Interleukin 12 Potentiates the Curative Effect of a Vaccine Based on Interleukin 2-transduced Tumor Cells

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Abstract

The purpose of these studies was to determine whether systemic administration of recombinant interleukin 12 (rIL-12) is able to potentiate an initial, but insufficient T-cell antitumor response. Mice challenged with carcinoma cells engineered to release interleukin 2 (IL-2) and displaying such a response received single or multiple i.p. injections of rIL-12. This combination of systemic rIL-12 and local IL-2 increased the percentage of mice that rejected two different IL-2 gene-transduced tumors. In another set of experiments more closely resembling a clinical situation, IL-2 gene-transduced tumors were used as vaccines in an attempt to cure mice bearing wild-type parental tumors. The combination of these vaccines with systemic rIL-12 cured mice more effectively than rIL-12 and IL-2 gene-transduced tumor vaccines alone.

Introduction

New immunization methods and new cytokines have led to recent advances in tumor immunology. The former include tumor cells engineered with genes encoding for cytokines (1) and costimulatory signals (2) to be used as an immunogen to activate specific antitumor immunity. The latter mostly rely on IL-12, which activates a strong antitumor activity and is much less toxic than IL-2 (3, 4).

In vivo, most cytokines secreted by engineered tumors of a different histotype and origin trigger an efficient antitumor reaction involving both nonspecific and specific immune mechanisms that markedly impair the oncogenicity of the engineered cells (1). When used as a vaccine, these cells elicit an immune memory which effectively inhibits a subsequent challenge with the parental tumor. By contrast, when used to cure mice harboring established tumors, their effect is usually marginal (5). Tumor cells engineered to release IL-2 are among the most effective (5, 6). There is thus a need for other treatments that can act in conjunction with IL-2 gene-engineered cells and widen the spectrum of curable tumors.

IL-12 is particularly interesting since its systemic administration improves the survival of mice bearing a great variety of tumors (4), although not the C-26 colon adenocarcinoma (7). The immune reaction it elicits stems from the induction of IFN-γ (8) and is dominated by T and NK cells. Moreover, IL-12 seems to act chiefly where T cells have been activated and accumulated, as shown in the case of experimental autoimmune diseases (9).

Systemic administration of IL-12 could thus be particularly effective against tumors engineered to release cytokines acting on infiltrating T lymphocytes and NK cells. This article assesses the extent to which such administration impairs the residual growth of tumors engineered to release IL-2, and enhances their potential when used to cure mice with established tumors.

Materials and Methods

Tumors and Mice. C-26 and C-51 are colon adenocarcinoma cell lines derived from BALB/c mice treated with N-nitroso-N-methylurethane and 1,2-dimethylhydrazine, respectively (10). TSA is a cell line established from the first in vivo transplant of a moderately differentiated mammary adenocarcinoma that arose spontaneously in a multiparous BALB/c mouse (11). Tumor cells were cultured in DMEM (GIBCO, Paisley, United Kingdom) supplemented with 10% FCS (GIBCO). BALB/cAnCr mice (Charles River, Calco, Italy) were maintained at the Istituto Nazionale Tumori under standard conditions according to institutional guidelines. Mice were challenged with 1 × 10⁶ parental or IL-2-transduced C-26 or TSA cells injected s.c. into the left flank. To obtain lung metastasis, mice were given i.v. injections of 10⁶ cells of C-51. Mouse rIL-12, kindly provided by Dr. Michael Brunda and Maurice Gately (Hoffman LaRoche, Nutley, NJ) was injected i.p. (0.5 or 0.1 µg in saline per day) in two courses of 5 days with 2-day intervals, starting at different times after challenge as indicated. Control animals received saline only. Differences in tumor take were evaluated using the χ² method.

Cytokine Gene Transduction. IL-2 cDNA from normal human peripheral blood lymphocytes was inserted into the retroviral vector LXSN (12), and producer cells were obtained by transfection as previously described (13). C-26 target cells (10⁶) were infected by exposure to undiluted supernatant for 3 h in the presence of 8 µg/ml polybrene, then selected in 0.5 mg/ml G418. Single G418-resistant colonies were isolated and expanded to determine their IL-2 production (Biotrak; Amersham International, Buckinghamshire, UK). The best colony, producing from 2.6 to 17 ng IL-2/ml (10⁴/ml/48 h), was selected. The retroviral vector carrying the murine GM-CSF cDNA has been described elsewhere (14). Transduced C-26 gene-transduced cells produced 200 ng/ml GM-CSF. Transfection of TSA cells with the IL-2 gene and selection of clones expressing variable amounts of IL-2 has been previously described (15).

IFN-γ Production. SPCs were restimulated in vitro with anti-CD3 mAb (10 µg/ml in Tris-Cl, 0.05 M, pH 9.5; clone 145–2C11; Pharmingen) coated on 96-well flat-bottomed plates (2 × 10⁵ cells/well). Supernatants harvested after 18-h incubation at 37°C were collected and tested in an ELISA for IFN-γ production. Purified mAb to IFN-γ (clone R4–6A2; Pharmingen) was diluted to 2 µg/ml in coating buffer (0.1 M NaHCO₃, pH 8.2), and 50 µl were incubated in wells of 96-well plates (Maxisorp Nunc-immuno module; Nunc) overnight at 4°C. Plates were washed with PBS containing 0.05% Tween 20 (Sigma) and blocked with PBS/10% FCS for 2 h at room temperature. After washing with PBS/Tween 20, 50-µl samples and standard dilutions were added and incubated for 2 h at 37°C. The wells were washed and incubated with 50 µl biotinylated anti-IFN-γ mAb (clone XMG1.2; Pharmingen), 1 µg/ml at room temperature, for 45 min. Fifty µl diluted avidin-peroxidase (1:400 of 1 mg/ml solution; Sigma) were added after washing again with PBS/Tween 20. After 30 min of incubation at room temperature and washing, 100 µl substrate solution (ABTS, 2.2'-azonio-bis(3-ethylbenzthiazoline-6-sulfonic acid); Sigma) were added to develop color reaction. The plate was read at absorbance 405 nm to determine the IFN-γ concentrations.
Mixed Lymphocyte Tumor Culture and CTL Assays. Mixed lymphocyte tumor culture was performed in RPMI 1640 medium (Whittaker Bioproducts, Walkersville, MD) supplemented with 10% FCS (Hyclone, Logan, UT) using a modification of a reported procedure (16). Responder SPCs from variously treated mice were stimulated by γ-irradiated (20,000 rad) C-26 cells. Responders and stimulators were suspended to 2.5 × 10^6 and 2.5 × 10^6 cells/ml, respectively, and mixed in a total volume of 2 ml in 24-well plates (Costar, Cambridge, MA). Cultures were incubated in a humidified atmosphere of 5% CO₂ in air. In CTL assays, C-26 cells were the specific target, YAC-1 cells were used as controls for NK cell-mediated lysis, and Fl-transformed fibroblasts (BALB/c) were the negative controls for C-26 tumor-specific lysis.

Results and Discussion

We have previously shown that growth of the C-26 colon carcinoma is not impaired by systemic administration of rIL-12 (7). Here, we show that the in vivo growth rate of C-26 cells engineered to release IL-2 (C-26/IL-2) is markedly reduced as compared with parental C-26 cells and about 20% of mice completely reject them, whereas that of cells engineered to release GM-CSF (C-26/GM-CSF) is not affected (Fig. 1). To determine whether rIL-12 can boost the weak reactivity triggered by C-26/IL-2 cells or impair the growth of C-26/GM-CSF cells, a few mice...
Fig. 3. Immunotherapy of mice bearing artificial lung metastasis of C-51 colon adenocarcinoma. At day 0, all mice were challenged i.v. with \(1 \times 10^6\) C-51 cells. At the indicated times, a few mice were vaccinated s.c. with irradiated C-26/IL-2 cells, others were vaccinated and received rIL-12 i.p., and others received rIL-12 only. □, C-26/IL-2 [3 \times 10^6 cells s.c. (days 3, 6, 9, and 13)]; ▪, C-26/IL-2 + rIL-2 (0.1 µg i.p. (days 3, 6, 9, and 13)); ○, C-26/IL-2 + rIL-12 (0.1 µg i.p. (days 6–9 and 13–16)); □, rIL-12 (0.1 µg i.p. (days 6–9 and 13–16)).

SPCs from individual C-26/IL-2-challenged mice treated or not treated with rIL-12 were collected and tested for both IFN-γ production following stimulation with anti-CD3 mAb coated to plastic and CTL activity against C-26 and YAC-1 targets following in vitro restimulation with irradiated C-26 cells. Fig. 24 shows that SPCs received 0.5 or 0.1 µg/day rIL-12 i.p. on days +7 to +11 and +14 to +18 after tumor challenge. Both doses greatly reduced the final takes of C-26/IL-2 cells, but had no effect on the growth of C-26/GM-CSF and C-26 parental cells (Fig. 1). A single 0.5-µg injection on day 0 or +14 was equally ineffective (data not shown).

SPCs from individual C-26/IL-2-challenged mice treated or not treated with rIL-12 were collected and tested for both IFN-γ production following stimulation with anti-CD3 mAb coated to plastic and CTL activity against C-26 and YAC-1 targets following in vitro restimulation with irradiated C-26 cells. Fig. 24 shows that SPCs collected after the first rIL-12 course (day +11) are high producers of IFN-γ, whereas those collected after the second course (day +18) and from untreated mice produced little IFN-γ (Fig. 2A). In contrast, CTL activity was not enhanced after either the first or the second course of rIL-12 treatment. The use of SPCs from single mice instead of pooled spleens produced a very wide range of individual responses. The absence of any correlation between the level of CTL reactivity and either the presence of a tumor or the treatment with IL-12 (Fig. 2B) suggests that IFN-γ production is more critical than CTL activity in the regression of C-26/IL-2 stemming from cooperation between the locally released IL-2 and systemic rIL-12.

Translation of this cooperation into a clinical setting requires experiments in which tumor cells engineered to produce IL-2 are used in conjunction with systemic rIL-12 to cure tumor-bearing mice. This is rendered particularly difficult in the case of C-26 by its early induction of cachexia (17). Use was therefore made of an i.v. challenge with C-51, a less wasting colon carcinoma that shares transplantation and CTL-recognized antigens with C-26 (18). The use of irradiated C-26/IL-2 cells vaccine to cure mice with C-51 metastases is an attractive approach, since this setting is close to clinical situations where most antigens thus far identified are common to tumors from several patients. Mice challenged with C-51 cells i.v. at day 0 received s.c. injections of 3 \(10^6\) irradiated C-26/IL-2 cells at days +3, +6, +9, and +13. A few also received 0.1 µg rIL-12 either on the same days or from days +6 +9 and then from days +13 +16. Other challenged mice received these two courses only (Fig. 3). Vaccination with C-26/IL-2 cells was ineffective, 20% of the mice survived when treated with rIL-12 given alone or in conjunction with C-26/IL-2 cells, and 80% survived when C-26/IL-2 vaccination was combined with the two 4-day courses. This greater susceptibility of C-51...
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to IL-12 makes it clear that C-26 cells are exceptionally resistant to the antitumor reaction it elicits. Since C-26 and C-51 are antigenically related, this difference in response may have a nonimmunological explanation.

In another set of experiments, cells from TSA, a spontaneous mammary adenocarcinoma, were used. In vivo, TSA cells engineered to release IL-2 (TSA/IL-2) are inhibited by the reaction elicited by the secreted IL-2 in proportion to the amount released. The clone that produces about 30 units IL-2/10^6 cells/48 h (TSA/IL-2^{300}) grows in about 50% of the challenged mice, whereas that releasing 3600 units IL-2 (TSA/IL-2^{3600}) is quickly rejected by all mice (15). Administration of 0.5 μg/day rIL-2 in two 5-day courses (days +7 to +11 and +14 to +18) protected all mice against a TSA/IL-2 challenge. These courses inhibited 40% against a parental TSA challenge (Fig. 4A).

Finally, we explored the ability of rIL-2 to cooperate with a TSA/IL-2^{3600} cell vaccine. Mice were challenged s.c. in the left flank at day 0 with TSA parental cells. Ten percent of the mice that received 1 × 10^7 TSA/IL-2^{3600} cells in the right flank during the next 3 weeks were cured. Administration of rIL-2 alone in two courses starting at day +1 cured 40% of the mice. Its association with TSA/IL-2^{3600} cured 60% and significantly extended the latency period of the TSA parental tumor that eventually appeared (Fig. 4B).

When rIL-2 administration was postponed to day +7, IL-2 alone unexpectedly cured 70% of the mice, and this percentage was not enhanced by the association of TSA/IL-2^{3600} vaccination (Fig. 4C). When rIL-2 administration was commenced on day +14, IL-2 alone was still able to cure 10%. On this occasion, however, its association with vaccination cured 20% of the mice, and some tumors presented an extended latency time (Fig. 4D).

It is interesting to note that rIL-2 cures more mice when its administration begins on day +7 (3.5 mm, mean diameter) rather than day +1 (only about 5 × 10^{-4} mm). At day +14, TSA tumors have a mean diameter of about 5 mm and are fully evident (19). This is in line with the finding of Nagouchi et al. (20) that the effects of IL-12 are more striking against established tumors than incipient tumors (20), a situation that may imply a dependence on the number of infiltrating T and NK cells. Accordingly, the efficacy of IL-12 on C-26 and TSA tumors is highly improved on IL-2 gene-transduced tumor cells showing an increased number of tumor-infiltrating leukocytes stained by anti-CD3 and asialo-GM-1 monoclonal and polyclonal antibodies, respectively (15).

Systemic rIL-12 appears to be particularly effective when an established tumor displays a certain number of infiltrating cells but has not yet reached the stage in which a tumor-activated suppression takes place. The additional effects observed when systemic rIL-12 is combined with locally released IL-2-engineered target tumor cells or vaccination with IL-2-engineered tumors may principally depend on the greater number of preactivated leukocytes recruited at the tumor site. On the strength of these findings, the association of rIL-12 with IL-2 gene-transduced melanoma cell vaccination (21) will be considered for use in ensuing clinical protocols.

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References

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