CCL5-Mediated Endogenous Antitumor Immunity Elicited by Adoptively Transferred Lymphocytes and Dendritic Cell Depletion

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Abstract

Adoptive transfer of antitumor T cells is a promisingly effective therapy for various cancers, but its effect on endogenous antitumor immune mechanisms remains largely unknown. Here, we show that the administration of naive T cells *de novo* primed for only 7 days against tumor antigens resulted in the durable rejection of otherwise lethal ovarian cancers when coupled with the depletion of tumor-associated immunosuppressive dendritic cells (DC). Therapeutic activity required tumor antigen specificity and perforin expression by the adoptively transferred T cells, but not IFN-γ production. Importantly, these shortly primed T cells secreted large amounts of CCL5, which was required for their therapeutic benefit. Accordingly, transferred T cells recruited CCR5+ DCs into the tumor, where they showed distinct immunostimulatory attributes. Activated CCR5+ host T cells with antitumor activity also accumulated at tumor locations, and endogenous tumor-specific memory T cells remained elevated after the disappearance of transferred lymphocytes. Therefore, persistent, long-lived antitumor immunity was triggered by the administration of *ex vivo* activated T cells, but was directly mediated by immune cells of host origin. Our data unveil a CCL5-dependent mechanism of awakening endogenous antitumor immunity triggered by *ex vivo* expanded T cells, which is augmented by tumor-specific targeting of the cancer microenvironment. [Cancer Res 2009;69(15):6331–8]

Introduction

Adoptive cell transfer therapy (ACT), the *ex vivo* activation, expansion, and subsequent administration of tumor-reactive T cells, is a vastly successful therapy against certain cancers. In fact ACT is currently the most effective therapy against metastatic melanoma, with objective regressions reported in 50% of patients (1–4). The therapeutic effects of ACT are commonly attributed to the *in vivo* expansion and antitumor activity of transferred lymphocytes. Correspondingly, major efforts have been focused on promoting long-term persistence of adoptively transferred T cells (5). Thus, recent studies indicate that compared with more differentiated effector lymphocytes, memory T cells or early effectors have a higher capacity for *in vivo* expansion, which is associated with enhanced therapeutic effects against melanoma (6).

Most ACT studies in mouse cancer models are performed with transgenic T cells (7), and their effect on activating endogenous (host-derived) protective immune mechanisms is not usually addressed. However, although their function in the tumor microenvironment is suboptimal without treatment, antitumor T cells exert spontaneous immune pressure against cancer progression (8). Upon pharmacologic inhibition of tumor-associated immunosuppressive leukocytes, endogenous T cells spontaneously and therapeutically expand, become activated (9), and contribute to the control of cancer after conventional chemotherapy (10). Correspondingly, changes in the tumor microenvironment caused by ACT and/or lymphodepletion may also release endogenous antitumor immune mechanisms from suppression. Therefore, the relative contribution of enhanced endogenous antitumor immunity to the persistent, long-lived therapeutic benefit elicited by ACT remains unknown.

Despite inducing major clinical responses in melanoma patients, ACT strategies attempted against significantly more lethal epithelial tumors have not yet yielded similar positive results. Recent studies in ovarian cancer, one of the most aggressive and frequent forms of epithelial cancer, indicate that, although their persistence is short-lived, large numbers of tumor-reactive T cells can be safely given to patients (11). Still, there is strong rationale for developing ACT strategies against ovarian cancer, which may be applicable to other aggressive epithelial tumors. Firstly, chemotherapies implemented in the last 30 years have led to a 5-year survival rate of 30%, at best, for patients with metastatic ovarian carcinoma, the stage at which most cases are diagnosed (12). As a result, epithelial ovarian cancer will claim the lives of >15,000 women in the United States alone in 2008 (12). Secondly, seminal studies by Coukos and colleagues (13, 14) showed that the infiltration of ovarian tumor islets by T cells strongly predicts a better clinical course, which was supported by subsequent studies that restricted the favorable prognostic effect to intraepithelial CD8+ cytotoxic T cells (15, 16). Thirdly, several cancer antigens recognized by antibodies and/or CD8 T cells have been found in many ovarian cancers (17–20) and could be harnessed for potent therapies.

To design clinically effective immunotherapies against aggressive epithelial tumors, multiple immunosuppressive networks orchestrated by regulatory T cells and myeloid-derived suppressor cells (9, 21–23) need to be overcome. Additionally, regulatory MHC-II+DEC205+CD11c+CD14− dendritic cells (DC), the most abundant leukocyte subset in solid ovarian cancer specimens (24), contribute to tumor vascularization and immunosuppression (24, 25). Thus, DCs in ovarian cancer present an additional barrier to successful immunotherapies, and their elimination from ovarian cancer–bearing mice significantly delays cancer progression by boosting antitumor immunity (24).

Here, we show that the adoptive transfer of *de novo* primed tumor-reactive T cells coupled with depleting immunosuppressive
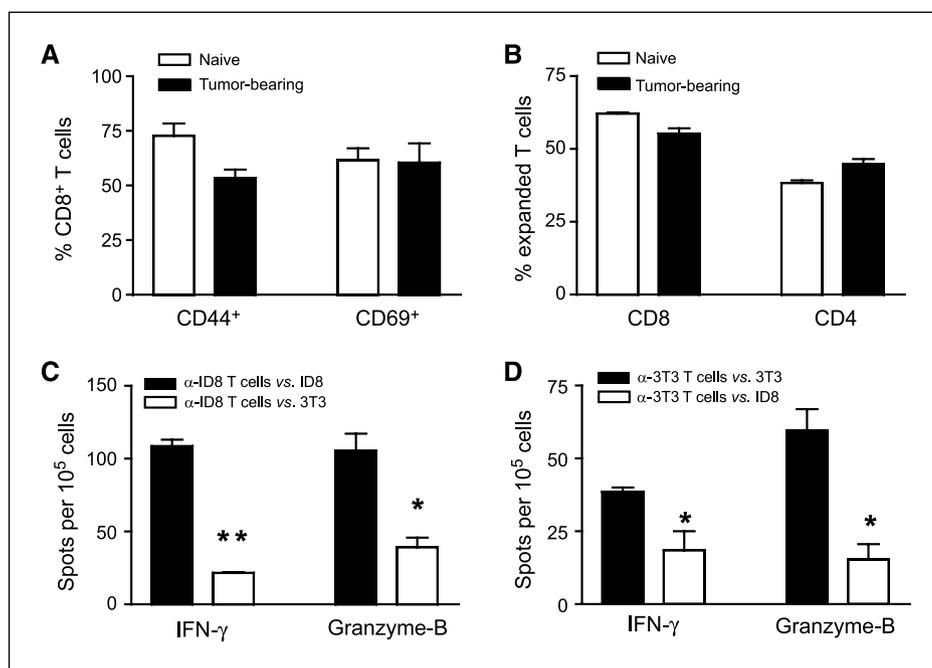
Figure 1. Phenotypic characterization of tumor-reactive and irrelevantly primed T cells. *A,* activation status of T cells from either healthy (*Naive*) or ID8-*Defb29/Vegf-a* tumor-bearing (*Tumor-bearing*) mice primed to ID8-*Defb29/Vegf-a* antigens. Representative of three independent experiments. *B,* T cells expanded from tumor-bearing or healthy mice show comparable proportions of CD8 versus CD4 T cells. *C,* IFN-γ and granzyme B ELISPOT analyses of naive T cells primed to ID8-*Defb29/Vegf-a* antigens, in response to their cognate tumor antigen (α-ID8 T cells versus ID8) or NIH-3T3 fibroblasts (α-ID8 T cells versus 3T3). Representative of two independent experiments (*n* = 6/group, total). *D,* similar ELISPOT analysis performed with naive T cells primed to irrelevant NIH-3T3 fibroblasts (*, *P* < 0.05; **, *P* < 0.01).

DCs results in durable regression of established ovarian cancer. Interestingly, this combination therapy triggered the sustained activation of endogenous, host-derived antitumor T cells in a CCL5-dependent manner. Our data provide a mechanistic rationale for differential targeting of the microenvironment of distinct aggressive epithelial cancers for enhanced immunotherapies and show that adoptively transferred T cells induce a previously unrecognized, therapeutically relevant, tumor-specific host response.

Materials and Methods

Mice and tumor lines. C57BL/6 (B6) and CD45.1 mice were purchased from National Cancer Institute. B6.129S7-Ifn[tm/Ts]/J (IFN-γ^{−/−}), perforin-deficient C57BL/6-Prf1[tm/Sdz]/J (Pfp^{−/−}) mice, and red fluorescent C57BL/6-Tg(ACTB-MAP2K1*K97M)1Stl/J mice were purchased from The Jackson Laboratory. Experiments were conducted in accordance with the Dartmouth Medical School guidelines. ID8-*Defb29/Vegf-a*, ID8-luciferase, NIH-3T3, and cultured splenocytes and DCs were maintained in RPMI medium containing 10% fetal bovine serum.

T-cell expansion, adoptive immunotherapy, and DC depletion. Bone marrow–derived DCs were produced (26) and then pulsed overnight (10:1) with irradiated (10,000 rad) and UV-light exposed ID8-*Defb29/Vegf-a* or NIH-3T3 cells. Splenocytes were harvested from CD45.1[+] mice and cultured for 7 d with ID8-*Defb29/Vegf-a*/NIH-3T3–pulsed DCs and 10 units/mL recombinant human interleukin-2 (IL-2; Peprotech). Mice (6–8 wk old) were injected i.p. with 1.5×10^6 ID8-*Defb29/Vegf-a* cells and treated on days 7 and 14 with i.p. adoptive transfer of 1.5×10^6 *in vitro* activated splenic T cells. Lymphopenia was induced by sublethal TBI (300 rad) of tumor-bearing mice on day 7 at 5 h before ACT. DCs were eliminated by i.p. administration of 10 μg/mouse of an anti-CD11c immunotoxin (IT; ref. 24) on the day before T-cell transfer (day 6) and thrice weekly for 2 wk thereafter.

Results

Optimization of a clinically relevant system for *de novo* priming of tumor-reactive T cells. We first developed a clinically relevant system for briefly arousing polyclonal naive antitumor T cells, exploiting the increased capacity of early effector T cells for *in vivo* expansion (6). Splenic lymphocytes were stimulated with DCs pulsed with UV plus γ-irradiated ID8-*Defb29/Vegf-a* cancer cells, which generate aggressive and widely disseminated ovarian tumors (27). After only 7 days of *de novo* priming, >68% of (naive) splenic T cells from healthy mice showed activation markers (Fig. 1*A*), with a final CD8/CD4 ratio of 3:2 (Fig. 1*B*). Surprisingly, similarly activated splenic T cells from tumor-bearing mice did not show stronger activation, higher cytotoxic/helper T-cell ratio, nor superior therapeutic effects (Fig. 1*A* and *B*; Supplementary Fig. S1*A*). Subsequent studies were performed with naive lymphocytes, as this approach was more clinically reproducible with peripheral blood T cells from cancer patients.

Although the priming regimen lasted only 7 days, T cells specific to ID8-*Defb29/Vegf-a* tumor cells were selectively activated, as significantly more lymphocytes produced IFN-γ and granzyme B in response to their cognate antigen than to nonspecific NIH-3T3 fibroblasts (Fig. 1*C*; *P* < 0.01, *P* < 0.05). Correspondingly, significantly fewer T cells similarly expanded to irrelevant NIH-3T3 cell antigens produced IFN-γ and granzyme B in response to ID8-*Defb29/Vegf-a* tumor antigens compared with their cognate NIH-3T3 cell antigen (Fig. 1*D*; *P* < 0.05). Importantly, T cells primed to these irrelevant antigens showed comparable CD8/CD4 ratios and activation phenotype (Supplementary Fig. S1*B* and *C*), showing the feasibility of selectively priming resting antigen-specific T cells within 7 days.

Eliminating CD11c^{+} regulatory DCs enhances the therapeutic effects of adoptively transferred tumor-reactive T cells. Because eliminating ovarian cancer–associated DCs boosts antitumor immunity (24), we hypothesized that their depletion from tumor locations could augment the effect of ACT. Thus, ID8 ovarian cancer–bearing mice were treated with an anti-CD11c IT. As we published, this DC depleting tool consists of a mouse CD11c-specific single-chain antibody fused to a *Pseudomonas* exotoxin-A

lacking the cell-binding domain (24) and elicits its lethality to the cytoplasm of tumor-associated CD11c⁺ cells, but not to other cells or splenic DCs (24). Confirming our previous observations, mice depleted of tumor-associated DCs showed a significant life span increase of 34% without additional treatment (Fig. 2*A* and *B*; *P* < 0.05). ACT alone led to a similar therapeutic benefit but did not produce durable curative responses (Fig. 2*B*; *P* < 0.05). In contrast, eliminating regulatory DCs before ACT (ACT + IT) led to the elimination of any obvious disease in 50% of treated mice, which remain healthy >485 days later (Fig. 2*B*; *P* < 0.01). Accordingly, ACT + IT significantly diminished tumor burden after 8 weeks of cancer progression (Supplementary Fig. S1*D, right*, quantification; *P* < 0.01). Therefore, eliminating regulatory tumor-associated DCs transforms the administration of shortly primed antitumor T cells from a simply palliative intervention to a durable curative approach.

To confirm the therapeutic potential of combining ACT with regulatory DC depletion in a more aggressive model of ovarian cancer, we next treated mice growing i.p. ID8-*Defb29/Vegf-a* tumors. These tumors show enhanced angiogenesis, immunosuppression, and accelerated progression (27). To boost the homeostatic expansion of adoptively transferred T cells, mice were sublethally irradiated before treatment. Confirming our previous results, mice depleted of tumor-associated DCs showed a modest but significant life span increase of 11%, whereas ACT alone statistically significantly enhanced survival by 22% in this aggressive model (Fig. 2*C*; *P* < 0.05 for both). Importantly, in agreement with previous reports (28), irradiation did not delay tumor progression of untreated or individually treated mice (Supplementary Fig. S2*A*). Furthermore, eliminating tumor-associated regulatory DCs promoted increased *in vivo* expansion of subsequently transferred tumor-reactive T cells (Fig. 2*D*; *P* < 0.05) and induced dramatically increased survival compared with individual treatments and a 53% increase in life span compared with untreated mice (Fig. 2*C*; *P* < 0.01 for both).

The therapeutic effects of ACT are mediated by cytotoxic T cells homing to tumor locations in an antigen-specific, IFN-γ–independent manner. Because administered T cells were derived from *de novo* primed lymphocytes, it was possible that the therapeutic effects resulted from antigen-independent, nonspecific secretion of proinflammatory factors. To define whether transferred T cells required tumor-specific priming, splenic T cells from healthy, congenic (CD45.1) mice were expanded against either irradiated ID8-*Defb29/Vegf-a* cells or irradiated irrelevant NIH-3T3 fibroblasts and then transferred into ID8-*Defb29/Vegf-a* tumor-bearing mice. Interestingly, irrelevantly primed T cells exerted some
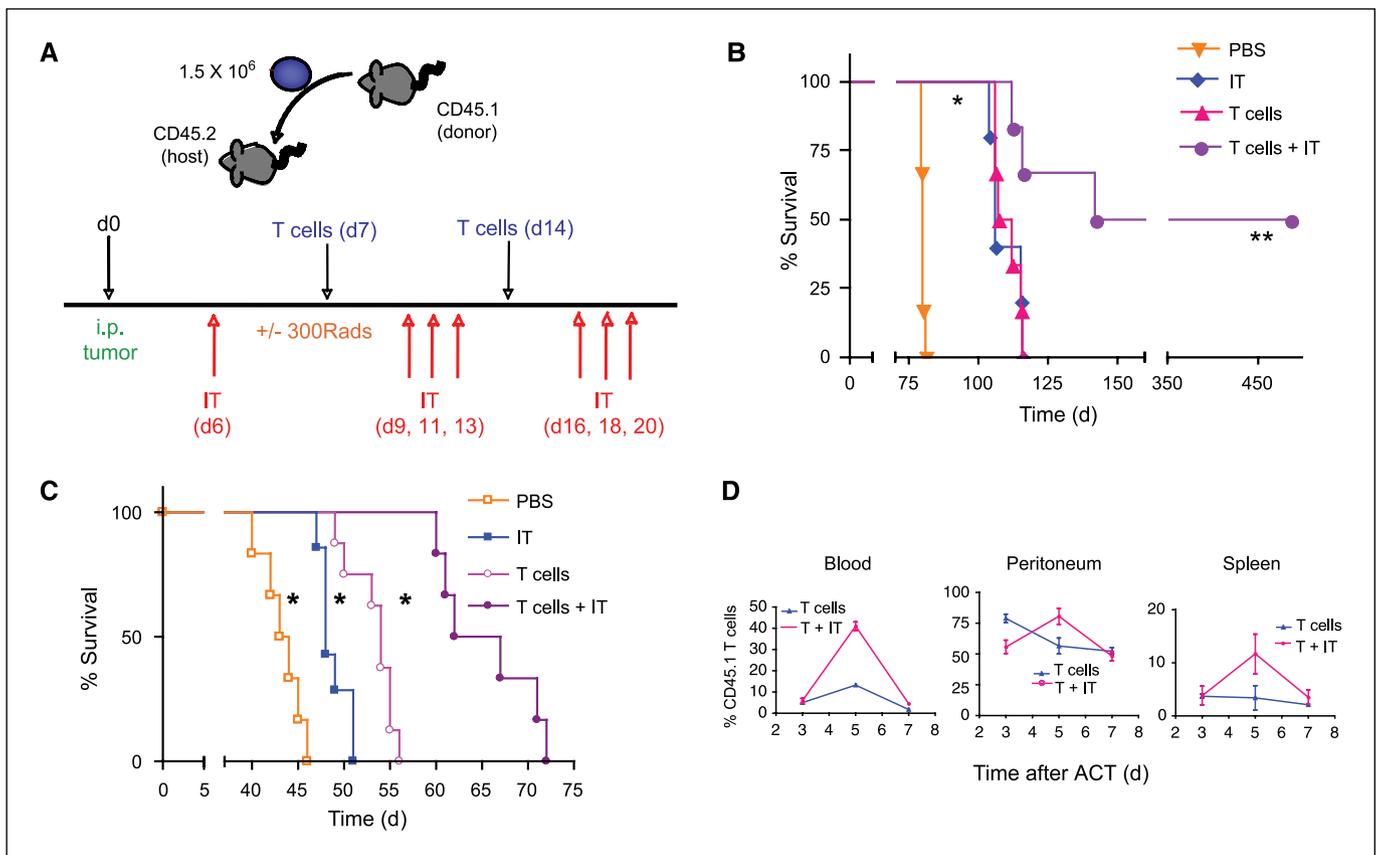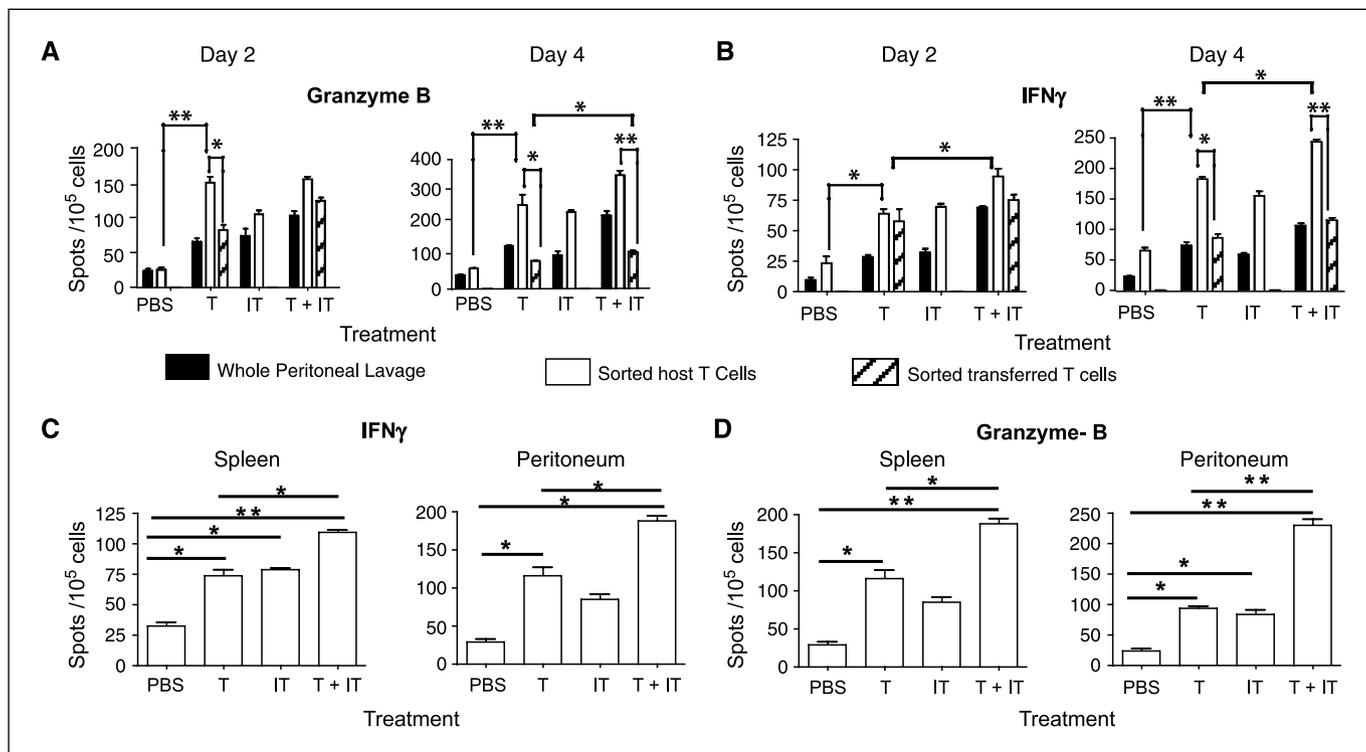

Figure 2. Elimination of CD11c⁺ cells enhances the efficacy of ACT (*, *P* < 0.05; **, *P* < 0.01). *T cells,* T-cell transfer; *IT,* anti-CD11c IT. *A,* experimental design. CD45.2⁺ mice were inoculated i.p. with tumor and treated on days 7 and 14 of tumor progression with 1.5 × 10⁶ CD45.1⁺ tumor-primed T cells (*T cells*). When relevant, tumor-bearing mice were depleted of CD11c⁺ cells with the anti-CD11c IT the day before the initial T-cell transfer and thrice weekly for 2 wk thereafter. *B,* ACT plus anti-CD11c IT, but not individual treatments, induced the regression of established i.p. ID8-luciferase tumors (*n* = 12 per group in two independent experiments). *C,* ID8-*Defb29/Vegf-a* tumor-bearing mice receiving ACT plus IT survived significantly longer than untreated mice or mice receiving individual treatments (*n* = 24 mice per group, total in four independent experiments). *D, in vivo* proliferation of transferred congenic (CD45.1⁺) T cells with (*T + IT*) or without (*T*) IT administration. The percentage of transferred cells at specific locations over time was determined (*n* = 8 mice per group in two independent experiments).
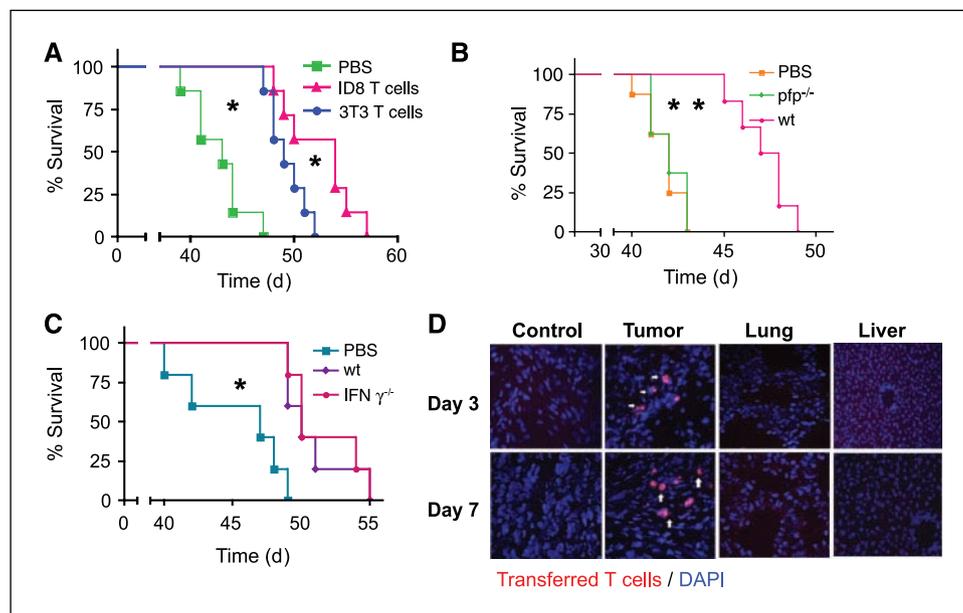
Figure 3. The therapeutic effects of ACT require perforin and antigen-specific priming, but not IFN-γ (*, $P < 0.05$; **, $P < 0.01$). *A,* survival of ID8-*Defb29/Vegf-a* tumor-bearing mice receiving T cells primed either against ID8-*Defb29/Vegf-a* tumor cell antigens (*ID8 T cells*) or irrelevant NIH-3T3 fibroblasts (*3T3 T cells*) was established (n = 8 mice per group). *B,* ID8-*Defb29/Vegf-a* tumor expanded splenic T cells from CD45.2⁺ perforin-deficient (*pfp⁻/⁻*) or wild-type (*wt*) mice were transferred into ID8-*Defb29/Vegf-a* tumor-bearing, congenic (CD45.1) mice (n = 16 mice per group in two independent experiments). Survival was determined. *C,* survival induced by IFN-γ–deficient (*IFN-γ⁻/⁻*) T cells (n = 16 mice per group in two independent experiments) was compared in an identical experiment. Mice were not irradiated in *B* or *C*. *D,* mice bearing 14-d-old flank ID8-*Defb29/Vegf-a* tumors received ID8-*Defb29/Vegf-a* primed red fluorescent T cells i.p., and immunofluorescent microscopy was performed (magnification, 200×; n = 6 mice per group).

therapeutic activity in this system (Fig. 3*A*; $P < 0.05$), comparable with that induced by naive lymphocytes (not shown). Nevertheless, tumor-primed T cells significantly prolonged survival compared with irrelevantly primed (NIH-3T3) T cells (Fig. 3*A*; $P < 0.05$), supporting the requirement for antigen specificity in eliciting robust therapeutic effects.

Transferred T cells also required tumor-specific cytotoxicity for successful ACT as expanded perforin-deficient T cells did not prolong survival in tumor-bearing hosts with (Supplementary Fig. S2*B*; $P < 0.05$) or without (Fig. 3*B*; $P < 0.01$) IT. The absence of effect was not caused by a functional deficit in perforin-deficient T cells, because they secrete similar amounts of IFN-γ as wild-type

Figure 4. Adoptive T-cell therapy induces host immune responses that are augmented by the elimination of CD11c⁺ cells (*, $P < 0.05$; **, $P < 0.01$). *T,* T-cell transfer; *IT,* anti-CD11c IT. Beginning on day 2 after T-cell transfer, peritoneal host (CD45.2⁺), and transferred (CD45.1⁺) T cells were independently sorted by fluorescence-activated cell sorting. Whole peritoneal wash samples, sorted host cells, and sorted transferred cells (n = 6/group in two independent experiments) were then analyzed by ELISPOT for secretion of (*A*) granzyme B and (*B*) IFN-γ in response to tumor antigen. IFN-γ (*C*) and granzyme B (*D*) ELISPOT analyses were performed on peritoneal wash samples and spleens obtained from mice at day 26 of tumor progression, when transferred T cells are no longer detectable (n = 9/group in three independent experiments).
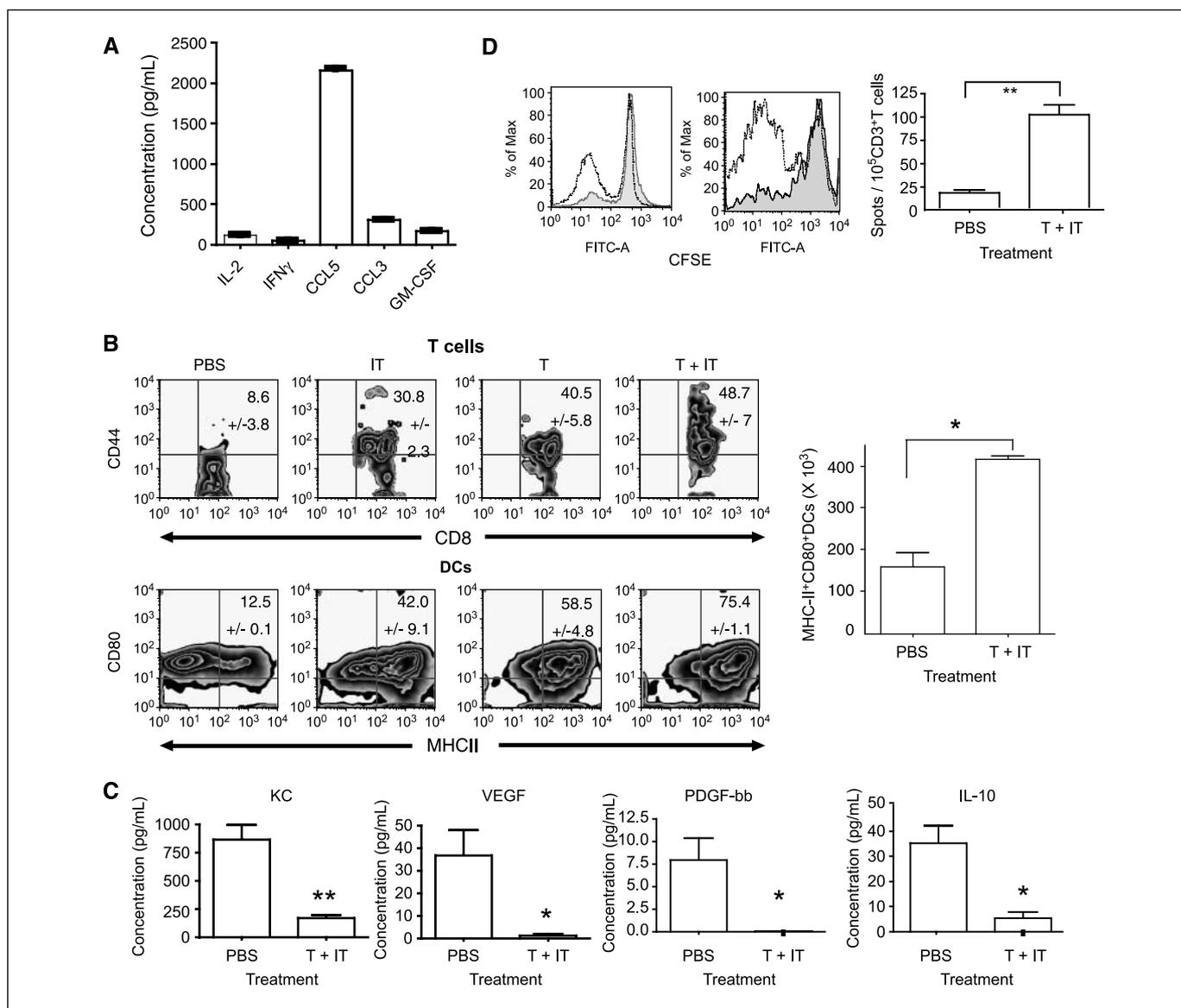
Figure 5. Expanded T cells secrete CCL5 and activate host immune cells (*, $P < 0.05$; **, $P < 0.01$, Mann-Whitney). *T*, T-cell transfer; *IT*, anti-CD11c IT. *A*, supernatants from naive splenic T cells primed for 7 d against ID8-*Defb29/Vegf-a* tumor cell antigens were examined by Luminex assays for cytokine production. *B, top,* phenotypic analysis of host (CD45.2+CD3+) T cells accumulated at the tumor site 3 d after ACT; *bottom,* expression profile of host CD11c+ (DCs) at tumor sites 3 d after treatment. Values represent mean percentages ± SEM. *Right,* quantification of MHCII+CD80+CD11c+ DCs in the peritoneum of mice treated with ACT and anti-CD11c IT (*T+IT*) compared with control mice. *C,* luminex analysis of phorbol 12-myristate 13-acetate/ionomycin–stimulated peritoneal MHCII+CD80+CD11c+ DCs from pretreatment and posttreatment mice. *D,* proliferation of CFSE-labeled magnetically purified allogeneic BALB/c splenocytes [*left*; proliferation indices, 2.84 (treated) versus 1.07 (untreated)] and CD8+ OT-1 splenocytes [*center*; proliferation indices, 4.68 (treated) versus 1.56 (untreated)] upon culture with sorted peritoneal MHCII+CD80+CD11c+ DCs from pretreatment (*shaded*) and posttreatment (*unshaded*) mice. *Right,* granzyme B ELISPOT analysis of CD3+ splenocytes from untreated tumor-bearing mice cultured with sorted peritoneal MHCII+CD80+CD11c+ DCs from pretreatment and posttreatment mice ($n = 9$ in three independent experiments).

T cells when cultured with tumor cells (29). In contrast, administering IFN-γ–deficient T cells prolonged survival in tumor-bearing mice as effectively as wild-type T cells with (Supplementary Fig. S2C; $P < 0.05$) or without (Fig. 3C; $P < 0.05$) IT.

De novo activated tumor-reactive T cells specifically homed to tumor locations, as tumor-primed fluorescently labeled T cells administered i.p. accumulated at distal (axilar) ID8-*Defb29/Vegf-a* tumors but were undetectable in other nonimmune organs at different temporal points (Fig. 3D). Together, these data indicate that the therapeutic activity of transferred T cells required selective tumor homing, tumor-specific priming, and perforin secretion but not IFN-γ production.

Adoptive administration of shortly primed tumor-reactive T cells boosts persistent tumor-specific host immune responses that are further augmented by depleting tumor-associated DCs. Although early effector T cells persist the longest *in vivo* (6), shortly primed tumor-reactive T cells started contracting after only 5 days in i.p. ovarian cancer–bearing hosts, supporting previous reports (11). Administered lymphocytes were undetectable at lymphatic locations, in the tumor or bone marrow after 12 days (Supplementary Fig. S2D). We therefore hypothesized that the persistent, long-lived therapeutic benefit elicited by ACT could be mediated by endogenous, host-derived antitumor immune responses rather than only resulting from direct killing by administered T cells.

Supporting this proposition, the number of endogenous (CD45.2[+]), host-derived granzyme B ($P < 0.01$) and IFN-γ ($P < 0.05$ at day 2; $P < 0.01$ at day 4) producing T cells was significantly higher in ACT-treated mice than untreated mice (Fig. 4A and B). Surprisingly, the number of endogenous (CD45.2^{+}) T cells producing IFN-γ and granzyme B in response to tumor antigens was significantly higher than their *ex vivo* primed transferred (CD45.1[+]) counterparts at early time points (Fig. 4A and B; $P < 0.05$). The activation of host antitumor T cells occurred within only 2 days of ACT, suggesting that the release from tumor-induced immunosuppression (awakening), rather than *de novo* priming, prompted these effects. Notably, exogenous and endogenous antitumor lymphocytes remained concurrently activated at tumor locations for at least 4 days after ACT. Most importantly, depletion of CD11c^{+} cells before ACT induced significantly higher numbers of endogenous (CD45.2^{+}) T cells producing IFN-γ and granzyme B in response to tumor antigens as early as 2 days after treatment (Fig. 4A and B; $P < 0.05$).

This awakening of endogenous antitumor immunity persisted longer than the direct effect of exogenous tumor-reactive T cells in tumor-bearing mice, because 18 days after their adoptive administration, after their elimination from the host, the number of endogenous, host-derived (tumor-associated and splenic) lymphocytes producing IFN-γ and granzyme B in response to tumor antigens remained significantly increased compared with untreated mice (Fig. 4C and D; $P < 0.05$). Depleting immunosuppressive tumor-associated DCs magnified this endogenous antitumor immune response elicited by ACT as shown by IFN-γ and granzyme B production 18 days after ACT (Fig. 4C and D; $P < 0.01$).

Confirming this awakening of endogenous antitumor immunity by ACT, tetramer analyses showed significantly increased proportions of host-derived (CD45.2^{+}), activated (CD69^{+}), and antigen-experienced (CD44^{+}) T cells specifically recognizing an H-2D[b]-restricted mesothelin epitope expressed by ID8 tumor cells (30) at splenic and tumor locations of ACT-treated mice and further increased by prior elimination of regulatory DCs (Supplementary Fig. S3A and B; $P < 0.05$ for both). Furthermore, the proportion of endogenous (CD45.2^{+}), tumor antigen (mesothelin)–specific T cells exhibiting a central memory (CD44^{+} CD62L^{+}) phenotype in the bone marrow was significantly increased in ACT-treated mice compared with untreated mice (Supplementary Fig. S3C; $P < 0.05$) and was augmented by depleting DCs ($P < 0.05$). Therefore, transferred T cells trigger durable and potent antitumor host responses that persist after their initial cytotoxic effect and subsequent disappearance, are partly mediated by endogenous memory T cells, and are significantly enhanced by regulatory DC depletion, resulting in prolonged survival.

Adoptive lymphocyte transfer induces the accumulation of activated host immune cells at tumor locations. To define the mechanisms mediating the host antitumor immunogenic boost elicited by ACT, cytokine and chemokine production by tumor-primed T cells were analyzed and high levels of the proinflammatory cytokine CCL5 were observed (Fig. 5A). CCL5 correlates with a better clinical outcome in ovarian cancer patients (14) and recruits lymphocytes and DCs through CCR5 and CCR8. Correspondingly, six times more host (CD45.2^{+}) T cells accumulated at tumor sites of ACT-treated mice compared with untreated mice (Supplementary Fig. S4A; $P < 0.01$). Leukocyte recruitment began 2 days after ACT and lasted up to 7 days later. Importantly, the recruitment of host T

cells induced by ACT was independent of the depletion of tumor-associated DCs, although the proportion of antigen-experienced (CD44^{+}) cytotoxic T cells in this host-derived population was augmented upon DC depletion (Fig. 5B, top; $P < 0.05$). Additionally, ACT significantly increased the activation of APCs at tumor locations (Fig. 5B, bottom; $P < 0.05$), specifically MHC-II^{+}CD80^{+}CD11c^{+} DCs. Thus, tumor-associated DCs expressed significantly higher levels of costimulatory CD86, CD70, and CD40 in mice treated with ACT + IT compared with untreated mice (Supplementary Fig. S4B; $P < 0.05$, $P < 0.01$). This increased APC maturation was not due simply to the elimination of immature DCs by IT administration, as IT administration occurred at least 2 days before analysis, allowing for repopulation, and did not completely eradicate host DCs (24). Moreover, the total number of activated APCs was significantly higher in mice receiving ACT + IT (quantification in Fig. 5B, right; $P < 0.05$).

Notably, CD45.2^{+}MHCII^{+}CD11c^{+} DCs sorted from treated mice secreted significantly lower levels of proangiogenic (KC, VEGF, and PDGF-bb) and immunosuppressive (IL-10) factors compared with untreated mice (Fig. 5C), supporting our previous observations surrounding the critical role of ovarian cancer–associated DCs in maintaining tumor vasculature and immunosuppression (24). Furthermore, these results suggested that posttreatment tumor-associated DCs are significantly more effective at amplifying immune responses against antigens spread by the direct cytotoxic activity of *ex vivo* activated T cells. Correspondingly, CD45.2^{+} MHCII^{+}CD11c^{+} cells sorted from (ACT + IT)–treated mice were significantly more effective than those from untreated mice at inducing the allogeneic expansion of CD3^{+} splenocytes (Fig. 5D, left; $P < 0.05$, proliferation indices). In addition, (host) tumor CD45.2^{+}MHCII^{+}CD11c^{+} DCs from treated mice induced greater antigen-specific proliferation of OVA-specific transgenic CD8 T cells (Fig. 5D, center; $P < 0.05$, proliferation indices). To test the actual therapeutic relevance of these findings, the capacity of sorted pretreatment and posttreatment CD45.2^{+}MHCII^{+}CD11c^{+} DCs to directly activate negatively purified CD3^{+} T splenocytes from untreated tumor-bearing mice was tested in ELISPOT analyses. Most importantly, the number of splenic T cells from untreated tumor-bearing mice producing granzyme B directly in response to unpulsed (returning) tumor-associated CD45.2^{+}MHCII^{+}CD11c^{+} DCs from treated mice was dramatically increased compared with that induced by tumor DCs sorted from control mice (Fig. 5D, right). Together, these data indicate that transferring tumor-specific lymphocytes combined with depleting regulatory tumor-associated DCs leads to the recruitment of more immunostimulatory and less proangiogenic host DCs that elicit host tumor-reactive T-cell activation.

Interestingly, irrelevantly (NIH-3T3 cells) primed T cells produced similar levels of CCL5 (Supplementary Fig. S5A) and initially recruited comparable numbers of T cells and DCs (Supplementary Fig. S5B). However, their ability to up-regulate the costimulatory CD80 on recruited CD11c^{+}MHC-II^{+} host DCs after ACT was significantly impaired compared with mice receiving tumor-primed T cells (Supplementary Fig. S5C; $P < 0.05$ for both). Correspondingly, mice receiving irrelevantly primed T cells had significantly diminished numbers of endogenous (CD45.2[+]) tumor-associated T cells producing IFN-γ in response to tumor antigens compared with mice treated with tumor-reactive T cells (Fig. 6A; $P < 0.05$). Together, these data indicate that adoptively transferred T cells need to be primed specifically against tumor antigens for the awakening of host-dependent

Figure 6. Tumor-specific priming and CCL5 are required for successful ACT. *A,* ELISPOT analysis of the number of sorted endogenous (CD45.2$^+$) T cells producing IFN-γ in response to tumor antigen 7 d after ACT with tumor-specific or irrelevantly primed T cells (*n* = 6 mice per group, total for *A* and *B* in two independent experiments). *B,* adoptive transfer of tumor-reactive T cells after anti-CD11c IT administration (*T + IT*) increases the total number of host CCR5$^+$ (CD45.2$^+$) CD3$^+$ T cells and CD11c$^+$ DCs (*n* = 6 in two independent experiments). *C,* administration of a neutralizing antibody to CCL5 concurrently with ACT, but not an irrelevant IgG, diminished the ability of transferred T cells to increase survival of tumor-bearing mice in the presence (*right*) or absence (*left*) of CD11c cell depletion (*P* < 0.05, *n* = 12 in two independent experiments).
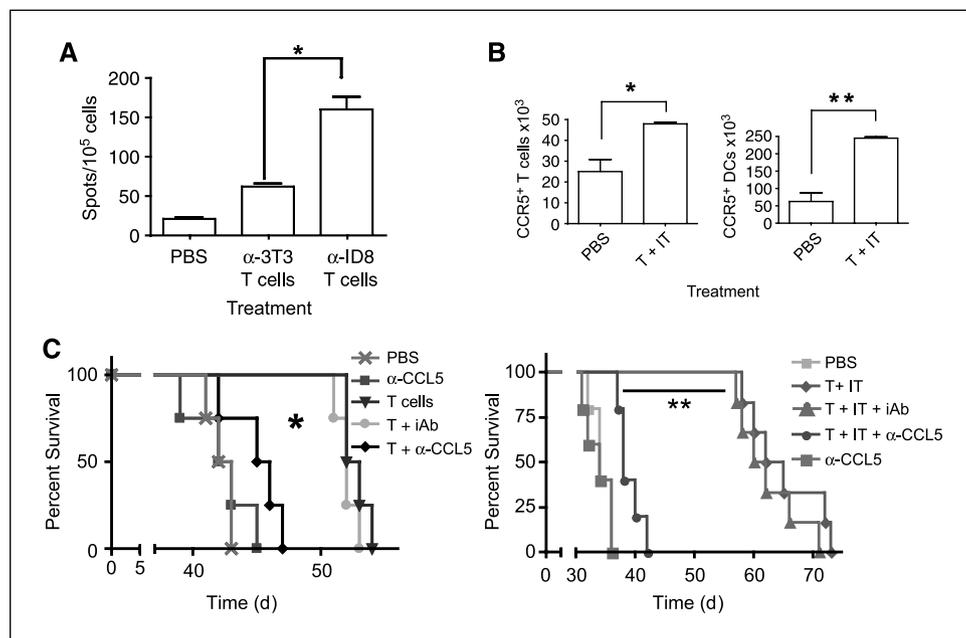
antitumor immune mechanisms, which are mediated by the accumulation of antigen-experienced T cells and activated APCs at tumor locations.

CCL5 is required for the therapeutic effects elicited by ACT. Because adoptively transferred T cells produced high levels of CCL5 and induced leukocyte recruitment, we next confirmed that most endogenous (CD45.2$^+$) tumor-associated lymphocytes and DCs in mice receiving ACT expressed surface CCR5 (Supplementary Fig. S5*D*). Additionally, ACT + IT significantly increased the number of host (CD45.2$^+$) T cells and DCs at tumor locations specifically expressing CCR5 compared with control mice (Fig. 6*B*; *P* < 0.05 and *P* < 0.01, respectively).

Because transferred T cells produce large amounts of CCL5 and induced the accumulation of CCR5$^+$ target cells, we hypothesized that CCL5 may also be required for the therapeutic effects triggered by ACT. Correspondingly, a neutralizing anti-CCL5 antibody injected concurrently with ACT significantly impaired the therapeutic effect (Fig. 6*C*; *P* < 0.05), whereas identical concentrations of an irrelevant IgG did not influence the effect of ACT (Fig. 6*C*; *P* > 0.05). Importantly, neither the neutralizing anti-CCL5 antibody nor the irrelevant IgG affected the survival of i.p. ID8-*Defb29/Vegf-a* tumor-bearing mice not receiving ACT (Fig. 6*C*; data not shown). Notably, this CCL5-dependent effect was also absolutely required for the beneficial effects observed with ACT combined with regulatory DC depletion, as eliminating CCL5 abrogated its therapeutic effect. Together, these data suggest that CCL5 produced by the transferred T cells, rather than endogenous CCL5, is at least partially required for the accumulation of host lymphocytes, which cooperatively function in delaying tumor progression.

Discussion

Here, we show that depleting regulatory DCs from the tumor microenvironment transforms tumor-reactive T-cell adoptive therapy from a palliative to a curative strategy in mice bearing ovarian cancers. Most importantly, long-term antitumor protection was mediated by endogenous CCL5-dependent host immune mechanisms, which remained activated after the disappearance of exogenously administered T cells.

The clinical effects elicited by adoptively transferred tumor-specific T cells in melanoma patients are usually attributed to their expansion and capacity to persist *in vivo*, but their ability to arouse host-derived protective immune mechanisms has not been addressed. Our ovarian cancer systems recapitulate recent findings suggesting that tumor-reactive T cells do not persist in patients with aggressive epithelial cancers, despite the fact that large numbers of lymphocytes can be safely administered (11). Strategies that maximize the awakening of host-mediated antitumor immunity, rather than the direct antitumor activity of transferred T cells, could be more valuable against these tumors.

Early effector T cells are more effective for *in vivo* tumor treatment than more differentiated effector T cells (6). Aiming to optimize a system for the early priming of tumor-reactive T cells that can be replicated using DCs generated from peripheral blood from ovarian cancer patients, we used DCs pulsed with doubly irradiated cancer cells. We hypothesized that polyclonal T cells would be better equipped to prevent the development of T cell–resistant tumor variants through immunoediting, than lymphocytes engineered to react against a single antigen. Surprisingly, we found that shortly primed lymphocytes from healthy or tumor-bearing mice induced comparable therapeutic effects, suggesting that naive peripheral blood T cells from ovarian cancer patients could also be converted into effective antitumor effectors through *de novo* priming.

Adoptively administered T cells rapidly expanded upon elimination of tumor-associated DCs but disappeared within 2 weeks. They required perforin, but not IFN-γ, for their therapeutic effects, supporting the requirement for cytotoxic killing. Shortly primed antitumor T cells also required tumor-specific recognition, as irrelevantly primed T cells induced reduced survival increases, despite producing comparable levels of measured cytokines/chemokines. The therapeutic effects induced by ACT were

abrogated by CCL5 neutralization. Correspondingly, tumor-reactive T cells induced rapid accumulation of CCR5^{+} antigen-experienced (CD44^{+}) T cells and antigen-presenting cells. Importantly, both tumor-specific and irrelevantly primed T cells produced similar levels of CCL5. However, the latter were inefficient at activating DCs translating to reduced accumulation of T cells at tumor sites and, correspondingly, their inability to stop tumor progression.

The blatant activation of host cells after ACT, as presented here, has never been previously reported. Antitumor T cells primed as described produced proinflammatory cytokines that recruit host immune cells to the tumor site. Tumor-reactive T cells, but not T cells expanded to irrelevant antigens, also induced the *in situ* maturation of host DCs and accumulation of antigen-experienced lymphocytes, as seen by their increased CD44 expression and production of IFN-γ and granzyme B. Combining ACT with regulatory DC depletion recruited antigen-presenting cells capable of stimulating T-cell expansion and their production of the cytotoxic granzyme B. Most importantly, host-derived lymphocytes acquired a memory phenotype, and their activity persisted after the elimination of exogenous lymphocytes. Therefore, long-term protection is mediated by host-dependent immune mechanisms rather than by the persistent effect of transferred T cells.

Because CCL5, perforin, and specific priming were required for successful ACT, we propose a mechanism whereby transferred lymphocytes briefly primed against tumor antigens mediate the specific lysis of tumor cells. Simultaneously, the release of large quantities of CCL5 by these cells precipitates the recruitment of host antitumor T cells and APCs. APCs mobilized to the tumor site become activated by tumor antigens made available by the perforin-mediated lysis and activate host T cells. Irradiation may further boost this process by facilitating the release of tumor antigen and providing space for tumor-reactive T-cell expansion. Although T cells expanded to irrelevant antigens secreted similar levels of CCL5, they were unable to effectively activate tumor-associated APCs. This implies that, whereas CCL5 is crucial for successful ACT, specific lysis of tumor cells is likely required to release antigens necessary to mature accumulated DCs and induce long-lasting antitumor effects.

In summary, our results show the feasibility of eliciting sustained, host-mediated, protective immunity against ovarian carcinoma by administering previously naive T cells shortly primed against tumor antigens after depleting tumor-associated regulatory DCs. These findings bring us closer to successfully using ACT therapies in ovarian cancer and beyond.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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