In Vivo Antitumor Activity of T Cells Redirected with Chimeric Antibody/T-Cell Receptor Genes


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ABSTRACT

In an effort to broaden the applicability of adoptive cellular immunotherapy toward nonmelanoma cancers, we have designed chimeric antibody/T-cell receptor genes composed of the variable domains from mAbs joined to T-cell receptor-signaling chains. We have demonstrated that T cells retrovirally transduced with these genes can recognize antibody-defined antigens and that this recognition leads to T-cell activation, specific lysis, and cytokine release.

In this study, we have examined the in vivo activity of murine T cells transduced with a chimeric receptor gene (MOv-y) derived from the mAb MOv18, which binds to a folate-binding protein overexpressed on most human ovarian adenocarcinomas. Nude mice that were given Lp. implants of human ovarian cancer (IGROV) cells were treated 3 days later with Lp. murine tumor-infiltrating lymphocytes (TIL). TIL that had significantly increased survival compared to mice treated with saline only, nontransduced TIL, or TIL transduced with a control anti-trinitrophenyl chimeric receptor gene (TNP-TIL). In another model, C57BL/6 mice were given Lp. injections of a syngeneic methylcholanthrene-induced sarcoma transduced with the folate-binding protein (FBP) gene. Three days later, mice were treated Lp. with retrovirally transduced murine TIL. (derived from an unrelated tumor), followed by low-dose systemic interleukin 2. Eleven days after tumor injection, mice were sacrificed, and lung metastases were counted. In multiple experiments, mice receiving MOv-TIL had significantly fewer lung metastases than mice treated with interleukin 2 alone, nontransduced TIL, or TNP-TIL.

These studies indicate that T cells can be gene modified to react in vivo against tumor antigens, defined by mAbs. This approach is potentially applicable to a number of neoplastic and infectious diseases and may allow adoptive immunotherapy against types of cancer not previously amenable to cellular immunotherapy.

INTRODUCTION

T-cell based therapies utilizing IL-2 and tumor-reactive T cells such as TILs have been developed for patients with metastatic melanoma, resulting in significant regression of tumor in some patients (1, 2). The nature of T-cell recognition of melanoma is being elucidated, with the cloning and characterization of several melanoma-associated antigens recognized by T cells (3–8). However, applying these principles to other, more common types of cancer such as breast cancer and colon cancer has remained elusive, largely due to difficulty in obtaining T cells with specific reactivity against these cancers.

mAbs have been generated that recognize tumor-associated antigens common to certain cancers. Therapy with mAbs alone has largely been ineffective due to weak effector responses, inadequate tissue penetration, or the development of human anti-mouse antibody responses (9). To combine the tumor recognition capabilities of antibodies with the potent antitumor effector abilities of T cells, we have designed chimeric receptor genes encoding the variable domain from mAbs joined to T-cell-signaling chains. Previously, we demonstrated that retroviral transduction of T cells with these chimeric receptor genes could enable T cells to recognize, lyse, and specifically secrete cytokines upon contact with cells expressing the appropriate, antibody-defined antigen in vitro (10, 11).

In this report, we have examined the ability of murine T cells expressing these chimeric antibody/T-cell receptors to function in vivo in tumor-bearing mice. The ability of these transduced T cells to successfully treat established tumors in vivo has significant implications in the generalization of this approach to the treatment of a variety of human cancers and infectious diseases.

MATERIALS AND METHODS

Construction of Chimeric Receptor Genes. Chimeric receptor genes composed of single chain variable regions from mAbs joined to the Fc receptor y chain, which is capable of mediating TCR signal transduction (12–16), were constructed as described previously (10, 11). Chimeric receptors derived from MOv18 (17, 18), a mAb that binds a M, 38,000 FBP highly expressed on most ovarian adenocarcinomas, and Sp6 (19, 20), an anti-2,4,6 TNP mAb, were engineered as described (11) (MOv-y and Sp-y receptors, respectively).

Retroviral Vectors. The MOv-y or Sp-y chimeric receptor genes were cloned into the LXSN or GEn (11, 21, 22) retroviral backbones under the transcriptional control of the long terminal repeat from Moloney murine leukemia virus. The retroviral constructs also contained the neomycin phosphotransferase gene (NeoR) as a selectable marker.

The gene encoding FBP was obtained from L. Coney (Apolloin, Malverna, PA) and cloned into the LXSN retroviral backbone. The retroviral constructs were then transfected with the use of CaPO4 into the PA317 amphotrophic packaging cell line as described previously (11, 23).

Tumor Transduction and Cell Culture. Tumor cell lines were cultured in RPMI 1640 with 10% heat-inactivated FCS and glutamine (all from Biofluids, Rockville, MD). 24JK tumor cells, a clone from the 3-methylcholanthrene-induced poorly immunogenic MCA 102 murine sarcoma (24, 25), were transduced with the FBP gene by incubation in retroviral supernatant in the presence of 8 μg/ml polybrene (Aldrich Chemical Co., Milwaukee, Wisconsin) to yield the 24JK-FBP tumor line. Media was replaced with fresh retroviral supernatant and polybrene every 12 h for 3 days. Seventy-two h after the final supernatant change, tumor cells were selected in 400 μg/ml of the neomycin analogue G418 (GIBCO, Grand Island, NY). After G418 selection, successful transduction was demonstrated by FACS analysis of tumor cells with MOv18 mAb.

Lymphocyte Transduction and Cell Culture. Murine TILs, derived from the diphenylhydrazine-induced MC38 murine colon adenocarcinoma, were grown in IL-2 as described (26). For retroviral transduction with MOv-y and Sp-y chimeric receptor genes, (to generate MOv-TIL and TNP-TIL, respectively), antigen-stimulated TIL were pelleted and resuspended at 3 X 10⁵ TIL/ml in retroviral supernatant containing 30 IU/ml human recombinant IL-2 and 20 μg/ml protamine sulfate (Eli Lilly & Co., Indianapolis, IN). Media was partially exchanged with fresh retroviral supernatant and protamine every 12 h for 2–3 additional exposures. Forty-eight h after the final supernatant change, TILs were selected in 0.3 mg/ml G418 for 5 days. This was followed by 1 week of expansion without G418, and then another 5-day selection in 0.3 to 1 mg/ml G418. After G418 selection, successful transduction was confirmed by Northern analysis of total RNA as described (27).

mIFN-y ELISA. TIL (5 X 10⁴) and stimulator (5 X 10⁵) cells were cocultured for 24 h at 37°C in a final volume of 1 ml RPMI with 10% FCS and 10% P/S. Supernatants were collected and assayed for IFN-y by ELISA.
30 IU/ml of IL-2. Supernatants were then aspirated, centrifuged at 2000 rpm to remove cells, decanted, and frozen at -70°C. Thawed aliquots were tested by ELISA for murine IFN-γ. The ELISA used a solid-phase rat IgG2A mAb specific for murine IFN-γ (Life Technologies, Gaithersburg, MD). After addition of either sample or recombinant IFN-γ standard, a biotinylated rat IgG1 mAb specific for IFN-γ (PharMingen, San Diego, CA) was used, followed by avidin-peroxidase. Color reaction was performed with the addition of H2O2 and ABTS substrate [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); Sigma Chemical Co., St. Louis, MO]. The plates were then read at A405.

Mice. C57BL/6 mice were obtained from Charles River Breeding Laboratories (Raleigh, NC) and the Frederick Cancer Research Facility (Frederick, MD) and were used at 8–16 weeks of age. Athymic nude mice were obtained from the Frederick Cancer Research Facility, maintained in laminar flow housing, and used at 6–12 weeks of age.

Pulmonary Metastasis Tumor Therapy Model. C57BL/6 mice received 500 cGy total-body irradiation (to minimize any host antitumor immune response), followed by i.v. injection of 5 × 10^6 to 1 × 10^7 24JK or 24JK-FBP tumor cells. On day 3, mice were treated i.v. with 2-3 × 10^6 nontransduced or nontransduced TIL cells derived from the MC38 tumor, followed by 30,000 to 60,000 IU IL-2 i.p. 3 times a day for 9 doses. Eleven to 16 days after initial tumor injection, mice were ear tagged and randomized, and the lungs were removed; the number of pulmonary metastases was evaluated in a coded, blinded fashion as described previously (28). Lungs with >250 metastases were scored as ≥250 because this was the largest number that could be accurately counted. Numbers presented are the mean numbers of pulmonary metastases ± SE. The significance of differences between groups was determined with the Wilcoxon rank Sums test. All P values are two-tailed.

RESULTS

Gene Transfer of FBP Antigen into Nonimmunogenic Murine Sarcoma. The nonimmunogenic murine fibrosarcoma 24JK was retrovirally transduced with the gene encoding FBP, the antigen recognized by MOv18. After selection with the neomycin analogue G418, FBP-transduced 24JK tumor (24JK-FBP) displayed high levels of FBP, as did the human ovarian carcinoma IGROV-1, as measured by FACS analysis with MOv18 (Fig. 1).

In Vitro Function of Murine TIL Transduced with Chimeric Receptor Genes. Murine TIL derived from the MC38 colon adenocarcinoma (38 TIL) were transduced with chimeric receptor genes derived from either the anti-ovarian cancer mAb MOv18 (MOv-γ) or the anti-TNP mAb Sp6 (Sp-γ; Ref. 11), and selected in G418. To assess in vitro activity, transduced, G418-selected TILs were cocultured with tumor lines for 16–24 h. Supernatants were then harvested and analyzed for mIFN-γ by ELISA. All TIL cultures produced large amounts of mIFN-γ when cocultured with MC38 tumor cells (their native antigen) or in anti-CD3-coated plates. When cocultured with IGROV-1 or 24JK-FBP tumor cells, both expressing large amounts of FBP, mIFN-γ production by MOv-γ-transduced TIL (MOv-TIL) increased by 54- and 14-fold, respectively, compared to MOv-TIL alone. In contrast, mIFN-γ production by nontransduced TIL and TIL transduced with the anti-TNP Sp-γ receptor (TNP-TIL) increased by only 2–4-fold upon coculture with the FBP-expressing cell lines, and was not different compared to coculture with the FBP-nonexpressing cell lines. None of the TIL cultures produced substantial amounts of mIFN-γ upon coculture with nontransduced 24JK cells or 888 human melanoma cells (Table 1). These data indicate that the MOv-γ receptor gene can confer to murine TIL the capability to specifically recognize FBP-expressing tumor cells.

Treatment of Pulmonary Metastases. To determine whether MOv-TIL had antitumor activity in vivo, C57BL/6 mice were given injections via the tail vein of 1 × 10^7 24JK tumor cells that were either nontransduced or transduced with the FBP gene. Three days later, mice were treated with 2.7 × 10^7 TIL, followed by 60,000 IU IL-2 every 8 h for 9 doses. Eleven days after the initial injection of tumor cells, mice were sacrificed, and lung metastases were counted. Only treatment with MOv-TIL in combination with IL-2 resulted in a significant reduction in lung metastases (P < 0.0004 compared to all other treatment groups), whereas treatment with IL-2 alone or nontransduced TIL in combination with IL-2 did not significantly reduce the number of 24JK-FBP pulmonary metastases (Table 2 and Fig. 2). MOv-TIL did not reduce the number of nontransduced 24JK tumor cells (Table 2) thus demonstrating their specificity for FBP-expressing tumors. These findings were corroborated in 2 replicate experiments.

Treatment of Human Ovarian Cancer Cells in Nude Mice. To assess whether MOv-TIL had significant in vivo activity against human ovarian carcinoma cells, 2.5 × 10^6 IGROV-1 cells from fresh ascites were implanted i.p. in nude mice. Three days later, mice were treated with a single i.p. injection of TIL and then followed for survival. Histopathological evaluation of sample mice at the time of treatment revealed that significant amounts of disease were present and invading structures within the murine peritoneal cavity 3 days after tumor injection (Fig. 3). Mice treated with MOv-TIL had significantly increased survival (median survival = 90 days; P < 0.0002) compared to mice treated with saline only, nontransduced TIL, or TNP-TIL (median survivals = 31, 37, and 31 days, respectively; Fig. 4). This study was repeated with similar results.

DISCUSSION

The ability to alter T-cell specificity with the use of chimeric receptor genes has the potential to broaden the applicability of adoptive cellular immunotherapy to include the treatment of common cancers such as breast cancer and colon cancer, as well as viral diseases such as hepatitis B or HIV. In addition, by eliminating the need to isolate naturally occurring T cells with a particular antigen specificity, T cells can be selected for a certain phenotype, function, or cytokine profile, regardless of the specificity of the native TCR. Indeed, specific patterns of cytokine production by T cells, such as...
TH1 versus TH2 cells, have been shown to dictate different immunological responses and therapeutic outcomes (31–35). This study demonstrates that T cells transduced with chimeric receptor genes are active in vivo against tumor cells bearing the receptor-defined antigen. Previous studies using nontransduced murine and human TIL (36, 37) have correlated specific cytokine production in vitro with function in vivo against tumor cells bearing native tumor-associated antigens. The present results, using T cells expressing chimeric receptors, also demonstrate that T cells that are therapeutically effective in vivo specifically secrete cytokines in vitro. This suggests that chimeric antibody/TCRs may function similarly to native TCRs with regard to induction of cytokine secretion and in vivo activity. Besides specific cytokine release, MOv-y-transduced T-cells have been shown to specifically lyse FBP-expressing target cells in vitro (11). Further studies are needed to assess the relative contributions of cytokine release and lysis to the observed in vivo antitumor activity.

Because antibody-based recognition of tumor is dependent on expression of tumor-associated antigens, one potential escape mechanism for tumor cells is the down-regulation of antigen expression. In the present study, i.p. injection of IGROV tumor cells into nude mice, followed by i.p. therapy with MOv-TIL, resulted in a significant increase in survival. Although survival was enhanced 3-fold, all mice eventually died from tumor ascites. FACS analysis of these tumor cells showed continued presence of FBP expression, unchanged from the tumors used to obtain the tumor cells. This suggests that antigen down-regulation was not the mechanism of escape in this particular model. Complete tumor eradication may require repeated treatments, combinations of i.p. and i.v. therapy, or combinations with other treatment approaches. Tumor therapy using a variety of chimeric receptors targeting different antigens may also be necessary should antigen down-regulation or in vivo immunoselection of antigen-negative cells become evident.

An i.p. tumor model is particularly appropriate for ovarian cancer because the most common and earliest mode of dissemination of this disease in cancer patients is by exfoliation of cells that implant along the surfaces of the peritoneal cavity (38). Alternative tumor models may be more appropriate when studying chimeric receptors targeted against other types of cancers.

Although MOv-TIL specifically secreted IFN-y upon coculture with FBP-expressing cells, more IFN-y was released upon coculture with the human ovarian cancer cell line (IGROV) compared to the FBP-transduced murine tumor cell line (24JK FBP), despite the fact that the 24JK FBP line expressed more FBP by FACS analysis. The reason for this difference is not clear. One possibility is that free FBP is shed by the high-expressing 24JK FBP line, thereby inhibiting reactivity by the MOv-TIL, as we have demonstrated previously that free antigen can block reactivity in this system (11).

This study has utilized primary, differentiated lymphocytes as effector cells. However, with the ability to dictate the specificity of any given lymphocyte by retroviral transduction with a particular chimeric receptor gene, other hematopoietic cells could potentially be used as effectors. For example, retrovirally transduced bone marrow stem cells could be used, thereby providing a constant supply of differentiated cells expressing the chimeric receptor. In addition, by this method, not only would lymphocytes contain the gene, but other cells such as monocytes and granulocytes might also express the chimeric receptor, thereby providing a continuous supply of multiple types of effector cells directed against the tumor. Because TCR and Fc receptor-signaling chains share common activation motifs (12–14, 16), a variety of effector cells, expressing either Fc receptors or TCRs, may be capable of functioning via the same chimeric receptor gene. The chimeric receptor genes we have used for this study have utilized the Fc receptor y chain for signal transduction. We are currently evaluating receptors that utilize the TCR 3 chain for signal transduction, although preliminary results suggest that the Fc-3 chain constructs are equivalent or superior to the 3 chain constructs in our system. This may be due to differences in surface expression between the two types of receptors.

For this study, we used a murine TIL line that we could readily transduce. However, we have found that some primary T-cell lines are

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* Mice were sacrificed on day 11 after tumor injection, and lung-metastases were counted in a blinded fashion. Metastases which were too numerous to count are arbitrarily designated as >50,000.

* IL-2 was given beginning on day 3 after tumor injection at 6 IU i.p. 3 times a day for 9 doses.

* TIL (2 x 10^7, either unmodified or transduced with the MOv-y chimeric receptor) were given once on day 3 after tumor injection and followed by systemic IL-2 given as described above.

* Significantly less compared to other groups; P < 0.0004.
Ovarian cancer cells can be seen invading the murine omentum.

Fig. 3. Histopathological evaluation of peritoneal cavity 3 days after i.p. injection of 2.5 × 10^6 human ovarian cancer IGROV cells into nude mice. Ovarian cancer cells can be seen invading the murine omentum.

Fig. 4. Survival of nude mice after i.p. injection with human ovarian cancer (IGROV) cells. On day 3 after tumor injection (see Fig. 3 for histopathological evaluation on day 3), mice were treated with HBSS, unmodified murine TIL, or TIL transduced with either the MOv-y receptor or the control Sp-y receptor (MOv-TIL and TNP-TIL, respectively). Mice treated with MOv-TIL demonstrated a significant increase in survival compared to the other groups.

ACKNOWLEDGMENTS

We thank L. Coney, S. Canevari, D. Perry-Lalley, A. Mixon, D. Jones, and P. Spiess for helpful reagents and discussions.

REFERENCES


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