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Immuno-gene Therapy Using Colon Cancer Cells Transfected with Mature-interleukin-18 cDNA and the Igκ Leader Sequence

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Abstract Interleukin (IL)-18 is a novel cytokine that induces interferon (IFN)-γ secretion and plays an important role in anti-tumor immunity. In the present study, we constructed plasmid vectors encoding the murine mature-IL-18 cDNA linked with the Igk leader sequence and the pro-IL-18 cDNA in order to estimate the efficacy of the mature-IL-18 vector and to evaluate IL-18-producing tumor cells as a tumor vaccine. Colon 26 cells were transfected with the above mentioned vectors or with vector alone (mock). The ability of the culture supernatants of mature-IL-18 transfectants to induce IFN-γ secretion was extremely high in comparison to that of pro-IL-18 transfectants. When injected into syngeneic BALB/c mice, the growth of mature-IL-18 transfectants, but not pro-IL-18 transfectants, was significantly less than that in mock transfected cells ($p < 0.01$, by ANOVA and analysis of covariance). Depletion of natural killer cells did not affect the growth of transfectants. The growth inhibitory effects were partially abrogated following treatment with anti-CD4+ and anti-CD8+ antibodies. These data suggest that the rejection of mature-IL-18/colon 26 cells was mediated through T cell activation. Gene therapy using mature-IL-18 transfectants containing a plasmid vector and the Igκ leader sequence may be a useful tumor vaccine.

Introduction

Interleukin (IL)-18 is a novel cytokine identified as interferon-γ inducing factor (IGIF).[1] Recent studies have shown that IL-18 stimulates T-cell proliferation, augments natural killer (NK) cell lytic activity, and induces interferon-g (IFN-γ) production in type 1 T-helper (Th1) cells.[2-7] Thus, local IL-18 production is likely to increase antitumor activity of host immune cells via altered IFN-γ levels. IL-18, like IL-1, lacks a typical leader sequence.[8] IL-18 is processed into the mature active form by caspase-1, which is an IL-1β converting enzyme (ICE).[9] Because of the difficulty in engineering secreted active IL-18, there have been few studies of gene therapy using IL-18.[10]

In the present study, we constructed plasmid encoding the murine mature- or pro-IL-18 cDNA, and then estimated the efficacy of mature-IL-18-producing tumor cells as a tumor vaccine. This is the first study to examine the efficacy of the Igκ leader sequence to stimulate secretion of active IL-18 by cells transfected with plasmid vectors and the possible use of mature-IL-

18-producing tumor cells as cancer vaccines.

Expression of IL-18 mRNA in transfectants

Six of 7 clones expressing pro-IL-18 mRNA and 7 of 8 clones expressing mature-IL-18 mRNA were selected for bioassay. Endogenous-IL-18 mRNA was not detected in either colon 26 cells transfected with vector alone (mock 1 and 2) or in untransfected colon 26 cells.

Bioassay for IL-18 produced from transfectants

The levels of IFN-γ induction by the culture supernatants were between 4 to 18 pg/ml in the pro-IL-18 transfectants (10^6 cells/24 hr), between 40 and 140 pg/ml in the mature-IL-18 transfectants , and as 2 to 4 pg/ml in parent cell and vector-alone transfectants (Fig. 1). We selected mature 2 (IFN-low-inducing clone; 40 pg/ml), mature 6 (IFN-γ high-inducing clone; 140 pg/ml), and pro 2 (pro-IL-18/colon 26; 10 pg/ml) for further examination.

Inhibition of tumor growth in vivo

The tumorigenicities of pro 2, mature 2, mature 6, and mock 1 cultures were examined by subcutaneous injections into BALB/c mice in 3 independent experiments. The mean tumor volumes of both the mature 2 and mature 6 clones were significantly reduced (n=10), whereas mock 1 grew progressively until the animals died (n=10). The mean tumor volumes of both the mature 2 and mature 6 clones were also significantly reduced compared with pro 2 (Fig. 2).

Rechallenge with parental cell (colon 26) and Meth-A

Forty days after the disappearance of the initial mature-IL-18/colon 26 implants, 7 immunized mice were injected with 2 x 10^5 parental colon 26 cells in the lower left abdominal flank. Seven non-immunized mice were injected in the same manner as control. In all immunized mice, tumors were rejected, whereas in non-immunized mice tumors grew progressively until the animals died.

Depletion test

There was no difference in tumor growth between the NK-depleted group (n=10) and the control group (n=10). Tumors in mice treated either with anti-CD4+ (n=10) or anti-CD8+ (n=10) antibodies grew rapidly

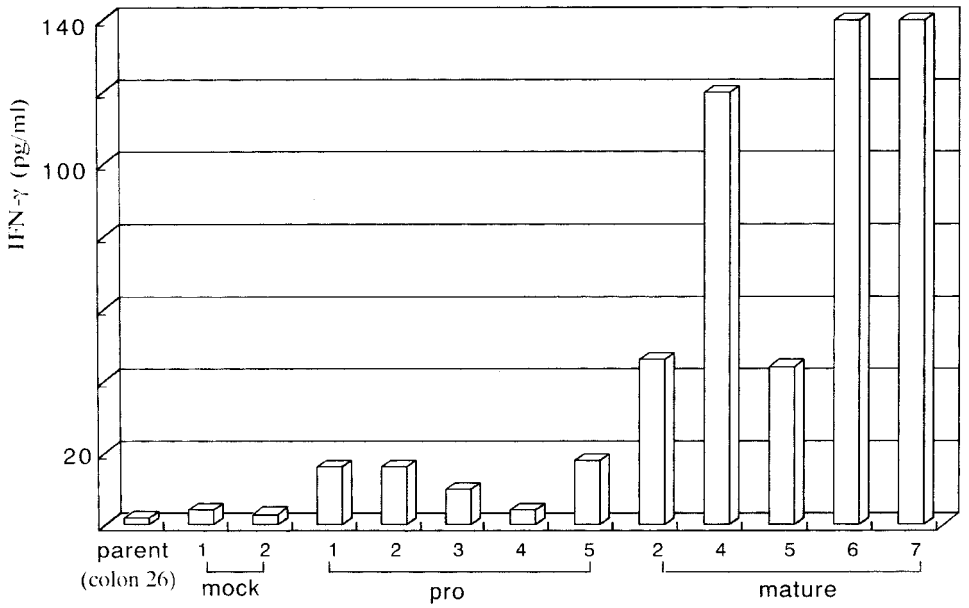

Fig. 1 Induction of IFN-γ secretion by culture supernatants from tumor cells. The levels of IFN-γ induction in the pro-IL-18 transfectants were between 4 and 18 pg/ml, while those levels in the mature-IL-18 transfectants were between 40 and 140 pg/ml, and those levels in parent cells and mock showed levels as low levels as 2 to 4 pg/ml.

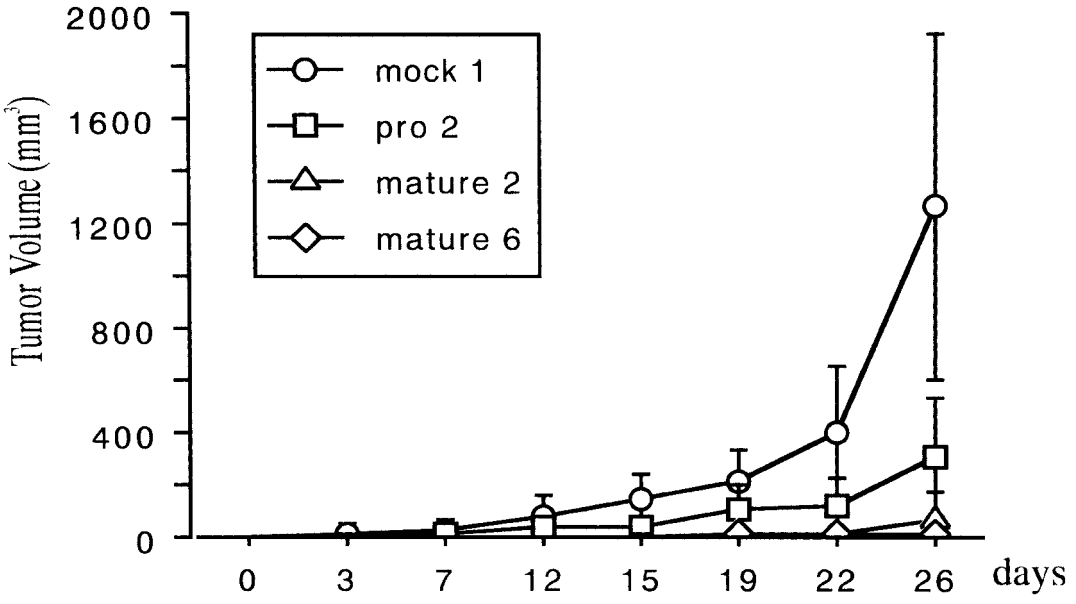

Fig. 2 Tumorigenicity of each pro 2, mature 2, mature 6, and mock 1 clone was examined by subcutaneous injection into BALB/c mice. Mean tumor volumes of both mature 2 and mature 6 clones were significantly smaller (n=10), while clone mock 1 grew progressively until it caused the death of the animals (n=10) ($p < 0.01$). Tumor volumes are mean ± SE.

when compared with those in mice treated with HBSS (control) ($p<0.01$).

Histology at the site of tumor cell injection

Immunohistochemical analysis of tumor implantation sites in BALB/c mice 14 days after subcutaneous injection of Mature-6 transfectant cells revealed a sparse infiltration of CD4+ T cells and a dense infiltration of CD8+ T cells in the tumor.

Expression of MHC class I, MHC class II, B7.1, and B7.2 on tumor cells

All cells expressed MHC class I, but, MHC class II, B7.1, and B7.2 were not detected on any cells. There was no difference between transfectants and parental cells.

DISCUSSION

IL-18, which is related structurally to IL-1β, is first synthesized as a leaderless precursor, and it requires ICE for cleavage into an active molecule. For gene therapy, a conventional signal sequence is necessary for extracellular secretion of mature-IL-18. In fact, our preliminary study demonstrated that pro-IL-18 cDNA transfectants of Meth-A cells which are highly immunogenic tumor cells, were completely rejected in all mice, while pro-IL-18 cDNA transfectants of colon 26 cells (pro 2), which are weakly immunogenic tumor cells failed to show such antitumor effects. We therefore constructed a mature-IL-18 expression vector that contains the Igκ leader sequence, and transfected it into colon 26 cells. Secretion of bioactive IL-18 by mature-IL-18 transfectants was significantly higher than that by pro-IL-18 cDNA transfectants. Furthermore, the mature-IL-18 transfectants were rejected more strongly than pro-IL-18 transfectants.

The levels of secretion of bioactive IL-18 should be important. It has been reported that the dose of IL-18 administered correlates well with the resulting level of serum IFN-γ and the strength of its antitumor activity.[11] Therefore, for an effective gene therapy it is essential to construct a vector that secretes high levels of bioactive IL-18. Indeed, the abilities of our mature-IL-18 transfectant to induce IFN-γ secretion were well correlated with the rate of the complete rejection. Osaki et al.[10] constructed a adenoviral vector that contains

the mature-IL-18 sequence and the leader sequence of the human parathyroid hormone (PTH) gene. The transfectants in the study induced secretion of about 40 pg/ml of IFN-γ. We constructed a clone using a new vector that contains the Igκ leader sequence and induces secretion of large amounts of IFN-γ (140 pg/ml). This result suggests that the Igκ leader sequence may be more suitable to induce secretion of IFN-γ than that of PTH. Because a plasmid vector is easy and relatively safely to handle, it is not necessary to set up an anti-viral unit or to worry about toxic viral infection.

Different mechanisms have been demonstrated in cells transfected with different cytokines. Transfectant with either IFN-γ, IL-2, IL-6, IL-12, or IL-15 induces primarily specific CTL activity,[12–14] while transfectant with either TNF-α, IL-4, MIP-1α, or IL-8 induces non-specific anti-tumor activity and granulocyte infiltration.[12,15] It is known that tumor rejection induced by local secretion of cytokines is mediated by a dual mechanism involving specific and non-specific anti-tumor effectors. With regard to the specific effects, our results indicate that in vivo IL-18 stimulates the sensitization of memory T lymphocytes, which is responsible for specific long-lasting antitumor protection. BALB/c mice, which rejected IL-18/colon 26 cells, were protected against parental colon 26 tumors, but not against Meth-A tumors. There was no difference in tumor growth between the NK-depleted group and the control group. Tumors in mice treated either with anti-CD4+(n=10) or anti-CD8+(n=10) antibodies grew rapidly when compared with those in mice treated with HBSS (control) ($p<0.01$). These results suggest that the main effector cells are T cells, but not NK cells. However, these results are not consistent with results from recombinant IL-18 i.v. studies and IL-18 gene therapy studies using MCA205.[10,11] Although the reason of the differences was unclear, we thought that secreted bioactive IL-18 at the tumor sites may induce T cell mediated antitumor host reactions. Our data also showed that IL-18 transfectants induced successfully IFN-γ production. Several studies have demonstrated the efficacy of cancer vaccines using IFN-γ-producing tumor cells.[16–18] It is unknown that IL-18 gene therapy is better than IFN-γ gene therapy. Since there is a variety of tumors, it may be important to create a variety of gene therapies. We therefore think that IL-18 gene therapy may be one of the effective strategies for cancer therapy.

In conclusion, our study demonstrates that a mature-IL-18 expression vector with the Igκ leader sequence is useful for construction of clones that secrete a high amount of bioactive IL-18 and that IL-18 secreting tumor cells can elicit a specific antitumor immune response. Mature-IL-18-secreting tumor cells may be useful as vaccines for cancer therapy.

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