REPORTS

Administration of Interleukin 12 With Pulse Interleukin 2 and the Rapid and Complete Eradication of Murine Renal Carcinoma

Jon M. Wigginton, Kristin L. Komschlies, Timothy C. Back, José L. Franco, Michael J. Brunda, Robert H. Wiltrout*

Background: Interleukin 2 (IL-2) and interleukin 12 (IL-12) are potent immunoregulatory cytokines that exhibit antitumor activity. Preliminary evidence suggests that combined administration of IL-2 and IL-12 may yield greater antitumor activity than that observed with either agent alone.

Purpose: We evaluated the ability of combination regimens of IL-2 and IL-12 to induce regression of established primary and metastatic murine renal carcinoma (Renca) tumors.

Methods: BALB/c mice were given either subcutaneous or intrarenal injections of 10^5 Renca cells; tumor cell injections were given to 10-12 mice for each treatment group. Mice bearing subcutaneous primary tumors were treated with chronic IL-2 (300 000 IU given on a daily basis) or pulse IL-2 (300 000 IU given twice daily one day per week) alone, IL-12 alone (0.5 μg given on a daily basis), or IL-12 in combination with either chronic or pulse IL-2. Mice with metastatic tumors (arising from intrarenal implants; animals were nephrectomized to remove the primary tumors) were treated with IL-12 plus or minus pulse IL-2; in these experiments, IL-12 was given at doses of either 0.5 or 1.0 μg. In most experiments, treatment was continued for at least 3 weeks. Two-sided statistical tests were used to evaluate the data.

Results: Most mice with subcutaneous Renca tumors treated with the combination of IL-12 and chronic IL-2 died of treatment-related toxic effects within 7-14 days. In contrast, treatment with IL-12 plus pulse IL-2 was well tolerated, and six of 10 mice experienced complete tumor regression; none of the mice treated with either IL-12 alone or pulse IL-2 alone experienced a curative response. Seven of eight and nine of nine mice with metastatic tumors experienced complete tumor regression after treatment with 0.5 μg IL-12 plus pulse IL-2 or 1.0 μg IL-12 plus pulse IL-2, respectively; two of 12 mice treated with pulse IL-2 alone and 10% or less of mice treated with IL-12 alone were cured of metastatic tumors (with 0.5 μg IL-12, none of 10 mice; with 1.0 μg IL-12, one of 10 mice). Five of 10 mice with metastatic tumors treated with a short-course regimen of IL-12 and pulse IL-2 (two pulses of IL-2 flanking 5 days of 0.5 μg IL-12) experienced complete tumor regression, while only one of the 12 mice treated with IL-2 alone and none of the mice treated with IL-12 alone experienced complete tumor regression. Virtually all curative response frequencies obtained with IL-12 and pulse IL-2 combination regimens differed significantly (P<.05) from those obtained with corresponding single-agent treatments.

Conclusions: IL-12 administered in combination with pulse IL-2 induced rapid and complete regression of primary and metastatic Renca tumors and displayed greater antitumor activity than that observed with either IL-12 or IL-2 alone. [J Natl Cancer Inst 1996;88:38-43]

Interleukin 12 (IL-12) is a recently discovered immunoregulatory cytokine that may provide an important link between nonspecific immune surveillance mechanisms and the initiation and development of a specific T-cell-mediated immune response (1-4). In particular, IL-12 may enhance proliferation, cytokine production, and cytotoxic activity of T lymphocytes and natural killer (NK) cells (1-4), and it may influence the development of a T-helper type 1 (Th1) (i.e., IL-2 and interferon gamma [IFN γ]) versus a T-helper type 2 (i.e., IL-4 and IL-10) pattern of cytokine production during the evolution of an immune response (5,6). In mice infected with Leishmania major, promotion of a Th1 response via an IFN γ-dependent mechanism appears to account for the ability of IL-12 to successfully induce clearance of the infection (7,8). Given the ability of IL-12 to modulate T-cell-mediated immune responses, its therapeutic potential has been evaluated in several preclinical models of infectious (7,9-11) and malignant (12,13) disease. IL-12 has exhibited antitumor activity in a variety of murine cancer models (including renal cancer, B16 melanoma, M5076 reticulum cell sarcoma, and C26 colon carcinoma) (12-14), thus making it an attractive candidate for combination therapy with IL-2, another potent immunoregulatory cytokine with antitumor activity (15-18).

Although IL-2 has shown some efficacy in the treatment of metastatic renal carcinoma and melanoma in humans, the frequency of complete responses is low;
its use at high doses has been limited by the occurrence of cardiovascular and/or renal toxic effects. In this regard, IL-12 displays substantial preclinical antitumor activity in vivo at lower, potentially less toxic doses than those necessary for the optimal induction of antitumor immune responses with IL-2. At these lower doses, IL-12 substantially prolongs the survival of mice bearing well-established tumors, and a portion of the mice are cured of their tumors.

Preliminary in vitro evidence suggests that the combined administration of IL-12 and IL-2 may produce additive or even synergistic immunomodulatory activity and may yield greater antitumor activity than that exhibited with either agent alone. IL-12 and IL-2 use parallel intracellular signaling pathways (19), and they complement each other in promoting the proliferation, IFN γ production, and cytotoxic activity of T and/or NK cells (20-28). IL-2 also enhances the expression of IL-12 receptors on T and NK cells (29). Furthermore, human peripheral blood mononuclear cells (PBMC) treated with IL-12 and IL-2 kill neuroblastoma target cells in vitro more effectively than PBMC treated with either cytokine alone (30). In view of these findings, we evaluated the ability of various regimens of IL-2 administered in combination with IL-12 to induce the regression of established primary and/or metastatic tumors.

Materials and Methods

Mice and Tumor Cells

BALB/c mice were obtained from the Animal Production Area of the National Cancer Institute-Frederick Cancer Research and Development Center. They were maintained in a dedicated pathogen-free environment and used between 8 and 10 weeks of age. Renca, a BALB/c mouse renal adenocarcinoma of spontaneous origin (31), was the tumor cell source for these experiments. The Renca cell line was maintained in BALB/c mice by serial transplantation. They were maintained in a dedicated Production Area of the National Cancer Institute-Frederick Cancer Research and Development Center. Mice were given daily intraperitoneal injections of IL-2 (or vehicle alone) was administered at a dose of either 0.5 or 1.0 μg as described above on days 13-16, 19-23, and 26-30.

Last, we evaluated the antitumor activity of a short-course IL-12 plus pulse IL-2 regimen in the same metastatic Renca model. Again, mice were nephrectomized 12 days after tumor implantation. Pulse IL-2 (or vehicle alone) was administered only on the day of nephrectomy and on day 19. IL-12 (or vehicle alone) was administered at a dose of 0.5 μg on days 12-16.

Statistical Analysis

The proportions of mice experiencing curative responses in the various treatment groups were compared using Fisher's exact test. Tumor volumes were estimated by measuring the smallest and largest dimensions of each tumor and calculating the product of the square of the smallest dimension multiplied by the largest dimension. Calculated tumor volumes were compared between groups using the nonparametric Wilcoxon rank sum test. The Jonckheere test for trend was used to evaluate the trend in tumor volumes when comparing control mice with mice receiving single-agent therapy (IL-2 or IL-12) and with mice receiving combination therapy (IL-2 and IL-12). For some groups, survival duration was compared by use of the Kaplan-Meier method. All P values were obtained from two-tailed tests of statistical significance and were considered significant when P < 0.05.

Results

In initial experiments, IL-12 was administered daily, alone or in combination with IL-2 that was given either daily (chronic regimen) or weekly (pulse regimen), to mice bearing established subcutaneous Renca tumors. At the doses used, chronic IL-2 administered with IL-12 appeared to be extremely toxic; 80% of the treated mice died between day 14 and day 21 after tumor cell implantation (7-14 days after treatment initiation, where the IL-2 and IL-12 treatments overlapped), compared with no deaths among the untreated controls by as late as day 20 (data not shown). Some modest improvements in survival were noted among mice treated with chronic IL-2 or with IL-12 alone compared with untreated control mice (data not shown).
Curative responses were seen in three of 10 mice treated with chronic IL-2 compared with none of 10 mice treated with IL-12 alone and one of two mice surviving the drug-related toxicity seen with the combination of IL-12 and IL-2.

In contrast, daily administration of IL-12 in combination with weekly pulses of IL-2 was well tolerated, with no apparent drug-related deaths. In addition, this treatment regimen produced considerably improved antitumor activity, with 60% of the mice (i.e., six of 10) treated with IL-12 plus pulse IL-2 achieving a curative response, while no curative responses were noted among mice treated with either agent alone ($P = .011$, IL-12 plus pulse IL-2 versus IL-12 alone; $P = .011$, IL-12 plus pulse IL-2 versus pulse IL-2 alone) (Fig. 1). A substantial reduction in median tumor volume was noted after completion of only two pulses of IL-2 and 5 days of IL-12 (21 days after tumor implantation) (Fig. 2). This reduction in tumor volume was statistically significant in mice treated with the combination of IL-12 and pulse IL-2 compared with untreated controls ($P = .003$), with marginally significant reductions among mice treated with pulse IL-2 ($P = .04$) or IL-12 ($P = .052$) alone compared with controls. When tumor volumes of mice treated with either IL-2 or IL-12 alone were compared with one another or with the tumor volumes of mice treated with the combination of IL-12 and pulse IL-2, they were not significantly different ($P = 1.0$, pulse IL-2 versus IL-12; $P = .31$, pulse IL-2 versus IL-12 plus pulse IL-2; and $P = .13$, IL-12 versus IL-12 plus pulse IL-2). Further analysis, however, indicated a highly significant ($P = .0026$) trend toward reduction in tumor volume in mice receiving the combination of IL-12 and pulse IL-2 versus those receiving only IL-12 or IL-2.

Given the potent antitumor activity of the combination of IL-12 and pulse IL-2, we next evaluated it in a more therapeutically challenging model designed to assess activity against established, widespread metastatic disease. In an attempt to further optimize the antitumor activity of the cytokine combination, we also compared use of IL-12 at the original dose of 0.5 μg with use at a dose of 1.0 μg, administered alone or in combination with pulse IL-2. In these experiments, we observed an even more striking effect with the combination of IL-12 plus pulse IL-2 (Fig. 3). IL-12 given alone at doses of 0.5 or 1 μg prolonged median survival (in comparison with controls) by 8 days ($P = .2$, not significant) and 17 days ($P = .035$), respectively, although most mice died as a consequence of tumor progression after the discontinuation of therapy. However, curative responses were seen in 100% and 88% of mice treated with pulse IL-2 and the 1.0- or the 0.5-μg dose of
IL-12 (nine of nine mice versus seven of eight mice), respectively. Only 17% of the mice (two of 12) treated with pulse IL-2 alone and 10% (one of 10) and 0% (none of 10) of the mice treated with the 1.0- and 0.5-μg doses of IL-12 alone, respectively, experienced curative responses (P = .00012, 1.0 μg IL-12 plus pulse IL-2 versus 1.0 μg IL-12; P = .00022, 1.0 μg IL-12 plus pulse IL-2 versus pulse IL-2; P = .00025, 0.5 μg IL-12 plus pulse IL-2 versus 0.5 μg IL-12; and P = .0045, 0.5 μg IL-12 plus pulse IL-2 versus pulse IL-2). Among mice cured of their original tumors, five of seven (71%) mice treated with the 0.5-μg dose of IL-12 plus pulse IL-2 and four of nine (44%) mice treated with the 1.0-μg dose of IL-12 plus pulse IL-2 rejected a rechallenge with 1.0 × 10^5 Renca cells that were administered subcutaneously; none of eight (0%) naive control mice were resistant.

Given the rapid and complete regression of tumors induced by IL-12 plus pulse IL-2, we initiated an examination of the antitumor activity of a short course of this regimen in the metastatic Renca model. Approximately 60 days after tumor implantation, we have found that seven (70%) of 10 mice treated with short-course 0.5-μg IL-12 plus pulse IL-2 are alive and five (50%) are tumor free (Fig. 4). In contrast, three (25%) of 12 mice treated with IL-2 alone are alive, with only one of these remaining tumor free (P = .056 for complete tumor regression, IL-12 plus IL-2 versus IL-2); among mice treated with IL-12 alone, only one (10%) of 10 has survived, and this mouse is harboring progressive, residual tumor (P = .033 for complete tumor regression, IL-12 plus pulse IL-2 versus IL-12).

Discussion

IL-2 has been used successfully to treat some patients with advanced cancer (15-18), but its use has been limited by the occurrence of severe toxic effects, especially in some high-dose regimens. It is clear that optimal expansion and activation of cytotoxic effector cells in vivo probably require an array of appropriately timed signals and cellular interactions. As a consequence, efforts have been directed toward the development of treatment regimens using combinations of biologic response modifiers in an attempt to optimize antitumor immune responses. Given evidence that IL-12 and IL-2 may interact favorably in the induction of an immune response (19-30), we have begun to evaluate the antitumor activity of combinations of these cytokines in vivo.

Treatment of well-established primary tumors and/or metastatic disease is the most relevant setting for preclinical evaluation of immunotherapy regimens. In models using rapidly growing murine tumors, such evaluation is often complicated by the relatively short time interval.
between tumor implantation and the development of a lethal tumor burden. However, it has been reported that IL-12 alone possesses substantial therapeutic activity against a variety of established murine tumors, including Renca (13,14). We have confirmed this observation and have developed a combination regimen consisting of treatment with IL-12 and IL-2 (currently a Food and Drug Administration-approved agent) that is not only well tolerated, but also exhibits dramatic activity against established Renca tumors. Although injection of 1 x 10^5 Renca cells is uniformly fatal to untreated mice by day 35 to day 45 after implantation, we have shown that the combination regimen (IL-12 plus pulse IL-2) may be initiated after tumors are well established and/or metastatic disease has developed, yet it can still induce complete regression of primary and/or metastatic disease in up to 88%-100% of treated mice.

The antitumor response induced by this combination of cytokines may be initiated very rapidly. Mice bearing subcutaneous Renca tumors that had received as little as 5 days of IL-12 treatment and two pulses of IL-2 exhibited significantly smaller tumor volumes compared with those of untreated mice. Furthermore, a highly significant trend toward smaller tumors was observed in mice treated with the combination compared with mice receiving either cytokine alone. In addition, in the metastatic Renca model, treatment with a total of only two pulses of IL-2 flanking 5 days of IL-12 (the short-course regimen) induced complete tumor regression in 50% of the treated mice. Thus, the combination regimen not only induces a substantial delay in tumor growth that is manifest early in therapy, but it also possesses a potent ability to engage antitumor mechanisms sufficient to achieve complete and sustained eradication of tumors, even with a very short course of application. To further limit potential toxicity, we are attempting to identify even less dose-intensive schedules of this cytokine combination that retain comparable antitumor activity.

Mice cured of Renca tumors by immunotherapy consisting of flavone acetic acid plus IL-2 are resistant to rechallenge (34). Thus, although most of the mice cured of tumors after treatment with IL-12 plus pulse IL-2 resisted tumor rechall-
The repository of the Biological Response Modifiers Program (BRMP), Division of Cancer Treatment (DCT), NCI, NIH, announces the availability of recombinant human lymphokines IL-1α, IL-1β, and IL-2; the monoclonal antibody 11B.11 against mouse IL-4; and the monoclonal antibody 3ZD against human IL-1β.

Use of these materials is limited solely to in vivo and in vitro basic research studies and is not intended for administration to humans. The lymphokine materials are aliquoted in 100 μg amounts (>10^6 units) and are available to investigators with peer-reviewed support. However, manufacturers' restrictions prohibit distribution of these materials to for-profit institutions or commercial establishments.

The monoclonal antibodies are available to peer-reviewed investigators, for-profit institutions or commercial establishments. The 11B.11 antibody is available in either 3 or 20 mg vials. The 3ZD antibody is available in 5 or 20 mg amounts.

Investigators wishing to obtain any of these materials should send requests to:

Dr. Craig W. Reynolds
Biological Response Modifiers Program
NCI-FCRDC
Building 1052, Room 253
Frederick, MD 21702-1201
FAX: 301-846-5429

Notes

We thank Dr. Dan L. Longo and John R. OrtaIdo for their critical review of the manuscript. Joyce Vincent for typing and editing. Charles RiggS for statistical support, and Dolly Graham for preparing the figures and technical assistance.

Manuscript received May 18, 1995; revised August 25, 1995; accepted September 21, 1995.