SD calculated from 5 experiments.

on the survival curve of irradiated kb/mil-12 cells (Figure 3), it was supposed that those injected cells lived for half a week, so we administered the treatment with peritumoral injections of those cells twice a week for 4 weeks (5 × 10⁶ cells). The tumors were measured in mice treated with kb/hil-2 and/or kb/mil-12 or kb/lacZ or kb/neo irradiated with 2000 rad (20 Gy) and in nontreated mice. The injection of both 5 × 10⁶ irradiated kb/hil-2 cells and 5 × 10⁶ irradiated kb/mil-12 cells twice a week significantly inhibited the growth of established tumors (P=.002) (Figure 5). The tumor volume was not significantly different between once-weekly and twice-weekly treatment with both kb/hil-2 and kb/mil-12 twelve weeks after tumor cell inoculation (data not shown).

CONCENTRATION OF SERUM IFN-γ

The serum murine IFN-γ levels in each treatment group (irradiated kb/hil-2 and/or irradiated kb/mil-12, 5 × 10⁶ cells, twice-weekly injections, 12 weeks after tumor cell inoculation) were determined by enzyme-linked immunosorbent assay. The serum IFN-γ levels of the mice treated with both irradiated kb/hil-2 and irradiated kb/mil-12 cells were significantly higher than those of the other groups (P<.05) (Figure 6).

COMMENT

The therapeutic efficacy of IL-2 gene–transfected tumor vaccine for head and neck carcinoma has been reported.23 It is suggested particularly as treatment for advanced and recurrent cases. Indeed, IL-2 gene therapy is effective to some degree, but used alone, it cannot attain a clinically complete response in advanced cases. Interleukin 12 is clearly an important regulator of immune systems and has many biological functions related to antitumor immunity, antimetastasis, and antiangiogenesis. In the present study, IL-12 gene–transfected vaccine therapy was combined with IL-2 gene therapy. Our goals were to determine the efficacy of the IL-2 and IL-12 double gene therapy for head and neck carcinomas and to elucidate the mechanism of the antitumor response with particular interest in the role of IFN-γ.

Interleukin 2 plays an important role in the amplification of immune responses. It stimulates the proliferation of CTLs, T helper cells, NK cells, lymphokine-activated killer cells, and macrophages.21 Our group has reported the therapeutic efficacy of IL-2 gene–transduced tumor vaccine for head and neck carcinoma and suggested that IFN-γ was secreted by NK cells and acti-
vated by IL-2 and that IFN-\(\gamma\) promoted the activation of CTLs and amplified the tumoricidal activity of NK cells.\(^{24}\) It has also been reported that IL-12 administration in vivo was associated with dramatically increased serum levels of IFN-\(\gamma\) and played a role of immunologic effects.\(^{25,26}\)

In the present study, the IL-2 and IL-12 double gene therapy was much more effective than IL-2 gene therapy alone. Twelve weeks after tumor cell inoculation, the murine serum IFN-\(\gamma\) level of the group treated with both IL-2 and IL-12 gene-transfected tumor vaccine was much higher than that of either the IL-2 or IL-12 gene therapy group alone. So serum IFN-\(\gamma\) level seems to be concerned with antitumor activities. And the IL-12 gene-transfected KB cells irradiated with 2000 rad (20 Gy) could not proliferate and survived only about 3 days. Therefore, irradiated KB/IL-12 cells might be clinically safe as an agent to inject. However, there was not a significant difference of antitumor effect between once-weekly and twice-weekly injection of both KB/hIL-2 and KB/hIL-12 cells. It seems that the antitumor effect of the double gene therapy was not merely based on each antitumor effect of IL-2 and IL-12, but on synergistic functions, including the antigenicity of the vaccine.

In conclusion, a new combined therapy using both IL-2 and IL-12 gene–transfected tumor vaccine has a much stronger antitumor effect than either IL-2 or IL-12 single gene therapy alone, and part of the antitumor effect seems to be related to activated CTLs, NK cells, and macrophages promoted by high-level secretion of IFN-\(\gamma\). Furthermore, in terms of inhibition on tumor growth, antiangiogenic effects of IL-12 might have important roles by down-regulating the expression of vascular endothelial growth factor and basic fibroblast growth factor.\(^{27}\)

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