Delivery of CCL21 to Metastatic Disease Improves the Efficacy of Adoptive T-Cell Therapy

Uma Thanarajasingam,[1,2] Laura Sanz,[1] Rosa Diaz,[1] Jian Qiao,[1] Luis Sanchez-Perez,[1,2] Tim Kottke,[1] Jill Thompson,[1] John Chester,[3] and Richard G. Vile[1,2]

[1]Molecular Medicine Program and [2]Department of Immunology, Mayo Clinic, Rochester, Minnesota and [3]Cancer Research UK Clinical Centre, St. James' University Hospital, Leeds, United Kingdom

Abstract

Adoptive T-cell transfer has achieved significant clinical success in advanced melanoma. However, therapeutic efficacy is limited by poor T-cell survival after adoptive transfer and by inefficient trafficking to tumor sites. Here, we report that intratumoral expression of the chemokine CCL21 enhances the efficacy of adoptive T-cell therapy in a mouse model of melanoma. Based on our novel observation that CCL21 is highly chemotactic for activated OT-1 T cells *in vitro* and down-regulates expression of CD62L, we hypothesized that tumor cell–mediated expression of this chemokine might recruit, and retain, adoptively transferred T cells to the sites of tumor growth. Mice bearing metastatic tumors stably transduced with CCL21 survived significantly longer following adoptive T-cell transfer than mice bearing non–CCL21-expressing tumors. However, although we could not detect increased trafficking of the adoptively transferred T cells to tumors, tumor-expressed CCL21 promoted the survival and cytotoxic activity of the adoptively transferred T cells and led to the priming of antitumor immunity following T-cell transfer. To translate these observations into a protocol of real clinical usefulness, we showed that adsorption of a retrovirus encoding CCL21 to OT-1 T cells before adoptive transfer increased the therapeutic efficacy of a subsequently administered dose of OT-1 T cells, resulting in cure of metastatic disease and the generation of immunologic memory in the majority of treated mice. These studies indicate a promising role for CCL21 in enhancing the therapeutic efficacy of adoptive T-cell therapy. [Cancer Res 2007;67(1):300–8]

Introduction

Adoptive T-cell therapy has emerged as a promising therapeutic strategy in the clinic to treat cancer (1, 2) having been validated in numerous animal models (3–5). In the clinical setting, adoptive transfer involves the recovery of autologous tumor-infiltrating lymphocytes, their expansion *ex vivo*, and subsequent re-administration into a patient where, ideally, they traffic to, and specifically lyse, antigen-expressing tumor cells. T-cell activation and expansion is achieved *ex vivo*, allowing for, at least in theory, the transfer of a large number of selected cells with high avidity for tumor antigens. Moreover, the patient's immune system can be further manipulated to optimize the therapy by using strategies such as prior vaccinations with tumor antigens and lymphodepletion (6, 7). However, adoptive transfer is not without its limitations. Notably, the persistence, trafficking, and maintenance of the effector functions of adoptively transferred cells *in vivo* can all be limiting (8). In this respect, because chemokines orchestrate the extravasation and directional migration of various leukocytes (9, 10), we hypothesized that expression of specific chemokines at a tumor site, in combination with adoptive T-cell transfer, might address some of the current limitations of this therapy.

CCL21, also known as secondary lymphoid chemokine or 6Ckine, has classically been known as a lymphoid chemokine that is mainly and constitutively expressed by lymphatic vessels (11, 12), stromal cells in the spleen and appendix, and by high endothelial venules in lymph nodes and Peyer's patches (13–16). CCL21 binds to the chemokine receptors CCR7 and CXCR3, and is chemotactic for mature dendritic cells, as well as naive and memory T cells (17–21). Of particular interest to its role as a potentiator of adoptive T-cell transfer therapies, CCL21 also mediates the extravasation of lymphocytes from the bloodstream into the periphery due to its ability to induce integrin-dependent arrest of rolling lymphocytes on high endothelial venules (22). Indeed, a critical role for CCL21 in lymphocyte homing is illustrated by the mutant mouse strain, paucity of lymph node T cells (*plt*), in which expression of CCL21 is abrogated, resulting in a failure of T cells and dendritic cells to migrate to lymph nodes, Peyer's patches, and the spleen (23, 24).

Given the ability of CCL21 to direct the migration of mature dendritic cells to colocalize with, and subsequently prime, naive T cells, engineering CCL21 expression has been used to enhance the priming of antitumor immune responses. Thus, intratumoral injections of dendritic cells modified to express CCL21 resulted in growth inhibition of murine lung cancers and B16 melanomas (25, 26). Colon carcinomas transduced to overexpress CCL21 also showed a CD8 T cell–dependent reduction in tumorigenicity in immunocompetent mice (27). Here, we report the novel observation that CCL21 is strongly chemotactic not only for naive T cells, but also for activated T cells. In the light of these findings, we hypothesized that it may be possible to exploit CCL21 expression *in vivo* to augment the effector arm of the antitumor response within the context of adoptive transfer of activated T cells for tumor therapy.

Specifically, we proposed that it would be possible to enhance the efficacy of adoptive T-cell therapy by overexpressing CCL21 intratumorally, thereby directing the migration of effector T cells to the tumor site, resulting in greater tumor cell killing. In this report, we used a murine model of adoptive T-cell transfer to treat malignant melanoma. The OT-1 mouse strain is on a C57BL/6 background (H2b) and expresses a transgenic T-cell receptor Vα2 specific for the SIINFEKL peptide of the chicken ovalbumin protein in the context of MHC class I, H2-Kb. B16ova tumor cells are derived from the B16 melanoma cell line and express the ovalbumin antigen. These cells present the SIINFEKL epitope of

Requests for reprints: Richard G. Vile, Molecular Medicine Program, Mayo Clinic, Guggenheim 1836, 200 1st Street Southwest, Rochester, MN 55902. Phone: 507-284-9941; Fax: 507-266-2122; E-mail: vile.richard@mayo.edu.

©2007 American Association for Cancer Research.

doi:10.1158/0008-5472.CAN-06-1017

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/1/300/2567206/300.pdf by guest on 14 December 2024

ovalbumin in the context of H2-Kb MHC class I molecules to OT-1 T cells (28). We show here that mice bearing established metastatic B16ova tumors engineered to overexpress CCL21 survived significantly longer than mice bearing B16ova metastases when treated with a single dose of adoptively transferred activated OT-1 T cells. However, in contrast to our *in vitro* data, CCL21 enhanced the efficacy of adoptively transferred T cells by means other than promoting trafficking to tumor sites. Thus, tumor cell–expressed CCL21 promoted the survival of OT-1 T cells *in vitro*, as evidenced by increased expression of Bcl-2, and enhanced their ability to kill B16ova target cells. Similarly, tumor expression of CCL21 increased the activity and longevity of adoptively transferred T cells *in vivo* and primed antitumor T-cell responses in combination with adoptive T-cell transfer.

Finally, we sought to translate these data into a protocol with potential clinical usefulness. We have previously shown that retroviral particles are able to adsorb to the surface of OT-1 T cells *in vitro* and be released upon T-cell activation *in vivo*, leading to direct infection of antigen-bearing target cells in a contact-dependent manner via a process we call "hitchhiking and handoff" (29, 30). Treatment of mice bearing metastatic B16ova disease with OT-1 cells hitchhiking CCL21 retroviral particles increased the therapeutic efficacy of a subsequently administered dose of OT-1 T cells compared with pretreatment with OT-1 T cells alone, and resulted in 90% of animals being cured; the majority of these cured mice also developed protective immunologic memory against rechallenge with tumor. Our data support a promising and novel role for CCL21 in enhancing the therapeutic efficacy of adoptive T-cell therapy in a clinically translatable manner.

Materials and Methods

Cell lines. The murine melanoma B16 line has been described previously (31). B16ova melanoma cells ($H2k^b$) were derived from B16 cells by transduction with a cDNA encoding the *ovalbumin* gene (32). B16ova served as the parental cell line to derive B16ovaCCL21. Briefly, the murine *CCL21* gene was cloned into the pCDNA3.1 Hygro plasmid and transfected into B16ova cells. Clones were grown in DMEM 10% fetal bovine serum (FBS) at 37°C 10% CO_2, selected in Hygromycin (200 μg/mL) and G418 (5 mg/mL), and screened by ELISA specific for CCL21 (R&D Systems, Minneapolis, MN). All cell lines were free of *Mycoplasma* infection.

Mice. C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) at 6 to 8 weeks of age. OT-1 mice are transgenic mice whose T cells recognize the SIINFEKL peptide from the chicken ovalbumin protein in the context of $H\text{-}2K^b$ (28).

Antibodies and flow cytometry. Anti-CD8b FITC, anti-CD8 phycoerythrin, anti-CD25 phycoerythrin, anti-CD44 phycoerythrin, anti-CD45 peridinin chlorophyll protein, anti-CD62L allophycocyanin, anti-Bcl-2 FITC, and the isotype controls were purchased from BD PharMingen (San Diego, CA). For flow cytometry, fluorescence data were collected on FACScan apparatus (Becton Dickinson, San Diego, CA) and analyzed using CELLQUEST software.

Intracellular staining for Bcl-2. OT-1 T cells were harvested, washed, and stained with anti-CD8 phycoerythrin. Cells were then permeabilized using the Cytofix/cytoperm kit (Becton Dickinson, PharMingen, San Diego, CA) as per manufacturer's instructions, washed with the perm/wash buffer provided, and then stained with Bcl-2 FITC. Isotype-matched, FITC-tagged control were also included for each individual sample.

***Ex vivo* CTL activation and adoptive transfer.** Single-cell suspensions of spleens and lymph nodes harvested from OT-1 mice were prepared by standard techniques. The cell suspension was treated with RBC lysis buffer, washed with PBS, counted, and plated at 2.5×10^6 cells/mL in OT-1 medium [Iscove's modified Dulbecco's medium (IMDM) 5% FBS, 50 μmol/L β-mercaptoethanol, 1% penicillin/streptomycin] plus 50 IU of recombinant human interleukin-2 (IL-2) per milliliter and 1 μg/mL of SIINFEKL peptide for 3 days at 37°C 5% CO_2. For adoptive transfer, cells were harvested, washed with PBS, layered over Ficoll (Lympholyte-M, Cedarlane, Canada), and centrifuged for 20 min at 2,000 rpm at 20°C. The cells were washed thrice with PBS and resuspended at the concentration required for the study. Phenotypic analysis by flow cytometry showed a population of activated CD8 T cells that were $CD3^{low}CD44^{high}CD25^{high}$, with minimal contamination from other cell types.

Chemotaxis of naive and activated T cells. Naive CD8 OT-1 T cells were isolated from spleens and lymph nodes of OT-1 mice; single-cell suspensions were prepared and purified using CD8 magnetic cell sorting beads (Miltenyi Biotec, Auburn, CA). Activated $CD8^+$ OT-1 T cells were prepared as described above. For the chemotaxis assay, 5×10^5 cells (naive or activated OT-1 T cells or B16ova tumor cells as a negative control) were resuspended in 50 μL of medium [DMEM or IMDM, supplemented with 0.5% bovine serum albumin (BSA)] and were loaded on the top filter of the chemotaxis chamber (96-well Chemotx, 5-μm pore size, Neuroprobe, Gaithersburg, MD). Increasing concentrations of rmCCL21 (1–100 ng/mL), or supernatants from the B16ova or B16ovaCCL21 cell lines collected in medium supplemented with 0.5% BSA, were placed in the bottom wells of the chemotaxis chamber in a final volume of 320 μL. After incubation for 2.5 h at 37°C 5% CO_2, 150 μL of medium were removed from the lower chamber, with precautions taken not to disturb the cells that had migrated. The viable cells migrating to the lower chamber were quantified using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) proliferation kit from Roche (Indianapolis, IN). Briefly, 10 μL of the MTT reagent 1 were added to the cells in the lower chamber. Four hours later, 100 μL MTT reagent 2 were added. The plate was read at 575 nm 24 h later. The chemotactic index (CI) was calculated as follows: CI = (absorbance of cell-type A migrating toward sample) / (absorbance of cell-type A migrating toward medium alone).

***In vitro* survival assay.** Three-day activated OT-1 T cells were collected, as described above, and resuspended in OT-1 medium alone, medium supplemented with 100 ng/mL rmCCL21, or supernatants from B16ova or B16ovaCCL21 grown in OT-1 medium at 5×10^4 cells per well in a 96-well plate. The cells were cultured for an additional 24, 48, and 72 h, and survival was quantified at each time point using the MTT proliferation kit as described earlier. The plate was read 1 day after the 72-h time point.

$Chromium^{51}$ release assay. For preparation of target cells, 5×10^6 B16ova or B16 cells were labeled with 100 μCi of Cr^{51} for 90 min at 37°C, washed thrice with medium, and resuspended at 5×10^5/mL. Targets were plated at 5×10^4 cells per well in 96-well V-bottomed plates. For preparation of effector cells, 3-day activated OT-1 cells were isolated and cultured with OT-1 medium alone; the medium was supplemented with 100 ng/mL rmCCL21; or supernatants from B16ova or B16ovaCCL21 were grown in OT-1 medium at 2.5×10^6 cells per well in a 24-well plate. After 48 h in culture, cells were collected, washed, and cocultured with targets in triplicate at effector-to-target ratios starting at 100:1. The cocultures were incubated for 4 h at 37°C 5% CO_2. Thirty-two microliters of coculture supernatants were then collected and added to Lumiplates (Packard, Pangbowne, United Kingdom). Plates were allowed to dry overnight and were then read. Percentage of lysis was determined as indicated: % lysis = ([sample lysis − spontaneous] / [maximum lysis − spontaneous]) × 100.

ELISPOT assay. Spleens and draining lymph nodes were harvested from mice challenged s.c. with 5×10^5 B16ova or B16ovaCCL21 tumor cells, 2 or 5 days after a single dose of 10^7 activated OT-1 T cells. Cells (2.5×10^5) were plated into each well of a 96-well plate and were restimulated for 48 h at 37°C 5% CO_2 under the following conditions: OT-1 medium alone, medium + 50 IU/mL recombinant human IL-2, medium + SIINFEKL peptide, medium + Trp2 peptide, and medium + gp100 peptide (all peptides at 5 mg/mL). Peptide-specific IFN-γ–positive spots were detected according to the manufacturer's protocol (Mabtech, Mariemont, OH) and were quantified using an ELISPOT plate reader.

***In vivo* experiments.** All procedures were approved by the Mayo Foundation Institutional Animal Care and Use Committee. Mice were age and sex matched. To establish s.c. tumors, 5×10^5 B16ova or B16ovaCCL21 tumor cells in 100 μL of PBS were injected into the flank of mice. To

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/1/300/2567206/300.pdf by guest on 14 December 2024

establish systemic lung metastatic disease, we i.v. injected mice with 5×10^5 B16ova or B16ovaCCL21 cells (100 μL). For adoptive transfer experiments, mice were i.v. given activated OT-1 T cells (up to 10^7 cells per injection) starting at 7 days after tumor establishment. For survival studies, tumor diameter in two dimensions was measured thrice weekly using calipers, and mice were killed when tumor size was ~1.0 × 1.0 cm in two perpendicular directions (s.c. tumors) or at the first sign of any distress (metastatic disease).

Generation of retroviral stocks. The C-type retroviral vectors used were MFG-GFP and MFG-CCL21, which express green fluorescent protein (GFP) or CCL21, respectively, from the MLV long terminal repeat. C-type vectors were packaged into viral particles by transfection of 5 μg of plasmid DNA into the 293INT cell line, which stably expresses the Mo-MLV *gag* and *pol* genes but no envelope (F. Loic-Cosset, Laboratoire de Vectorologie Retrovirale et Therapie Genetique, Institut National de la Sante et de la Recherche Medicale U412, Lyon, France.). 293INT cells were also transfected with 5 μg of plasmid encoding the amphotropic 4070A envelope proteins (33). Transfections were carried out using Effectene (Qiagen, Valencia, CA) according to the manufacturer's instructions. Cell supernatants containing enveloped virus 4070A-GFP or 4070A-CCL21 were recovered 48 to 72 h after transfection, filtered (0.45 μm), and then either used directly or were frozen at −80°C.

***In vitro* loading of OT-1 T cells with retroviral particles.** *In vitro* loading of OT-1 T cells with retroviral particles was carried out as previously described by our laboratory (29). Briefly, activated OT-1 T cells were pelleted and gently resuspended at 4°C for 4 h, in the smallest volume of serum-free viral supernatant possible. Cells were washed thrice in PBS at 4°C. Virus-loaded T cells were pelleted, and the retrovirally loaded T cells were then used for adoptive transfer *in vivo*.

Statistics. Survival data from the animal studies were analyzed using the log-rank test, and the two-sample unequal variance Student's *t* test analysis was applied for *in vitro* assays. Statistical significance was determined at the level of $P < 0.05$.

Results

CCL21 is chemotactic for activated OT-1 T cells and down-regulates CD62L expression. To determine whether CCL21 might be used to enhance adoptive T-cell therapy, we assessed its ability to attract both naive and activated OT-1 T cells *in vitro*. As expected from other reports (12, 13, 34), naive, CD8-positive OT-1 T cells migrated toward recombinant CCL21 protein in a concentration-dependent manner (Fig. 1*A*). However, surprisingly, OT-1 T cells activated for 3 days with SIINFEKL in the presence of IL-2 also migrated toward recombinant CCL21 (Fig. 1*A*), and more robustly than naive T cells at high concentrations of CCL21. Activated OT-1 T cells also migrated similarly toward supernatants from B16ova tumors engineered to overexpress CCL21 (data not shown). We also examined the response to CCL21 expression of various cell surface molecules on activated OT-1 T cells. Activated OT-1 T cells grown in CCL21-containing supernatants from a stable CCL21-expressing B16ova clone consistently down-regulated expression of CD62L, a cell surface molecule critical for homing to lymph nodes, compared with T cells cultured in B16ova supernatants (Fig. 1*B*). Other markers of activation, such as CD25 and CD44, were not affected by growth in CCL21 (data not shown). From these data, we hypothesized that CCL21 increases the efficacy of adoptively transferred T-cell therapy both by enhancing the trafficking of adoptively transferred T cells to tumors and by promoting their retention in the tumor by down-regulating CD62L.

CCL21 expression combined with adoptive transfer significantly prolongs survival of mice bearing lung metastases. To test this hypothesis, lung metastases were established in immunocompetent C57BL/6 mice by i.v. injection of either B16ova cells or of B16ovaCCL21 cells, engineered for stable expression of CCL21. Seven days later, mice received 1×10^6 activated OT-1 T cells i.v. Overexpression of CCL21 in lung metastases, even in the absence of adoptively transferred OT-1 T cells, significantly prolonged survival compared with mice bearing B16ova tumors ($P < 0.0001$; Fig. 2*B*). Adoptive transfer of OT-1 T cells improved therapy of mice bearing B16ova tumors compared with treatment with no T cells. However, mice bearing B16ovaCCL21 tumors treated with OT-1 T cells displayed highly significant prolongation in survival compared with mice treated with OT-1 T cells bearing B16ova tumors only ($P < 0.04$) or mice bearing B16ovaCCL21 tumors not treated with

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/1/300/2567206/300.pdf by guest on 14 December 2024

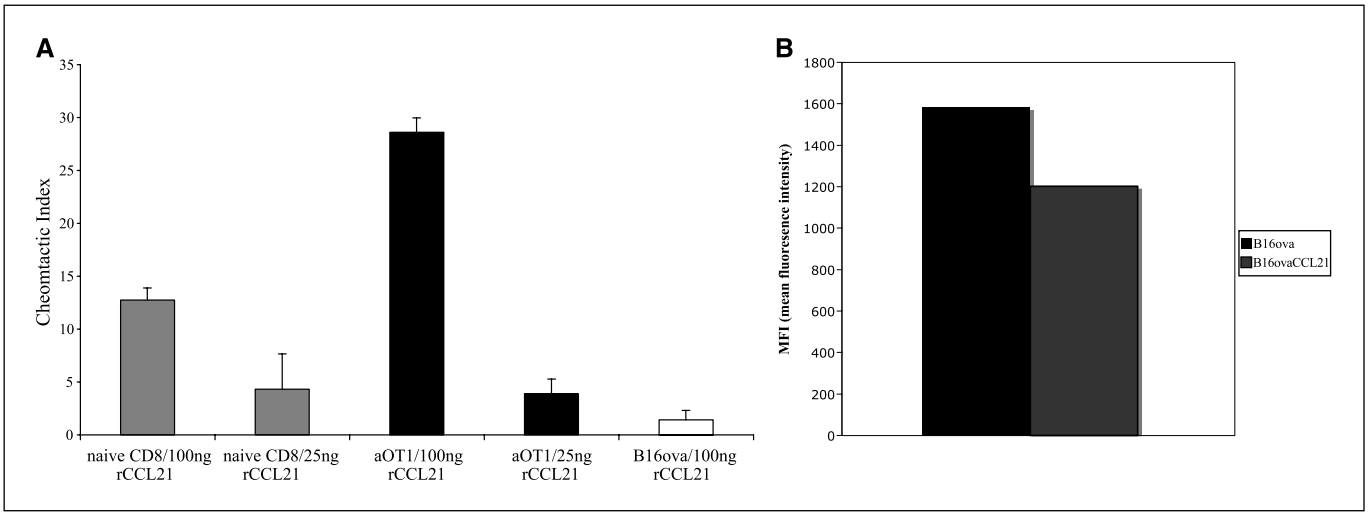

Figure 1. CCL21 is chemotactic for activated OT-1 T cells and down-regulates CD62L expression on activated OT-1 T cells *in vitro*. *A,* 5×10^5 naive or activated OT-1 T cells, or B16ova tumor cells (negative control), were loaded on the top filter of the chemotaxis chamber. Recombinant mCCL21 was placed in the bottom wells at the indicated concentrations. Viable cells migrating to the lower chamber after 2.5 h were quantified using the MTT proliferation kit. *Chemotactic Index,* fold migration over background. *Bars,* SD. *B,* 3-d–activated OT-1 T cells cultured with supernatants from the B16ova or B16ovaCCL21 cell lines, or medium alone, for 48 h and analyzed by flow cytometry. $CD8^+CD45^+$ cells were gated and expression of CD62L was assessed on this population by mean fluorescence intensity (*MFI*). Representative of three independent experiments.

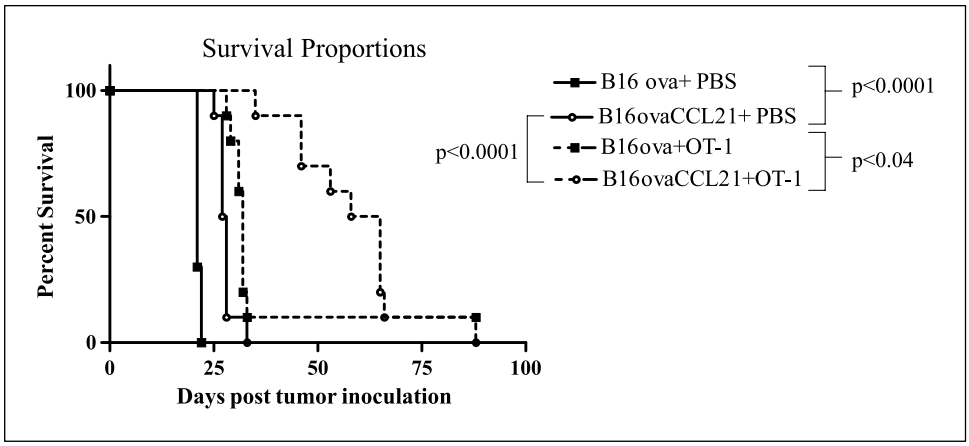

Figure 2. CCL21 expression combined with adoptive transfer of activated OT-1 T cells significantly prolongs survival in mice bearing lung metastases. Lung metastases were established in C57BL/6 mice (n = 10) by i.v. injection of B16ova or B16ovaCCL21 tumor cells. Seven days later, mice received a single dose of 5×10^5 activated OT-1 T cells or PBS as a control. Survival of mice is given as time in days following tumor challenge.

adoptive T-cell therapy (Fig. 2). These results indicate that, in the setting of metastatic disease, the combination of tumor cell–mediated CCL21 expression and adoptive transfer of activated T cells prolongs survival significantly compared with adoptive transfer or overexpression of CCL21 alone.

CCL21 increases the longevity of adoptively transferred T cells. We investigated whether CCL21 expression by tumor cells enhanced the efficacy of adoptive T-cell transfer by increasing trafficking of OT-1 T cells to B16ovaCCL21 tumors *in vivo* as suggested by our *in vitro* results (Fig. 1). Seven days following seeding of B16ova or B16ovaCCL21 metastases, mice received 1×10^7 activated carboxyfluorescein diacetate, succinimidyl ester (CFSE)–labeled OT-1 T cells or PBS; then, 24 or 48 h later, lung metastases were analyzed by flow cytometry for the presence of adoptively transferred cells. OT-1 T cells were not significantly increased in number at either time point in B16ovaCCL21 tumors compared with B16ova tumors (data not shown), suggesting that increased trafficking to CCL21-overexpressing tumors did not account for the observed therapeutic efficacy.

However, ELISPOT analysis on spleens and draining lymph nodes harvested from mice bearing B16ovaCCL21 tumors 24 h after adoptive transfer showed an increased proportion of IFN-γ–producing cells upon restimulation with SIINFEKL, compared with spleens and lymph nodes of mice bearing B16ova tumors (Fig. 3*A*). Splenocytes from mice that did not receive adoptively transferred cells showed no significant reactivity to SIINFEKL restimulation regardless of tumor type (data not shown). We observed similar results when mice bearing s.c. B16ova or B16ovaCCL21 tumors were treated in the same way (data not shown). These data suggest that expression of CCL21 intratumorally enhances the longevity and/or the proliferation of adoptively transferred OT-1 cells *in vivo*. Consistent with these observations, activated OT-1 T cells cultured in the presence of recombinant CCL21 survived, and/or proliferated, significantly better than activated OT-1 T cells grown in the absence of CCL21 (Fig. 3*B*). However, *in vitro* CFSE labeling of OT-1 cells did not indicate increased levels of proliferation in the presence of CCL21 (data not shown). Similar results were observed for OT-1 T cells grown in the presence of supernatants from the B16ovaCCL21 tumor cell line compared with supernatants from the B16ova cell line (data not shown). Thus, increased survival of T cells may, in part, account for the enhanced efficacy of adoptive therapy in the presence of CCL21 *in vivo*.

To investigate whether these effects were mediated through increased T-cell survival, levels of the antiapoptotic survival factor Bcl-2 were measured in OT-1 T cells cultured in the presence, or absence, of recombinant CCL21. OT-1 T cells grown in recombinant CCL21 at doses ranging from 50 to 1,000 ng expressed levels of Bcl-2 comparable with OT-1 treated with IL-15, a known survival factor for OT-1 T cells (a single representative histogram is shown in Fig. 3*C*). Bcl-2 expression was also increased when OT-1 were grown in supernatant from B16ovaCCL21 cells compared with supernatants from B16ova cells. Finally, coculture with B16ovaCCL21 tumor cells also increased levels of Bcl-2 in OT-1 T cells compared with OT-1 grown alone or with B16ova cells (Fig. 3*C*). These data suggest that CCL21 acts as a survival factor for activated T cells.

CCL21 and adoptive T-cell transfer primes endogenous T-cell responses against tumor antigens. Spleens and draining lymph nodes from mice bearing B16ovaCCL21 tumors, harvested 5 days after a single dose of activated adoptively transferred OT-1 T cells, contained significantly more IFN-γ–producing cells after restimulation with SIINFEKL than did spleens and lymph nodes from mice bearing B16ova tumors (Fig. 3*D*), consistent with our previous data. However, B16ovaCCL21 tumor-bearing mice treated with OT-1 T cells also developed T cells specific for the tumor-associated antigens Trp2 and gp100 (Fig. 3*D*). Importantly, no such reactivity was observed in spleens or lymph nodes from B16ova- or B16ovaCCL21 tumor-bearing mice in the absence of adoptive T-cell transfer (data not shown). These data indicate that tumor-expressed CCL21, combined with adoptive T-cell therapy, generates endogenous effector T-cell responses directed against tumor-associated antigens separate from those against which the adoptively transferred T cells are specific.

CCL21 enhances OT-1 cytotoxicity *in vitro*. OT-1 T cells activated for 3 days with SIINFEKL and IL-2, and then cultured with recombinant CCL21 for 48 h, lysed B16ova targets at high effector-to-target ratios more effectively than activated OT-1 grown in medium alone (Fig. 4). Therefore, in addition to promoting the survival of OT-1 T cells *in vitro* and *in vivo*, CCL21 also enhanced the cytotoxicity of T cells.

Systemic delivery of CCL21 by T cells increases efficacy of adoptive T-cell therapy. Our overall aim is to develop therapies that can be used in a truly systemic manner to treat metastatic disease, in which pretransduction of patient tumors with CCL21 will not be possible. We recently showed that retroviruses can be adsorbed to the surface of OT-1 T cells *in vitro* and be released upon T-cell activation *in vivo* to infect antigen-expressing tumor cells in a contact-dependent manner (a process we call

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/1/300/2567206/300.pdf by guest on 14 December 2024

hitchhiking and handoff; refs. 29, 30). Therefore, OT-1 T cells were loaded *in vitro* with retroviral vectors, encoding either the CCL21 or GFP transgenes, pseudotyped with the 4070A envelope protein. Under these conditions, <1% of the T cells themselves are directly infected with the retrovirus, and T-cell expression of the transgene is negligible (data not shown; refs. 29, 30). Mice bearing B16ova lung metastases in fully immunocompetent C57BL/6 mice were treated with OT-1, OT-1 (4070A-GFP), or OT-1 (4070A-CCL21) T cells and then received an additional dose of unmodified OT-1 T cells. A single dose of OT-1 (4070A-CCL21) T cells resulted in a small but significant ($P < 0.02$) prolongation in survival time compared with treatment with OT-1 (4070A-GFP)

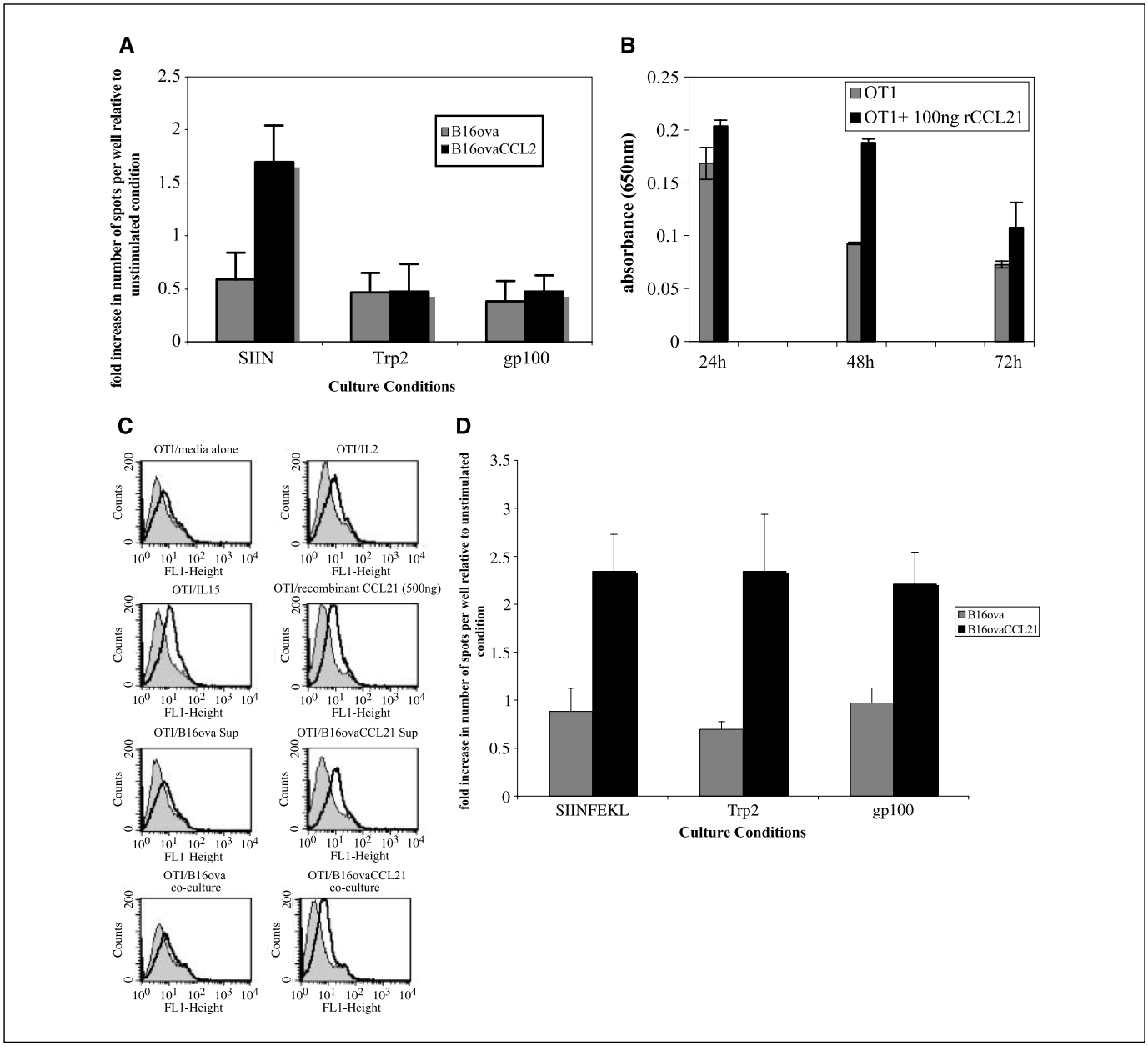

Figure 3. CCL21 increases the longevity of adoptively transferred T cells, by promoting their survival and maintaining Bcl-2 expression, and primes antitumor responses. *A,* C57BL/6 mice were challenged with 5×10^5 B16ova or B16ovaCCL21 tumor cells i.v. and received a single dose of 10^7 activated OT-1 T cells 7 days later. Twenty-four hours later, spleens and lymph nodes were harvested from mice ($n = 3$) and were cultured with IL-2 or the indicated peptides. Spots were developed and quantified on the ELISPOT plate after 48 h. *Columns,* fold increase in number of spots per well over number of spots from the unstimulated control. Average of three mice per group. *B,* 5×10^4 activated OT-1 T cells were plated per well in a 96-well plate in triplicate in the presence of 100 ng rmCCL21 protein or in medium alone for 24, 48, or 72 h. At each time point, cell viability was assessed using the MTT assay. *Columns,* absorbance, read at 650 nm, at each time point. Representative of at least three independent experiments. *C,* 10^6 activated OT-1 T cells were plated per well in a 24-well plate in OT-1 medium alone, IL-2, IL-15, rCCL21, supernatant from B16ova or B16ovaCCL21, or in the presence of 10^6 B16ova or B16ovaCCL21 tumor cells. OT-1s were harvested 48 h later, stained for intracellular Bcl-2, and analyzed by flow cytometry. Bcl-2 expression was assessed on $CD8^+$ T cells and depicted in *histogram form* compared with the isotype control for each sample (*shaded area,* isotype control; *black line,* intracellular Bcl-2). *D,* C57BL/6 mice were challenged with 5×10^5 B16ova or B16ovaCCL21 tumor cells s.c. and received a single dose of 10^7 activated OT-1 T cells 7 days later. Five days later, spleens and lymph nodes were harvested from mice ($n = 2$) and were restimulated with IL-2 or peptide under the indicated conditions. Spots were developed and quantified on the ELISPOT plate after 48 h. *Columns,* fold increase in number of spots per well over number of spots from cells from the unstimulated control. Average of two mice per group.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/1/300/2567206/300.pdf by guest on 14 December 2024

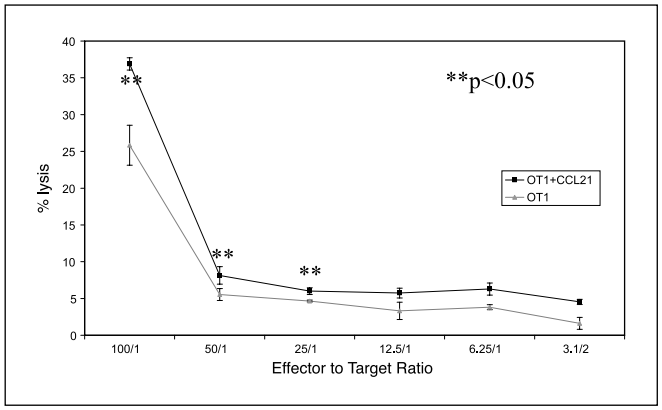

Figure 4. CCL21 enhances cytotoxicity *in vitro*. Three-day–activated OT-1 T cells, grown in the presence of 100 ng rmCCL21 or medium alone for 48 h, were used as effectors against B16ova targets in a Cr^{51} release assay. Supernatants were harvested and analyzed after a 4-h incubation. *Points,* % lysis from three duplicate experiments; *bars,* SD.

T cells; however, there were no long-term survivors (data not shown). In contrast, 90% (n = 10) of mice treated with two doses of OT-1 (4070A-CCL21) T cells survived the initial tumor challenge, compared with 10% of animals treated with two doses of OT-1 (4070A-GFP; Fig. 5). Five of the nine mice that survived the treatment with OT-1 (4070A-CCL21) cells also survived subsequent tumor rechallenge (Table 1), consistent with our previous data that CCL21 intratumoral expression confers long-term memory on cured mice. In addition, the four mice that succumbed to tumor rechallenge did so at a significantly delayed rate compared with controls (Table 1). These results show that expression of CCL21 can be transferred to metastatic tumors, in a fully immunocompetent host, using adoptive T-cell therapy; can both enhance the therapeutic efficacy of subsequently transferred, unmodified T cells; and can generate immunologic memory in the majority of responding mice.

Discussion

Previous studies reported that CCL21 provides survival signals to, and is chemotactic for, both mature dendritic cells and naive T cells that express the cognate receptor CCR7 (22–25). However, a role for CCL21 in the biology of activated effector T cells has not been previously reported. Indeed, it might be unexpected given that activated T cells are thought to down-regulate CCR7 expression. However, we observed that both recombinant CCL21 protein and supernatants from the B16ovaCCL21 tumor cell line were chemotactic for OT-1 T cells with an activated phenotype (i.e., $CD8^+CD3^{low}CD25^{hi}CD44^{hi}$; Fig. 1*A*). In addition, activated T cells cultured in the presence of CCL21 displayed a moderate, but reproducible, reduction of cell surface CD62L (L-selectin), a molecule critical for homing to lymph nodes (Fig. 1*B*; ref. 22). Therefore, we hypothesized that tumor cell expression of CCL21 may promote trafficking to, and retention of, effector cells at the tumor site.

By using the B16ova/OT-1 model of adoptive T-cell transfer therapy for metastatic melanoma, we established a suboptimal regimen of OT-1 dosing such that tumors were not cured by the T cells alone. In this model, but in the absence of adoptive T-cell therapy, stable expression of CCL21 in lung metastases significantly prolonged survival compared with mice bearing B16ova tumors alone, indicating that CCL21 exerts antitumor effects, consistent with previous reports (25–27). When we added a single, suboptimal dose of OT-1 therapy, mice bearing B16ovaCCL21 metastases survived significantly longer than mice bearing B16ova metastases (Fig. 2). Given our earlier *in vitro* findings, we hypothesized that CCL21 expression in lung metastases might act to attract, and retain, adoptively transferred T cells to the tumor site, leading to more efficient tumor cell killing. However, we were unable to detect any difference in the trafficking of CFSE-labeled $CD8^+$ OT-1 T cells to B16ovaCCL21, or B16ova, lung metastases *in vivo* either 24 or 48 h after adoptive transfer (data not shown). These data suggested that CCL21 was enhancing the efficacy of adoptive therapy by means other than promoting increased T-cell trafficking.

Signaling through the CCL21 receptor CCR7 promotes survival of both mature dendritic cells (15) and activated T cells (16) by maintaining the expression of the prosurvival protein Bcl-2. Therefore, we investigated whether exposure to CCL21 would enhance the survival of activated OT-1 T cells *in vivo*. ELISPOT analysis on spleens and draining lymph nodes harvested from mice bearing B16ovaCCL21 tumors 24 h after adoptive transfer showed an increased proportion of IFN-γ–positive cells upon restimulation with SIINFEKL (Fig. 3*A*) compared with mice bearing B16ova tumors alone following adoptive transfer. At the same time point, only background levels of IFN-γ–secreting cells were present in mice bearing either tumor type but not receiving adoptive transfer (data not shown). Therefore, we believe that this assay was detecting increased persistence/numbers of adoptively transferred cells in the context of a tumor expressing CCL21.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/1/300/2567206/300.pdf by guest on 14 December 2024

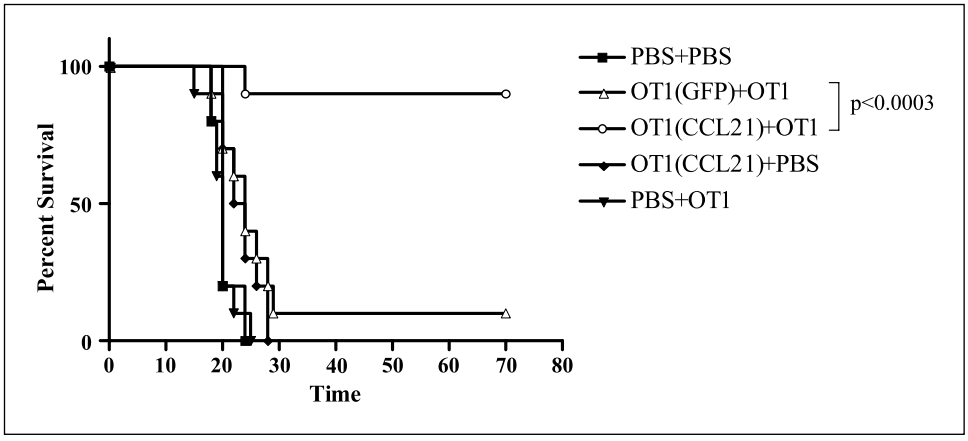

Figure 5. Hitchhiking of a retrovirus encoding CCL21 to OT-1 T cells increases the therapeutic efficacy of a subsequently administered dose of OT-1 T cells. Lung metastases were established in C57BL/6 mice by i.v. injection of 5×10^5 B16ova cells. Activated OT-1 cells (5×10^6) loaded with retrovirus or PBS control were administered 5 and 11 days after tumor inoculation. On day 13, mice received a single dose of 10^6 unmodified OT-1 or PBS control as indicated and were monitored for survival.

Table 1. Survival of mice following rechallenge

Treatment group	No. surviving primary tumor	No. surviving s.c. rechallenge	Time to death following rechallenge (d)
OT1(GFP) + OT1	1/10	0/1	22
OT1(CCL21) + OT1	9/10	5/9	30, 32, 39, 44
Naive control	NA	0/3	13, 20, 20

NOTE: Mice surviving initial tumor challenge and treatment with OT-1s modified to carry retrovirus encoding CCL21 or GFP (Fig. 5) were rechallenged with 5×10^5 B16ova tumor cells s.c. Mice were monitored for tumor growth. Naive mice were challenged with the same dose of tumor cells and served as negative control.
Abbreviation: NA, not applicable.

To address the issue of whether CCL21 was promoting increased T-cell proliferation or survival, we showed increased survival, without obvious proliferation, of activated OT-1 T cells in the presence of CCL21 *in vitro* by the MTT assay (Fig. 3*B*). Indeed, Bcl-2 expression in activated OT-1 T cells was detectable only in the presence of CCL21, at levels comparable with those seen in OT-1 T cells treated with IL-15 (Fig. 3*C*), a factor known to promote survival of the memory T-cell subset by increased Bcl-2 expression (35). Thus, increased survival of adoptively transferred T cells, through enhanced expression of Bcl-2, may in part account for the enhanced efficacy of adoptive therapy in the presence of CCL21. We also observed that OT-1 T cells grown in the presence of CCL21 displayed a modest, but significant, increase in target cell killing, which was dependent on the presence of the ovalbumin antigen. The biological significance of these results are not entirely clear, because increased cytotoxicity was observed at high effector-to-target ratios (which are unlikely to be achieved *in vivo* at the tumor site).

Therefore, taken together, our data suggest that it is likely to be the combination of direct effects of CCL21 on T-cell survival, longevity, and activity that leads to the overall potentiating effects that CCL21 has on adoptive T-cell therapy, rather than any of the relatively modest individual effects alone. However, our results with the use of stably transfected B16 clones expressing CCL21 also indicate that additional indirect effects of CCL21 expression by tumor cells may also contribute to the enhancement of adoptive T-cell therapy by this chemokine. Given the reported activity of CCL21 on the recruitment of antigen-presenting cells (17–21), both *in vitro* and *in vivo*, increased cross priming of host antigen-presenting cells with tumor antigens from CCL21-expressing tumor cells may play a critical role in enhancing the survival/longevity and activity of adoptively transferred T cells. This is consistent with our demonstration of long-term protection generated in mice treated with OT-1 (CCL21) T cells. Therefore, we believe that the results presented here support a role for CCL21 in both direct and indirect effects on enhancing the antitumor efficacy of adoptively transferred T cells.

Consistent with this conclusion, we also observed that 5 days following adoptive T-cell transfer, spleens and lymph nodes of mice bearing B16ovaCCL21 metastases contained T cells specific for the tumor antigens Trp2 and gp100, whereas no such cells were present in mice bearing B16ova metastases treated with adoptive transfer (Fig. 3*D*). Expression of CCL21 by tumors, without additional T-cell therapy, did not generate T cells reactive to these tumor antigens. Thus, overexpression of CCL21 at the tumor site, combined with the tumor cell killing induced by OT-1 T-cell therapy, induced the priming of endogenous T cell responses that were specific for antigens separate from that targeted by the adoptively transferred T cells themselves (36). We hypothesize that the increased survival and cytotoxic capacity of OT-1 T cells that are exposed to CCL21 at the tumor site leads to greater levels of tumor cell killing (as evidenced by the greater therapeutic effects in Fig. 2) compared with those levels of killing in the absence of CCL21. These higher levels of tumor cell killing would then provide sufficient levels of released tumor antigen to allow cross priming of host antigen-presenting cells, possibly recruited by the CCL21 expression. In turn, this would promote T-cell priming against endogenous antigens, in addition to the ovalbumin antigen, which is the original target of the OT-1 T cells. It seems probable that the induction of these antitumor T-cell responses would also play a role in the therapeutic effects that we observed in both the stable CCL21-expressing model (Fig. 2) and the *in vivo* delivery model (Fig. 5), and depletion studies to confirm this are currently under way.

Our long-term aim is to develop therapies that can be used in a truly systemic manner to treat metastatic disease, in which pretransduction of patient tumors with CCL21 will not be possible. We have previously shown that we can use OT-1 T cells to carry preadsorbed retroviral particles to the sites of (ovalbumin) antigen-positive tumor growth, where T cell activation–dependent release of the virus from the T-cell surface allows for direct infection of the tumor cells (29). By using the appropriate retroviral envelope, transduction of the OT-1 T cells is negligible and the therapeutic effects observed are due to handoff of the preadsorbed virus to tumor cells *in vivo* (29). In addition, handoff of the adsorbed virus to other tissues in the mice was undetectable by PCR (data not shown; refs. 29, 30). Therefore, this system allows for a truly systemic delivery of vectors to disseminated disease, in fully immunocompetent hosts, without the need to access tumors directly for gene delivery. We exploited this system by hypothesizing that adoptive transfer of OT-1 T cells engineered to deliver a retroviral vector encoding CCL21 to metastatic disease would improve the efficacy of a subsequent dose of unmodified OT-1 T cells, through the ability of CCL21 to improve the longevity and activity of the OT-1 T cells that reach the tumors.

Consistent with this hypothesis, 90% of mice bearing B16ova metastases, and treated with two doses of OT-1 (4070A-CCL21) T cells, survived their tumor load when they received one additional dose of unmodified OT-1 cells. In contrast, only 10% of mice treated with OT-1 (4070A-GFP) cells were cured by the therapy (Fig. 5). In the model where stable CCL21-expressing B16ova clones were used along with a single dose of OT-1 for therapy, we observed no cures (Fig. 2). In contrast, in the model using OT-1 cells to carry CCL21 vectors to tumor sites, we observed significant cures of metastatic disease using a total of three doses of OT-1 T cells. We believe that the difference in these two models is due to the increased total number of OT-1 T cells administered to the mice, based on the observations by ourselves as well as by others, that the efficacy of adoptive T-cell therapy is critically dose dependent (37, 38). This hypothesis is supported by our observations that only a single dose of OT-1 (4070A-CCL21) T cells, before a second dose of unmodified OT-1, resulted in a

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/1/300/2567206/300.pdf by guest on 14 December 2024

small, but significant, prolongation in survival time compared with treatment with OT-1 (4070A-GFP) T cells; however, there were no long-term survivors. Therefore, we believe that overall therapy is critically dependent on both dose of OT-1 and the presence of CCL21 expressed at the tumor site. We have previously shown that between 5% and 10% of the tumor cells that survive cytolysis *in vivo* by the adoptively transferred OT-1 T cells become transduced by the preadsorbed retroviral vector carried by the T cells (30). We have not yet determined the minimum levels of B16ova transduction with CCL21, which are required for effective therapy; however, this question will be addressed by seeding mixtures of different proportions of stable CCL21 transfectants along with parental B16ovacells as tumors *in vivo*. Furthermore, five of nine surviving mice survived subsequent tumor rechallenge, whereas mice in the control groups all succumbed (Table 1). Hence, and consistent with our previous results, the combination of tumor-expressed CCL21 and T-cell killing *in vivo* generated immunologic memory in the majority of responding mice. We are currently investigating whether this immunologic memory is directed solely against the ovalbumin antigen or if it encompasses other B16-specific antigens (such as TRP-2 and gp100), which would be suggested by the ELISPOT analyses of Fig. 4.

In the studies reported here, we used clonal populations of T cells (OT-1) with defined specificity for a model tumor-associated antigen (ovalbumin). Clearly, this animal model is rather artificial and, in general terms, animal models do not necessarily predict what will happen in the clinical setting; nonetheless, we do foresee a direct route to clinical translation of our results. Thus, polyclonal T-cell populations with often undefined specificities can be recovered from patient tumors, expanded *in vitro* to large numbers, and adoptively transferred back into the patient where they localize, at least in part, to tumor sites (1, 2). We have previously shown that, in addition to murine OT-1 T cells, human antigen-specific T cells can readily be loaded with retroviral particles, which can be delivered to cocultured tumor cells (29, 30). Hence, we believe that our results with the OT-1 model should be relatively simply transferred into clinical protocols using viral loading, and subsequent adoptive transfer, of polyclonal human antigen-specific T-cell populations recovered from patient tumors. This would both enhance the cytotoxic activity, and persistence (39), of conventional adoptive T-cell therapies (8) and will allow delivery of vectors to systemically distributed tumors.

In summary, we have shown here that tumor cell–mediated expression of CCL21 enhances the survival and effector functions of adoptively transferred T cells. In addition, in combination with adoptive T-cell transfer, CCL21 expression leads to levels of tumor destruction sufficient to prime endogenous antitumor immune responses *in vivo*. Moreover, we have shown that it is possible to deliver CCL21 in a truly systemic manner, in a fully immunocompetent host, using adoptively transferred, antigen-specific T cells, without the need to access any tumor sites for direct delivery of the *CCL21* gene. In this way, CCL21 can significantly enhance the therapeutic efficacy of subsequently administered doses of T cells. Studies are under way to investigate whether CCL21 needs to be expressed from tumor cells, which are the target of the T-cell therapy, or whether systemic levels of CCL21 can be achieved by other means to have the same T-cell supportive effects. Therefore, in addition to other manipulations, such as vaccinations and lymphodepletion (6, 7), CCL21 is a promising and novel reagent for use in enhancing the efficacy of adoptive T-cell transfer protocols.

Acknowledgments

Received 4/7/2006; revised 9/11/2006; accepted 10/12/2006.

Grant support: Mayo Foundation and NIH grants 1RO1CA94180 and 1RO1CA107082.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Toni L. Higgins for expert secretarial assistance.

References

1. Dudley ME, Wunderlich JR, Robbins PF, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 2002; 298:850–4.
2. Rosenberg SA, Dudley ME. Cancer regression in patients with metastatic melanoma after the transfer of autologous antitumor lymphocytes. Proc Natl Acad Sci U S A 2004;101 Suppl 2:14639–45.
3. Ho WY, Blattman JN, Dossett ML, Yee C, Greenberg PD. Adoptive immunotherapy: engineering T cell responses as biologic weapons for tumor mass destruction. Cancer Cell 2003;3:431–7.
4. Gattinoni L, Klebanoff CA, Palmer DC, et al. Acquisition of full effector function in vitro paradoxically impairs the in vivo antitumor efficacy of adoptively transferred $CD8^+$ T cells. J Clin Invest 2005;115:1616–26.
5. Gattinoni L, Finkelstein SE, Klebanoff CA, et al. Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific $CD8^+$ T cells. J Exp Med 2005;202:907–12.
6. Palmer DC, Balasubramaniam S, Hanada K, et al. Vaccine-stimulated, adoptively transferred $CD8^+$ T cells traffic indiscriminately and ubiquitously while mediating specific tumor destruction. J Immunol 2004;173: 7209–16.
7. Wang LX, Shu S, Plautz GE. Host lymphodepletion augments T cell adoptive immunotherapy through enhanced intratumoral proliferation of effector cells. Cancer Res 2005;65:9547–54.
8. Dudley ME, Rosenberg SA. Adoptive-cell-transfer therapy for the treatment of patients with cancer. Nat Rev Cancer 2003;3:666–75.
9. Sallusto F, Mackay CR, Lanzavecchia A. The role of chemokine receptors in primary, effector, and memory immune responses. Annu Rev Immunol 2000;18: 593–620.
10. Schall TJ, Bacon KB. Chemokines, leukocyte trafficking, and inflammation. Curr Opin Immunol 1994;6: 865–73.
11. Moser B, Loetscher P. Lymphocyte traffic control by chemokines. Nat Immunol 2001;2:123–8.
12. Cyster JG. Chemokines and cell migration in secondary lymphoid organs. Science 1999;286:2098–102.
13. Gunn MD, Tangemann K, Tam C, Cyster JG, Rosen SD, Williams LT. A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. Proc Natl Acad Sci U S A 1998;95:258–63.
14. Saeki H, Moore AM, Brown MJ, Hwang ST. Cutting edge: secondary lymphoid-tissue chemokine (SLC) and CC chemokine receptor 7 (CCR7) participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes. J Immunol 1999;162: 2472–5.
15. Sanchez-Sanchez N, Riol-Blanco L, de la Rosa G, et al. Chemokine receptor CCR7 induces intracellular signaling that inhibits apoptosis of mature dendritic cells. Blood 2004;104:619–25.
16. Kim JW, Ferris RL, Whiteside TL. Chemokine C receptor 7 expression and protection of circulating $CD8^+$ T lymphocytes from apoptosis. Clin Cancer Res 2005;11:7901–10.
17. Willimann K, Legler DF, Loetscher M, et al. The chemokine SLC is expressed in T cell areas of lymph nodes and mucosal lymphoid tissues and attracts activated T cells via CCR7. Eur J Immunol 1998;28: 2025–34.
18. Warnock RA, Campbell JJ, Dorf ME, Matsuzawa A, McEvoy LM, Butcher EC. The role of chemokines in the microenvironmental control of T versus B cell arrest in Peyer's patch high endothelial venules. J Exp Med 2000; 191:77–88.
19. Hedrick JA, Zlotnik A. Identification and characterization of a novel β chemokine containing six conserved cysteines. J Immunol 1997;159:1589–93.
20. Hromas R, Kim CH, Klemsz M, et al. Isolation and characterization of Exodus-2, a novel C-C chemokine with a unique 37-amino acid carboxyl-terminal extension. J Immunol 1997;159:2554–8.
21. Tanabe S, Lu Z, Luo Y, et al. Identification of a new mouse β-chemokine, thymus-derived chemotactic agent 4, with activity on T lymphocytes and mesangial cells. J Immunol 1997;159:5671–9.
22. Stein JV, Rot A, Luo Y, et al. The CC chemokine thymus-derived chemotactic agent 4 (TCA-4, secondary lymphoid tissue chemokine, 6Ckine, exodus-2) triggers lymphocyte function-associated antigen 1-mediated arrest of rolling T lymphocytes in peripheral lymph node high endothelial venules. J Exp Med 2000; 191:61–76.
23. Nakano H, Tamura T, Yoshimoto T, et al. Genetic

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/1/300/2567206/300.pdf by guest on 14 December 2024

defect in T lymphocyte-specific homing into peripheral lymph nodes. Eur J Immunol 1997;27:215–21.
24. Nakano H, Mori S, Yonekawa H, Nariuchi H, Matsuzawa A, Kakiuchi T. A novel mutant gene involved in T-lymphocyte-specific homing into peripheral lymphoid organs on mouse chromosome 4. Blood 1998;91: 2886–95.
25. Kirk CJ, Hartigan-O'Connor D, Nickoloff BJ, et al. T cell-dependent antitumor immunity mediated by secondary lymphoid tissue chemokine: augmentation of dendritic cell-based immunotherapy. Cancer Res 2001; 61:2062–70.
26. Yang SC, Hillinger S, Riedl K, et al. Intratumoral administration of dendritic cells overexpressing CCL21 generates systemic antitumor responses and confers tumor immunity. Clin Cancer Res 2004;10: 2891–901.
27. Vicari AP, Ait-Yahia S, Chemin K, Mueller A, Zlotnik A, Caux C. Antitumor effects of the mouse chemokine 6Ckine/SLC through angiostatic and immunological mechanisms. J Immunol 2000;165:1992–2000.
28. Hogquist KA, Jameson SC, Heath WR, Howard JL, Bevan MJ, Carbone FR. T cell receptor antagonist peptides induce positive selection. Cell 1994;76:17–27.
29. Cole C, Qiao J, Kottke T, et al. Tumor-targeted, systemic delivery of therapeutic viral vectors using hitchhiking on antigen-specific T cells. Nat Med 2005;11:1073–81.
30. Kottke T, Qiao J, Diaz RM, et al. The perforin-dependent immunological synapse allows T cell activation-dependent tumor targeting my MLV vector particles. Gene Ther 2006;13:1166–77.
31. Melcher A, Todryk S, Hardwick N, Ford M, Jacobson M, Vile RG. Tumor immunogenicity is determined by the mechanism of cell death via induction of heat shock protein expression. Nat Med 1998;4:581–7.
32. Linardakis E, Bateman A, Phan V, et al. Enhancing the efficacy of a weak allogeneic melanoma vaccine by viral fusogenic membrane glycoprotein-mediated tumor cell-tumor cell fusion. Cancer Res 2002;62:5495–504.
33. Markowitz D, Goff S, Bank A. Construction and use of a safe and efficient amphotropic packaging cell line. Virology 1988;167:400–6.
34. Weninger W, von Andrian UH. Chemokine regulation of naive T cell traffic in health and disease. Semin Immunol 2003;15:257–70.
35. Berard M, Brandt K, Bulfone-Paus S, Tough DF. IL-15 promotes the survival of naive and memory phenotype $CD8^+$ T cells. J Immunol 2003;170:5018–26.
36. Vanderlugt CL, Miller SD. Epitope spreading in immune-mediated diseases: implications for immunotherapy. Nat Rev Immunol 2002;2:85–95.
37. Hanson HL, Donermeyer DL, Ikeda H, et al. Eradication of established tumors by $CD8^+$ T cell adoptive immunotherapy. Immunity 2000;13:265–76.
38. Matsui K, O'Mara LA, Allen PM. Successful elimination of large established tumors and avoidance of antigen-loss variants by aggressive adoptive T cell immunotherapy. Int Immunol 2003;15:797–805.
39. Robbins PF, Dudley ME, Wunderlich J, et al. Persistence of transferred lymphocyte clonotypes correlates with cancer regression in patients receiving cell transfer therapy. J Immunol 2004; 173:7125–30.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/67/1/300/2567206/300.pdf by guest on 14 December 2024