Restoration of Tumor Immunosurveillance via Targeting of Interleukin-13 Receptor-α2

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Abstract

In previous studies, we described a “counter-immunosurveillance” mechanism initiated by tumor-activated, interleukin-13 (IL-13)–producing natural killer T cells that signal Gr-1+ cells to produce transforming growth factor-β1 (TGF-β1), a cytokine that suppresses the activity of tumor-inhibiting cytolytic CD8+ T cells. Here, we show that in two tumor models (the CT-26 metastatic colon cancer and the 15-12RM fibrosarcoma regressor models), this counter-surveillance mechanism requires the expression of a novel IL-13 receptor, IL-13Rα2, on Gr-1intermediate cells, because down-regulation of IL-13Rα2 expression or the activator protein-1 signal generated by the receptor via in vivo administration of specific small interfering RNA or decoy oligonucleotides leads to loss of TGF-β1 production. Furthermore, acting on prior studies showing that IL-13Rα2 expression is induced (in part) by tumor necrosis factor-α (TNF-α), we show that receptor expression and TGF-β1 production is inhibited by administration of a TNF-α–neutralizing substance, TNF-αR-Fc (etanercept). Taking advantage of this latter fact, we then show in the CT-26 model that counter-immunosurveillance can be inhibited. anti-CT-26–specific CD8+ cytolytic activity can be restored, and CT-26 metastatic tumor nodules can be greatly decreased by administration of TNF-αR-Fc. Corroborative data were obtained using the 15-12RM fibrosarcoma model. These studies point to the prevention of metastatic cancer with an available agent with already known clinically acceptable adverse effects and toxicity.

Introduction

Recent studies of the murine 15-12RM fibrosarcoma regressor and CT-26 colon carcinoma lung metastasis models have shown that the function of T cells engaged in the immunosurveillance of tumors can be undermined by tumor-driven immune counter-surveillance mechanisms. In these studies, it was shown that after a period of initial growth, the fibrosarcoma undergoes regression due to the development of cytolytic CD8+ T cells; however, the tumor later recurs and persists due to the appearance of cells producing TGF-β1 that inhibits the cytolytic T cells. The chain of cellular events that leads to such reversal of immunosurveillance involves first the tumor-induced expansion of NKT cells that produce interleukin-13 (IL-13), followed by IL-13 induction of TGF-β1 by a cell bearing a Gr-1 marker (most likely a monocyte cell; refs. 1–3).

An important question that arose from the above studies relates to how IL-13 signaling activates cells to produce TGF-β1. One possibility comes from studies of TGF-β1 production during inflammation that showed that IL-13 induction of TGF-β1 is a two-stage process whose first stage is the induction of an IL-13 receptor previously thought to be a decoy receptor without signaling function, IL-13Rα2; this event is then followed by a second stage involving IL-13 signaling through this receptor. The initial induction of IL-13Rα2 expression requires IL-4 or IL-13 signaling via the IL-13Rα1 receptor to generate activated Stat6 and tumor necrosis factor-α (TNF-α) signaling to generate nuclear factor-κB (NF-κB), whereas the induction of TGF-β1 secretion requires IL-13 signaling via IL-13Rα2 to generate an activator protein-1 (AP-1) variant composed of c-jun and Fra-2 (4).

In the present study, we determined whether TGF-β1 production arising from IL-13 signaling via IL-13Rα2, as described above, applies to counter-immunosurveillance in the syngeneic CT-26 colon cancer and 15-12RM fibrosarcoma regressor experimental tumor models.

Materials and Methods

TNF-αR-Fc and control IgG1. TNF-αR-Fc (Enbrel, etanercept) was purchased from Amgen. It consists of a dimeric fusion protein of the human (75 kDa) TNF receptor linked to the Fe portion of human IgG1. Control IgG1 consisted of human polyclonal IgG1 obtained from Invitrogen or HuMik-p1 clinical grade human monoclonal IgG1 antibody obtained from Dr. T.A. Waldmann [National Cancer Institute (NCI), NIH, Bethesda, MD]. The amount of etanercept used was selected based on previous experience using this human agent in a mouse system (4, 5). The amount of 100 μg of etanercept administered every other day is slightly higher than the dose suggested for use in humans.

Mice. Female BALB/c mice (8–10 wk old) were used in studies of tumor development in both the CT-26 colon cancer and 15-12RM fibrosarcoma models. All mice were obtained from The Jackson Laboratory and were maintained in the National Institute of Allergy and Infectious Diseases (NIAID) animal holding facilities. Animal use adhered to NIH Laboratory Animal Care Guidelines and was approved by the NIAID and NCI Animal Care and Use Committee Review Boards.

Cell lines. The CT-26 cell line (a N-nitro-N-methylurethane–induced BALB/c murine colon carcinoma cell) was purchased from the American Type Culture Collection and maintained in RPMI 1640 complete medium supplemented with 10% FCS, 1-glutamine, sodium pyruvate, streptomycin, and penicillin. The 15-12RM fibrosarcoma cell line (BALB/c ST3 fibroblasts transfected with HIV-1 HIIB gp160, Bas and Myc) originally developed in this laboratory was maintained in RPMI 1640 complete medium with 200 μg/mL of G418 (6).

Assessment of CT-26 tumor cell pulmonary nodules. The CT-26 tumor model was initiated by tail vein injection of 0.5 × 106 tumor cells derived from the CT-26 cell line. Thereafter, mice were randomly separated...
into several groups depending on the experiment being conducted. Enumeration of pulmonary nodules was done at the time control mice had sufficient numbers of pulmonary tumor nodules to allow reliable quantitation. In effect, this occurred at day 21 after CT-26 cell injection in studies wherein treatment was initiated at the time of initial tumor cell injection and at day 28 in studies wherein treatment was delayed to a later point in time. CT-26 cell pulmonary nodes were enumerated by counting the number of macroscopically apparent nodules in dissected lungs (7).

Assessment of s.c. 15-12RM fibrosarcoma growth. One million 15-12RM cells were injected s.c. at a site on the right flank of a mouse under study. The size of tumor nodules was measured periodically by caliper gauge.

Encapsulation of small interfering RNA and oligonucleotides in HVJ envelope vector. Encapsulation of small interfering RNA (siRNA) or decoy oligonucleotides for in vivo transfection was done as previously described (8).

IL-13Rα2-specific siRNA. IL-13Rα2-specific siRNA and control (scrambled) siRNA for use in gene silencing studies was obtained from Dharmacon. The specific mRNA targeting sequence was 5′-GGAATC-TAAATTACAAGGA-3′. The specificity and efficiency of this siRNA has been previously shown (4, 9).

IL-13Rα2 decoy ODN. Decoy oligodeoxynucleotides (ODN) targeting AP-1 were prepared from complementary single-stranded ODN obtained from Qiagen by melting at 95°C 3 to 5 min followed by incubation for 3 h at ambient temperature. For in vivo transfection, 100 μg of AP-1 decoy ODN or scrambled oligonucleotides were administered via an intratracheal route following encapsulation in HVJ-E. The following sequences were used: AP-1 decoy ODN, 5′-CGCTTGTAGTACTGGCAGC- GAA-3′ and 3′-GGCAACTGATGTCGGCTCT-5′; scrambled decoy ODN, 5′- CATGCTGTACCTGGCCTAT-3′ and 3′-GATACGACGTTGGAG-5′.

CTL assays. CTL assays were done using cells obtained from single-cell suspensions of splenocytes isolated from CT-26 tumor-bearing mice. Splenocytes (2 × 10⁵ cells) were restimulated in vitro with CT-26 cells (5 × 10⁴) treated with mitomycin C. After 2 d, cytolytic activity against CT-26 cells was determined by the CellTitre-Glo Luminescent Cell Viability Assay (Promega).

Western blot. The cells were lysed by radioimmunoprecipitation assay buffer and the whole-cell lysates thus obtained were subjected to SDS-PAGE. The separated proteins obtained were transferred to a nitrocellulose membrane and immunoblotted. IL-13Rα1 (measured by RT-PCR) is expressed in the presence of previous experience in several different animal models (bleomycin induced lung fibrosis, chronic TNBS-colitis refs. 4, 9).

Results

Expression of IL-13 signaling components in the CT-26 tumor model. In previous studies, it was shown that IL-13 induces TGF-β1 production via a two-stage process involving first the induction of IL-13Rα2 expression and second IL-13 signaling via this receptor (4). To establish if this mechanism of IL-13 activity occurs in relation to tumor immunity, we determined whether it was operative in the syngeneic CT-26 colon cancer cell model and, as described below, in the 15-12RM fibrosarcoma regression model.

In initial studies of the CT-26 model, we determined whether cells of mice injected with the CT-26 tumor cells activate the first stage of the IL-13 induction process, namely the induction of IL-13Rα2 expression. To this end, we first showed that splenic CD4+ T cells and CD11b+ cells stimulated by anti-CD3/anti-CD28 and by SAC plus IFN-γ, respectively (see Materials and Methods), produced increased amounts of IL-13 and TNF-α when obtained from mice injected with tumor cells compared with un.injected mice (data not shown). Thus, the cells of tumor-bearing mice were capable of actively synthesizing components that up-regulate expression of IL-13Rα2. Next, to determine whether IL-13Rα2 expression was indeed up-regulated in the target organ of the tumor, the lung, we did Western blot studies on extracts of lung tissue. As shown in Fig. 1A, naïve mice not bearing tumor (day 0 mice) express little or no IL-13Rα2 in the lung, whereas mice express this receptor beginning as early as day 7 after tumor injection. In contrast, IL-13Rα1 (measured by RT-PCR) is expressed constitutively. Similar findings were obtained with Western blot studies of spleen cells (data not shown). Such differential expression of IL-13 receptors has been observed previously under in vivo conditions in which IL-13 is able to induce TGF-β1 (4, 9).

Characterization of cells responding to IL-13 and producing TGF-β1 in the CT-26 tumor model. Prior studies have disclosed that the TGF-β1-producing cell in a tumor-bearing mouse is a myeloid cell bearing surface CD11b and Gr-1intermediate but that neither the number of these cells nor the ratio of Gr-1high cells (granulocytes) to Gr-1intermediate (monocytes and immature granulocytes) change as a result of tumor cell administration (2). To determine whether this is also the case in the CT-26 tumor model, we subjected spleen cells of mice 7 days after CT-26 tumor injection to flow cytometric cell sorting to obtain purified populations of cells bearing these markers. These studies showed that once again the ratio of Gr-1high to Gr-1intermediate cells in the spleens of mice was the same in control mice and mice injected with tumor cells. In addition, as shown in Fig. 1B, we found that the cells bearing high amounts of CD11b sorted into two distinct Gr–1positive populations, one CD11bhigh/Gr-1high and the other CD11bhigh/Gr-1intermediate. To determine which of these cell populations is involved in TGF-β1 production, we measured TGF-β1 production and IL-13R expression in the purified (sorted) cells. As shown in Fig. 1C, after in vitro culture in the presence of
IL-13, only the CD11b<sup>high</sup>/Gr-1<sup>intermediate</sup> cells from tumor-bearing mice produced substantial amounts of TGF-β<sub>1</sub>. In addition, as shown in Fig. 1D, whereas both cell populations expressed constitutive levels of IL-13Rα<sub>2</sub> regardless of tumor burden, only the CD11b<sup>high</sup>/Gr-1<sup>intermediate</sup> cells expressed IL-13Rα<sub>2</sub>, and such expression was noted only after tumor cell injection. Although these studies were conducted with spleen cell populations, it is reasonable to assume that cells with similar properties are also present in the lung of the tumor-bearing mouse.

**Inhibition of IL-13 induction of TGF-β<sub>1</sub> in the CT-26 tumor model.** If indeed IL-13 induces TGF-β<sub>1</sub> production via IL-13Rα<sub>2</sub> signaling, one might predict that such production should be inhibited by administration of various agents that block this pathway at various stages of its development. Recognizing that TNF-α (and IL-4 or IL-13) stimulation was necessary to induce surface expression of IL-13Rα<sub>2</sub>, in one approach to test this prediction we administered TNF-αR-Fc (etanercept; 100 μg by i.p. injection) every other day beginning on day 0 of CT-26 tumor cell administration) to block TNF-α signaling. As shown in the Western blot of cell extracts of purified CD11b<sup>high</sup>/Gr-1<sup>intermediate</sup> splenocytes prepared on day 7 after CT-26 administration depicted in Fig. 2A, such treatment prevented the expression of the IL-13Rα<sub>2</sub> in these cells, whereas similar cells from mice treated with control IgG expressed this receptor. In contrast, expression of the IL-13Rα<sub>2</sub> was constitutively present and unchanged in the face of TNF-αR-Fc treatment (data not shown). In concomitant studies, shown in Fig. 2B, CD11b<sup>high</sup>/Gr-1<sup>intermediate</sup> cells obtained from the spleens of mice on day 7 after CT-26 administration and cultured with IL-13 produced increased amounts of TGF-β<sub>1</sub> if obtained from mice subjected to control IgG treatment, whereas those that were subjected to TNF-αR-Fc treatment did not produce increased amounts of this cytokine.

In a second approach along these lines, we administered IL-13Rα<sub>2</sub>-specific siRNA or control siRNA to mice to directly inhibit IL-13Rα<sub>2</sub> synthesis at the molecular level. In these studies, the siRNA was administered (at 100 μg siRNA) every other day by an intranasal route and the siRNA was encapsulated in a viral coat preparation to enhance cell entry in vivo (HVJ-E; see Materials and Methods). As also shown in Fig. 2A and B, the result was similar to that obtained with TNF-αR-Fc, in that again, treatment prevents IL-13Rα<sub>2</sub> expression and decreased the capacity of CD11b<sup>high</sup>/Gr-1<sup>intermediate</sup> cells to produce TGF-β<sub>1</sub>.

In a third and final approach to testing the prediction that blockade of IL-13Rα<sub>2</sub> signaling would block TGF-β<sub>1</sub> production, we blocked such signaling by administration of a decoy oligonucleotide that competitively inhibits the binding of the receptors downstream signal, AP-1, to its consensus target sequence. As in the case of the siRNA, the decoy oligonucleotide (or a scrambled control oligonucleotide) was administered by an intranasal route (at 100 μg ODN) following encapsulation in HVJ-E. As shown again in Fig. 2A and B, although the specific AP-1 decoy oligonucleotide had no effect on IL-13Rα<sub>2</sub> expression in CD11b<sup>high</sup>/Gr-1<sup>intermediate</sup> cells, it prevented IL-13-induced TGF-β<sub>1</sub> production, whereas a control (scrambled) decoy had no such effect.

In summary, blockade of IL-13 signaling via IL-13Rα<sub>2</sub> in the CT-26 tumor system using three distinct blocking strategies led to inhibition of IL-13–induced TGF-β<sub>1</sub> production and it thus seems reasonable to conclude that IL-13 induces TGF-β<sub>1</sub> in this system via this signaling pathway.
Anti-CT-26 cytotoxic activity of CD8+ T cells following inhibition of IL-13 induction of TGF-β1. In previous studies, it was shown that the induction of TGF-β1 by IL-13 (produced by NKT cells) leads to diminished CD8+ cell-mediated anti-tumor cell cytotoxicity and resultant counter-immunosurveillance (2). We therefore examined the effect of TNF-αR-Fc, IL-13Rα2-specific siRNA, or AP-1 decoy oligonucleotide administration as indicated above on CD8+ T-cell-mediated cytotoxic activity directed against CT-26 cells. As shown in Fig. 2C, whereas CD8+ spleen T cells from untreated mice 7 days after CT-26 administration exhibited little or no toxicity for CT-26 cells, CD8+ T cells from mice given all three inhibitors of IL-13 induction of TGF-β1 exhibited substantial cytotoxic activity against CT-26 tumor cell targets. Parallel studies of CD4+ spleen T cell showed that the latter population exhibited no cytotoxicity for CT-26. Finally, to establish the specificity of CD8+ T-cell cytotoxic activity for CT-26 tumor cells, we determined if these cells were cytotoxic for 4T1 cells (BALB/c mouse mammary tumor cells). We found that CD8+ spleen cells from mice inoculated with CT-26 cells did not exhibit cytotoxicity for 4T1 cells, indicating the specificity of the cytotoxicity against CT-26 cells (data not shown). Thus, the inhibition of IL-13 induction of TGF-β1 led to the acquisition of an immune function that could mediate immunosurveillance.

Effect of delayed administration of IL-13 signaling inhibitors on TGF-β1 production and CD8+ T-cell cytotoxic activity. As a further test of the effect of inhibitors of IL-13 induction of TGF-β1 on immune counter-surveillance, we determined the effect of delayed administration of TNF-αR-Fc (every other day, starting on day 0), IL-13Rα2-specific siRNA, or AP-1 decoy oligonucleotide (weekly, starting on day 7) after tumor cell injection and evaluation of effects on IL-13Rα2 expression, TGF-β1 production, and cytotoxicity of CD8+ cells on day 11 after tumor injection. As shown in the Western blot analysis of extracts of CD11bhigh/Gr-1intermediate cells isolated from the spleens of mice on day 11 after CT-26 cell injection depicted in Fig. 3A, the cells continued to express IL-13Rα2 after delayed treatment with both inhibitors. However, as shown in Fig. 3B, although CD11bhigh/Gr-1intermediate cells isolated on day 11 produced increased TGF-β1 in response to IL-13 after delayed treatment of mice with TNF-αR-Fc starting on day 7, they did not do so after delayed treatment with AP-1 decoy oligonucleotides. In addition, as shown in Fig. 3C, a similar dichotomy was observed with respect to the cytotoxic activity of CD8+ cells for CT-26 tumor cells: Delayed administration on day 7 of TNF-αR-Fc was associated with low-level cytotoxicity of CD8+ cells isolated on day 11, whereas administration of AP-1 decoy oligonucleotides was associated with robust cytotoxicity of CD8+ cells for tumor cells. Taken together, these studies show that delayed administration of TNF-αR-Fc had little or no effect on immune counter-surveillance in the time frame studied, probably because this inhibitor did not affect the function of cells already expressing IL-13Rα2. In contrast,
the AP-1 decoy interfered with the downstream signaling of the receptor even in cells already bearing the receptor. As in previous studies conducted at an earlier time point, CD11b^{high}/Gr-1^{high} cells isolated at day 11 did not express IL-13R_{\alpha 2} and did not produce TGF-\beta_1 in response to IL-13. Finally, it should be noted that treatment of mice with TNF-\alpha_R-Fc, IL-13R_{\alpha 2}-specific siRNA, or AP-1 decoy oligonucleotides did not influence the serum levels of soluble IL-13R_{\alpha 2} throughout the entire time course the mice were monitored in this animal model (data not shown).

**Clinical effect of inhibition of IL-13 induction of TGF-\beta_1 by targeting IL-13R_{\alpha 2} signaling.** The clinical effect of inhibition of IL-13 induction of TGF-\beta_1 by targeting IL-13R_{\alpha 2} in the CT-26 tumor model was subsequently determined by mouse mortality and enumeration of macroscopic pulmonary nodules at 21 days after tumor cell injection. Treatment was done by using 100 \mu g TNF-\alpha_R-Fc every other day starting on day 0, 100 \mu g IL-13R_{\alpha 2}-specific siRNA every other day starting on day 0, or 100 \mu g AP-1 decoy oligonucleotides once weekly starting on day 0. As shown in Fig. 4A and B, mice given tumor cells without any form of treatment or mice given control materials (IgG control, siRNA control, or scrambled oligonucleotide control) displayed >250 macroscopic pulmonary nodules and suffered a mortality rate of 50% by day 21. In contrast, treatment groups administered with TNF-\alpha_R-Fc, IL-13R_{\alpha 2}-specific siRNA, or AP-1 decoy oligonucleotides at the time of tumor cell injection exhibited greatly reduced numbers (and smaller size) of macroscopic tumor nodules and significantly lower mortality on day 21. It should be noted that because the number of pulmonary nodules in mice receiving control IgG did not differ from those not receiving the latter (siRNA control and scrambled oligonucleotide control mice), there was no evidence that the control IgG had any effect on tumor development.

In a more stringent test of the possible therapeutic benefit of targeting IL-13R_{\alpha 2} signaling in the CT-26 tumor model, we evaluated the effect of administration of inhibitors beginning at day 14 after initial tumor cell injection when pulmonary nodules already made their appearance. Treatment was done by using 100 \mu g TNF-\alpha_R-Fc every other day starting on day 14, 100 \mu g IL-13R_{\alpha 2}-specific siRNA every other day starting on day 14, or 100 \mu g AP-1 decoy oligonucleotides once weekly starting on day 14. As shown in Fig. 5A and B, untreated mice or mice treated with...
control materials beginning on day 14 again exhibited high
numbers of pulmonary tumor nodules and high mortality rates
on day 28. In addition, in this case, administration of TNF-α-Fc
every other day beginning at day 14 did not reduce the number
of pulmonary nodules at day 28 and had no effect on mortality,
whereas administration of AP-1 decoy oligonucleotides on day 14
and day 21 led to a significant decrease in the number of
pulmonary nodules at day 28 and greatly reduced mortality. In fact,
mice given AP-1 decoy oligonucleotides exhibited a similar number
of pulmonary nodules at day 28 as control, untreated mice did at
day 14, indicating that this treatment had almost completely
suppressed the progression of tumor formation and also greatly
improved survival of the mice even with delayed treatment.

These findings are compatible with the supposition that by day
14 after tumor cell injection, IL-13Rα2 expression had already been
induced and was therefore beyond the point when it could be
inhibited by TNF-α-Fc; at this time point, AP-1 decoy ODN could
still exert its negative effect on IL-13Rα2 downstream signaling,
thereby maintaining the continued clinical effectiveness of the latter blocker.

**Effects of targeting IL-13Rα2 signaling on the growth of**
**15-12RM fibrosarcoma.** To determine if the IL-13 induction of
TGF-β1 also requires signaling via IL-13Rα2 in a second tumor
model exhibiting counter-immunosurveillance, we turned to the
15-12RM fibrosarcoma tumor model. As noted above, this was in
fact the first tumor model in which immune counter-surveillance
via IL-13 secretion had been shown. As in the case of the CT-26
tumor cell model, tumor growth in this model was associated with
the expression of IL-13, TNF-α, and TGF-β1, and, as shown in
Fig. 6A, CD11b^{high}/Gr-1^{intermediate} spleen cells but not CD11b^{high}/Gr-1^{high} spleen cells isolated on day 14 after tumor cell injection
expressed IL-13Rα2. Repeated administration of TNF-α-Fc initiat-
ed at the time of tumor cell injection inhibited such expression.
In addition, as shown in Fig. 6B, administration of TNF-α-Fc greatly
reduced the production of TGF-β1 by CD11b^{high}/Gr-1^{intermediate}
spleen cells. Finally, and most importantly, as shown in Fig. 6C,
treatment of mice initiated at the time of tumor cell inoculation
with TNF-α-Fc (every other day with 100 μg or every day with 60 μg
TNF-α-Fc) greatly reduced tumor recurrence in two separate
studies: on day 60 or day 80 at the end of the observation period,
tumor recurrence was reduced by ~50% in TNF-α-Fc–treated
mice compared with control IgG-treated mice. These studies
therefore verified that the IL-13 induction via IL-13Rα2 is a central
pathway in counter-immunosurveillance in a second tumor cell
model.

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**Figure 4.** CT-26 tumor development in mice subjected to treatment with TNF-α-Fc, IL-13Rα2–specific siRNA, and AP-1–specific decoy oligonucleotides. Treatment started on the day of CT-26 injection. **A,** survival rates of mice until day 21 after CT-26 injection. Data shown are representative of three independent experiments containing 10 mice per group for TNF-α-Fc treatment and AP-1 decoy ODN treatment, and 8 mice per group for IL-13Rα2 siRNA treatment. *P < 0.05 comparing treatment group with control treatment group (Wilcoxon test). **B,** number of pulmonary tumor nodules present on day 21 after CT-26 injection. Points, mean (representative of three independent experiments each containing 10 mice per group); bars, SD. Each point represents a living mouse at the termination of the study on
day 21. ***, *P < 0.01 comparing treatment group with control treatment group (Student's t test).
Discussion

In a series of previous studies, it was shown that immunologic inhibition of tumor growth (immunosurveillance) could be undermined in several different tumor models by tumor-induced secretion of TGF-β1 and the inhibitory effect of this cytokine on tumor antigen-specific cytolytic T cells (2, 10–13). Furthermore, it was shown that such counter-immunosurveillance was initiated by NKT cells that secrete IL-13 and thereby induce Gr-1 cells to produce TGF-β1 (1–3, 13). These studies led to attempts to treat tumors by disarming counter-immunosurveillance. Although these included the use of antagonists that block the activity of either of the above cytokines, they did not include approaches that address key signaling mechanisms involved such as those mediating IL-13 induction of TGF-β1 because the latter were as yet poorly understood.

With regard to this latter point, previous studies showing that IL-13 signals via the type II IL-4R, a heterodimer of the IL-4Rα and IL-13Rα1 that responds to either IL-13 or IL-4, left unanswered the question of why stimulation of Gr-1 cells to produce TGF-β1 required IL-13 and thereby induce IL-3 and thereby induce IL-3 cells to produce TGF-β1 (1–3, 13). These studies led to attempts to treat tumors by disarming counter-immunosurveillance. Although these included the use of antagonists that block the activity of either of the above cytokines, they did not include approaches that address key signaling mechanisms involved such as those mediating IL-13 induction of TGF-β1 because the latter were as yet poorly understood.

Figure 5. CT-26 tumor development in mice subjected to delayed treatment with TNF-αR-Fc and AP-1–specific decoy oligonucleotides. Treatment started 14 d after CT-26 injection. A, survival rates of mice until day 28 after CT-26 injection. Data shown are representative of three independent experiments each containing 10 mice per group. P ≤ 0.05 comparing treatment group with control treatment group (Wilcoxon test). B, number of pulmonary tumor nodules on day 28 after CT-26 injection. Additional panel also shows baseline pulmonary tumor nodules of untreated mice on day 14 after CT-26 injection. Data shown are representative of three independent experiments each containing 10 mice per group. Each point represents a living mouse at the termination of the study on day 28. **, P ≤ 0.01 comparing treatment group with control treatment group (Student’s t test).

Further studies of the IL-13Rα2 receptor have shown that this receptor is not constitutively expressed on the cell surface (as is the IL-13Rα1) and for this reason the induction of TGF-β1 by IL-13, is, in reality, a two-stage process characterized by an initial receptor induction step that is only then followed by the receptor signaling step described above (4, 9). In addition, such induction requires a dual signal consisting of IL-4 or IL-13 acting through IL-13Rα1 and TNF-α acting through its receptor to generate activated Stat6 and NF-κB, respectively, the factors that transactivate the IL-13Rα2 promoter. With the elucidation of this induction step, the major shown that cells lacking cell surface IL-13Rα2 or expressing a mutant form of this receptor lacking the cytoplasmic tail could not be induced by IL-13 to produce TGF-β1. Second, it was shown that agents that blocked the induction of receptor expression also blocked IL-13 induction of TGF-β1. Third, and finally, it was shown that signaling via this receptor resulted in AP-1 generation, and blockade of AP-1 with an AP-1–specific decoy oligonucleotide also blocked IL-13 induction of TGF-β1. In summary, the evidence that IL-13Rα2 serves as a signaling receptor for IL-13 during TGF-β1 induction is quite strong and there appears to be little question that while this receptor can indeed function as a decoy under some circumstances, it also functions as a bona fide receptor for IL-13.

This conclusion is consistent with a recent study showing that the soluble and membrane-bound forms of IL-13Rα2 are actually different splice variants of the IL-13Rα2 gene, with the soluble form lacking the transmembrane domain of the molecule and therefore lacks signaling capability (22).

Further studies of the IL-13Rα2 receptor have shown that this receptor is not constitutively expressed on the cell surface (as is the IL-13Rα1) and for this reason the induction of TGF-β1 by IL-13, is, in reality, a two-stage process characterized by an initial receptor induction step that is only then followed by the receptor signaling step described above (4, 9). In addition, such induction requires a dual signal consisting of IL-4 or IL-13 acting through IL-13Rα1 and TNF-α acting through its receptor to generate activated Stat6 and NF-κB, respectively, the factors that transactivate the IL-13Rα2 promoter. With the elucidation of this induction step, the major
factors involved in IL-13 induction of TGF-β1 were now known and, indeed, it could now be shown that such induction was inhibited in several models of inflammation by administration of an agent that blocked IL-13Rα2 receptor induction, TNF-αR-Fc, an agent that down-regulated the IL-13Rα2 itself, IL-13Rα2-specific siRNA, and, finally, an agent that blocks the activity of IL-13Rα2 signaling, AP-1 decoy oligonucleotide.

In the present study, all of the major features of the model of IL-13 induction of TGF-β1 discussed above were noted in the context of tumor immunosurveillance. In particular, it was observed that inoculation of mice with CT26 tumor cells was promptly followed by increased IL-13 and TNF-α secretion as well as the appearance of the IL-13Rα2 on CD11b<sup>high</sup>/Gr-1<sup>intermediate</sup> cells and the secretion of TGF-β1 by such cells. Furthermore, in inhibition studies, it was shown that as in the various inflammatory models mentioned above, TNF-αR-Fc, IL-13Rα2–specific siRNA, and AP-1 decoy oligonucleotide had predicted effects on IL-13Rα2 expression on CD11b<sup>high</sup>/Gr-1<sup>intermediate</sup> cells and blocked TGF-β1 induction by IL-13; as a result, administration of these inhibitors restored the ability of CD8<sup>+</sup> T cell to kill tumor cells.

In actual studies of control of CT-26 growth via immunosurveillance, we, in effect, again verified the IL-13 signaling mechanism described above in an in vivo context by showing that the various inhibitors of the IL-13 signaling greatly reduced tumor burden in the lung and thereby reduced mortality from the tumor. Of interest, the response pattern seen was fully consistent...
with function of an inhibitor of the signal cascade in that delayed administration of TNF-α or Fc rendered this agent ineffective in treatment, presumably because an agent that blocks induction of IL-13Rα2 expression could not work after the receptor was already being expressed (at least in a short-term experiment). In contrast, AP-1 decoy oligonucleotide continued to exert a therapeutic effect in the face of delayed therapy, presumably because continued synthesis of TGF-β1 is necessary to maintain inhibition of cytotoxic CD8+ T cells. More limited studies of the 15-12RM fibrosarcoma were fully consistent with these results in that also in this case, administration of TNF-α-Fc inhibited expression of IL-13Rα2 on CD11b++/Gr-1intermediate cells as well as negative regulation of immunosurveillance.

A major outcome of this study is the discovery that administration of TNF-α-Fc, a relatively safe TNF-α inhibitor that is widely used in the treatment of inflammatory diseases, could have an antitumor effect by enhancing immunosurveillance (23, 24). This finding, as well as others, support the idea that TNF-α can have protumor activity and, thus, the treatment of tumors via TNF-α blockade is a reasonable approach to the treatment of tumors (25). It should be noted, however, that there is also a considerable body of evidence supporting the idea that TNF-α can have antitumor activity especially when present at pharmacologic concentrations. This is buttressed by studies of the effect of TNF-α on tumor cell line growth in vitro and studies of the treatment of patients with tumors with TNF-α in vivo (26–28). Thus, assuming that endogenous TNF-α may have an inhibitory effect on tumor growth in some circumstances, it is possible that treating cancer patients with TNF-α blockers may enhance tumor development. Nevertheless, it appears that the effect of anti–TNF-α therapy on any particular tumor is difficult to predict and the final outcome will depend on such factors as the inherent susceptibility of the tumor to immunosurveillance, its tendency to undergo TNF-α–induced apoptosis, and, finally, the ability of TNF-α to elicit protumor or antitumor effects of immune cells in the milieu of the tumor. These issues are best sorted out by appropriate studies of patients with tumors.

Acknowledgments


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References

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