Tumors Suppress In Situ Proliferation of Cytotoxic T Cells by Promoting Differentiation of Gr-1+ Conventional Dendritic Cells through IL-6

Jun Diao, Jun Zhao, Erin Winter and Mark S. Cattral

*J Immunol* published online 23 March 2011
http://www.jimmunol.org/content/early/2011/03/21/jimmunol.1004125

Supplementary Material

http://www.jimmunol.org/content/suppl/2011/03/23/jimmunol.1004125.DC1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Tumors Suppress In Situ Proliferation of Cytotoxic T Cells by Promoting Differentiation of Gr-1+ Conventional Dendritic Cells through IL-6

Jun Diao,* Jun Zhao,* Erin Winter,* and Mark S. Cattral*†

Cancers are often accompanied by inflammation, which can promote tumor growth, invasion, and metastases. We show that the tumor microenvironment induces the development of a Gr-1+ conventional dendritic cell (cDC) subpopulation that is functionally defective. Gr-1+cDCs differentiated from recruited immediate precursors of cDCs, a process supported by the inflammatory cytokine milieu in tumors. Inhibition of Gr-1+cDC differentiation enhanced intratumor expansion of cytotoxic CD8+ T cells (CTLs), resulting in suppression of tumor growth. Diphtheria toxin treatment of CD11c–diphtheria toxin receptor chimeras revealed the importance of intratumor cDCs in stimulating CTL proliferation in situ. Our study demonstrates a key role of intratumor cDCs in determining antitumor CTL responses and suggests that they may be an appropriate target for tumor immunotherapy. The Journal of Immunology, 2011, 186: 000–000.

Inflammation is a well-recognized component of various types of cancer in patients and in animal tumor models (1). Altered expression of chemokines, cytokines, and growth factors in tumors contributes to an influx of infiltrating cells and generation of various stromal elements; in these respects, tumors resemble a chronic “smoldering” wound (2). Cellular stress, aberrant growth, and death of cancer cells generates many tumor-associated factors that can enhance inflammatory responses including high mobility group box 1, matrix proteins (fibronectin, heparan sulfate, versican), heat shock proteins, ATP, and uric acid (3–5). Tumor-associated inflammation promotes tumorigenesis, angiogenesis, local invasion, metastases, and evasion of immune surveillance (4, 6).

Dendritic cells (DCs) provide a critical link between innate and adaptive immune responses (7). DCs capture and process Ags in peripheral tissues and migrate to lymphoid tissues where they stimulate Ag-specific lymphocytes. In addition, recent evidence suggests that tissue DCs stimulate effector and memory T cells during vaccination (8–10). Whether these factors alone account for DC dysfunction has been challenged, however (14, 15). Much remains unclear about the nature of DCs in tumors, the mechanisms by which tumors inhibit their function, and how they affect antitumor T cell responses in vivo.

In this study, we show that the intratumor inflammatory milieu skews differentiation of conventional DC (cDC) toward a Gr-1+ subpopulation. This transformation altered the composition and immunogenicity of tumor cDCs and was tumor dependent. These changes influenced the proliferation and expansion of Ag-specific CTLs in tumors. Further, inhibition of Gr-1+ cDC differentiation increased intratumor CTL expansion and the success of adoptive T cell immunotherapy.

Materials and Methods

Mice

Male C57BL/6 and BALB/c mice were purchased from Charles River (Wilmington, MA). C57BL/6.SJL congenic mice, OT-I, OT-II, and CD11c–diphtheria toxin receptor (CD11c-DTR) transgenic mice, and IL-6−/− mice were originally purchased from The Jackson Laboratory (Bar Harbor, ME) or Taconic Farms (Germantown, NY) and bred in our animal facility. Mice were maintained in pathogen-free conditions in accordance with institutional guidelines and used at 2–3 mo of age. The Animal Research Committee of University Health Network reviewed and approved the studies.

Tumor models

B16-F10 melanoma (B16), Lewis lung carcinoma (LLC), and CT26 colon carcinoma were purchased from American Type Culture Collection. For some studies, we used B16 tumors that express the model tumor Ag OVA [B16-OVA; kindly provided by R.W. Dutton, Trudeau Institute (16)]. To establish tumors in mice, 0.5 × 106 to 1 × 106 tumor cells in 50 μl PBS were injected s.c. into the flank or i.v. The diameter of s.c. tumors was measured by caliper, and tumor size was calculated by multiplying ⅔ length by width.

Abs and cytokines

Adenoviral vectors encoding OVA or GFP were prepared, purified, and titrated as described previously (19).
mice by flow cytometry. CD11c-enriched cells dissociated from tumors were stained with Abs to lineage markers (CD3, CD49b, B220, and CD19), CD11c, and MHC class II (MHC II). Consistent with previous reports (14), these tumors contained few B220+ plasmacytoid DCs. We defined cDC in the Lin− population based on the expression of CD11c and MHC II. We found that tumor cDCs consisted of two subsets that could be distinguished by cell surface expression of Gr-1 (Fig. 1A), which is an epitope of Ly6C and Ly6G (21). Further phenotypic analysis revealed that neither subset expressed CD115, Ly6G, Mac-3, CD31, or CD45RB; some cells from both subsets expressed Ly6c; and most cells from both subsets expressed CD11b and F4/80 (Fig. 1B). This analysis indicated that Gr-1+ cDCs were distinct from Gr-1+ myeloid-derived suppressor cells (MDSCs), which do not express CD11c and MHC II subsets expressed CD11b and F4/80 (Fig. 1C). In normal mice, Gr-1+ cDCs were undetectable in lung, liver, spleen, thymus, bone marrow, s.c. and mesenteric lymph nodes, and Peyer’s patches. However, we found that Gr-1+ cDCs appeared in draining lymph nodes and spleen of tumor-bearing mice; the frequency of Gr-1+cDCs in lymphoid tissues correlated directly with tumor size (Fig. 1D) and increased markedly when they contained tumor metastases (Fig. 1E). We also found Gr-1+ cDCs in lungs with B16 or LLC tumor metastases (data not shown). These data suggested that Gr-1+cDCs represent a novel population of cDC that is abundant in transplantable tumors irrespective of tumor type and location and mouse strain.

Characterization of Gr-1+ cDCs and Gr-1− cDCs

To understand the nature of Gr-1+ cDCs, we compared tumor Gr-1+ cDCs with Gr-1− cDCs sorted from the same tumors. Although similar in shape and size, Gr-1+ cDCs exhibited fewer and shorter dendrites than Gr-1− cDCs (Fig. 2A). After overnight culture, about half of the Gr-1+ cDCs had adhered to the plastic culture well with an irregular cell shape, whereas most Gr-1− cDCs floated freely with typical DC morphology (data not shown). Cell survival at the end of culture was ~60% for both populations.

To compare the phagocytic capacity of Gr-1+ cDCs and Gr-1− cDCs, we incubated them with OVA conjugated to allophycoerycyanin and measured uptake at 2 h by flow cytometry. Gr-1+ cDCs and Gr-1− cDCs acquired similar amounts of OVA (Fig. 2B). This result agreed with our finding that both populations isolated from B16 tumors contained a similar number of melanin-derived granules in their cytoplasm (data not shown).

We compared the stimulatory capacity of Gr-1+ cDCs and Gr-1− cDCs in mixed allogeneic lymphocyte reactions. Freshly isolated Gr-1− cDCs, but not Gr-1+ cDCs, stimulated BALB/c lymphocyte proliferation. Overnight culture increased the stimulatory capacity of Gr-1− cDCs significantly but had little effect on the potency of Gr-1+ cDCs (Fig. 2C). We examined the expression of costimulatory molecules by flow cytometry (Fig. 2D). CD80 expression was higher in Gr-1− cDCs (61 versus 29%), whereas both populations expressed similar levels of CD86 and CD40. In addition, both expressed similar levels of the coinhibitor molecule PD-L1. Both populations upregulated cell surface expression of MHC II and CD86 after overnight culture, but this change was much less in Gr-1+ cDCs (Fig. 2E). We obtained similar results by culturing the cells with LPS and TNF-α (data not shown).

Freshly isolated Gr-1+ cDCs expressed more IL-10 constitutively than Gr-1− cDCs (22 ± 5.9% versus 2 ± 0.5%; n = 6, p < 0.001) (Fig. 2F). IL-12p40 expression was low but marginally higher in Gr-1+ cDCs (8.6 ± 0.5% versus 1 ± 0.8%). Neither population expressed IFN-γ, IL-6, or TNF-α, and ~25% of both populations expressed iNOS (data not shown). We also assessed cytokine levels in supernatants after 18-h culture and found that Gr-1+ cDCs spontaneously produced higher amounts of IL-10 than Gr-1− cDCs; this difference persisted even after treatment with LPS (Fig. 2G). IL-6 production by Gr-1+ cDCs and Gr-1− cDCs was undetectable in basal conditions but increased to similar levels after LPS stimulation (Fig. 2H). We did not detect TNF-α, TGF-β, IL-12p70, or IFN-γ in culture supernatants from either population with or without LPS stimulation (data not shown). These findings indicated that tumor Gr-1+ cDCs were distinct from inflammatory Gr-1+ “tip” DCs, which typically produce large amounts of TNF-α and iNOS (24).

To determine whether preferential production of IL-10 by Gr-1+ cDCs contributes to T cell hyporesponsiveness, we stimu-
lated OT-II T cells with OVA-pulsed Gr-1+ cDCs or Gr-1− cDCs in the presence or absence of a neutralizing Ab to the IL-10R. Consistent with previous data (Fig. 2C), Gr-1+ cDCs stimulated lower levels of OT-II T cell proliferation than those stimulated by Gr-1− cDCs (Fig. 2I). IL-10R blockade significantly increased proliferation of OT-II T cells stimulated by Gr-1+ cDCs but had no effect on OT-II T cells stimulated by Gr-1− cDCs. These results suggest that IL-10 produced by Gr-1+ cDCs plays a role in regulating CD4+ T cell responses.

Tumors induce pre-cDCs to differentiate into Gr-1+ cDCs

The distribution pattern of Gr-1+ cDCs suggested that the tumor microenvironment was critical for their development. Recent studies indicate that circulating pre-cDCs (Lin−CD11c+MHCIId−Gr-1+Flt3+Sirpα−) are an important source of tumor cDCs (14). To test whether tumors could induce recruited pre-cDCs to differentiate into Gr-1+ cDCs, we adoptively transferred CFSE-labeled bone marrow CD45.1 pre-cDCs into tumor-bearing CD45.2 congenic mice and analyzed their progeny 3 d later. Consistent with previous reports (14), ~60% of the cDCs generated from pre-cDCs had divided, as determined by CFSE dilution. We detected de novo Gr-1 expression in 16.3 ± 8% (n = 6) of the cDCs arising from pre-cDCs in LLC (Fig. 3A); similar results were obtained in mice bearing B16 tumors. In normal mice and in mice bearing small s.c. tumors, pre-cDCs generated exclusively Gr-1− cDCs in spleen and lung (Fig. 3B), whereas Gr-1+ cDCs could be detected in lungs with B16 or LLC tumor metastases (Fig. 3C). The frequency of Gr-1+ cDCs in lungs correlated directly with tumor burden; Gr-1+ cDCs constituted 4% of the total number of cDCs in lungs with 100–150 macroscopic (1- to 3-mm diameter) B16 tumors. These findings indicated that tumors can induce pre-cDCs and/or their cDC progeny to differentiate into Gr-1+ cDCs.

Tumor-associated cytokines regulate Gr-1+ cDC differentiation

To understand how tumors drive differentiation of pre-cDCs into Gr-1+ cDCs, we performed a cytokine and growth factor screen of normal lungs, lungs containing LLC tumor metastases, and LLC tumors. Compared with normal lung tissue, we identified increased expression of various cytokines and growth factors in lungs with tumors and in LLC tumors (Supplemental Fig. 1). The expression levels of IL-6, IL-1β, IL-3, IL-10, G-CSF, GM-CSF, and M-CSF showed the greatest increase. We next cultured pre-cDCs for 3 d with LLC tumor supernatants in vitro in the presence of the cytokines and growth factors that showed the greatest change in vivo and with cytokines known to affect DC development (e.g., TGF-β and IL-4) (Fig. 4A). About 20–25% of the cells expressed Gr-1 de novo with tumor supernatant alone; this percentage increased with the addition of IL-6, IL-1β, IL-10, and G-CSF and decreased with the addition of GM-CSF, IL-3, IL-4, TGF-β, and TNF-α. We found that adding a neutralizing anti–IL-6 Ab to culture medium containing tumor supernatant alone decreased the frequency of Gr-1+ cDCs by 50% (Fig. 4B) and improved their capacity to stimulate allogeneic lymphocyte pro-
liferation (data not shown); by contrast, blocking Abs to G-CSF, IL-1β, and IL-10 had little or no effect. We therefore focused our attention on assessing the relevance of IL-6 in Gr-1+ cDC development in vivo.

We found a 3-fold reduction in the frequency of tumor Gr-1+ cDCs in IL-6−/− mice, which was associated with an increase in the MHC II expression level by the total tumor cDC population (Fig. 4C, 4D). This response appeared to be confined to the tumor because the frequency and phenotype of cDCs in secondary lymphoid tissue were similar in IL-6−/− mice and wild-type mice, as reported previously (25) (Supplemental Fig. 2). The absence of IL-6 did not affect the intratumor frequency of CD4+ T cells, CD8+ T cells, and NK cells (Fig. 4E). We also found no difference in the frequency of MDCs or Foxp3+ T cells in tumors (data not shown). Collectively, these data indicated that the absence of IL-6 inhibits Gr-1+ cDC differentiation in tumors.

Gr-1+ cDCs induce defective T cell responses in vivo

Because Gr-1+ cDCs produce IL-10 and were poor stimulators of lymphocytes in vitro, we investigated the effect of tumor Gr-1+ cDCs on T cell responses in vivo. OVA-pulsed tumor Gr-1+ and Gr-1− cDCs were injected s.c. into mice that had received CFSE-labeled OT-II CD4+ or OT-I CD8+ T cells 24 h earlier. We recovered lymph nodes at 3 d to analyze OT-I and OT-II T cell proliferation by CFSE dilution and cytokine production after in vitro restimulation. OT-II cells from Gr-1+ cDC-injected mice proliferated less than those from Gr-1− cDC-injected mice (Fig. 5A). Moreover, primed (CFSE-diluted) OT-II T cells from Gr-1+ cDC-injected mice produced 3-fold less IL-2 and IFN-γ (Fig. 5B). The proliferative response of OT-I T cells mirrored that of OT-II T cells, but the differences in IFN-γ production were more striking (Fig. 5C, 5D). These data indicate that T cells primed by Gr-1+ cDCs in vivo are functionally impaired.

Tumor cDCs promote intratumor CTL proliferation

Recent studies indicate that cognate interactions between Ag-experienced T cells and tissue DCs can enhance T cell proliferation and function (8, 9). To investigate whether the frequency of tumor Gr-1+ cDCs influences CTL activity, we transferred OT-I CTLs into wild-type and IL-6−/− mice with B16 or B16-OVA tumors and recovered tumors, lymph nodes, and spleen at various time points for analysis. We found preferential accumulation of OT-I CTLs and a corresponding increase in IFN-γ+ CD8+ cells in B16-OVA tumors but not in control tumors; this difference increased further by 3- to 4-fold in IL-6−/− mice compared with that in wild-type mice (Fig. 6A–D). Spleen and lymph nodes in
of cell division at 7 d as determined by CFSE dilution, which was more extensive in IL-6−/− mice (Fig. 7C). Ag-specific OT-I CTL proliferation in lymph nodes and spleen was limited in both IL-6−/− and wild-type mice, suggesting that proliferation occurred predominately in the tumor. Using a 1-h BrdU pulse assay, we confirmed that a high proportion of CTLs in tumors were actively dividing at 7 d (Fig. 7D).

To investigate whether tumor cDCs contribute to CTL proliferation, we generated CD11c-DTR bone marrow chimeras, in which CD11c+ cells can be ablated by treatment with DT. Preliminary experiments revealed that a higher dosage of DT was required to deplete cDCs in tumors compared with that in lymphoid tissues (Supplemental Fig. 4 and Ref. 20). We assessed the rate of OT-I CTL proliferation 3 d after transfer in tumor-bearing CD11c-DTR and B6 chimeras that were treated with DT 1 d before and 1 d after OT-I CTL injection. DT treatment caused a 50–60% reduction in the frequency of tumor cDCs in CD11c-DTR chimeras (Fig. 8A). Despite this incomplete depletion, the level of OT-I CTL proliferation decreased significantly in B16-OVA tumors from CD11c-DTR chimeras compared with that in those from control chimeras (Fig. 8B). A low level of OT-I CTL proliferation persisted in the lymph nodes and spleen of both chimeras, indicating that OT-I CTL proliferation in these tissues is mostly autonomous and independent of the presence of DCs. Notably, activated CD8+ T cells express CD11c (26); however, the adoptively transferred OT-I CTLs are unaffected by DT because they lack DTR.

To establish further the involvement of tumor cDCs in CTL proliferation, we selectively sorted DC-depleted CD45+ infiltrating cells from B16-OVA tumors, stained them with CFSE, and cultured them together with or without sorted cDCs from the same tumor. We found that OT-I CTLs proliferated only in the presence of tumor cDCs (Fig. 8C). We considered the possibility that tumor cDCs were providing non-Ag-specific support for T cell proliferation; however, normal spleen cDCs failed to stimulate CTL proliferation unless they were pulsed with OVA peptide (data not shown). Tumor cDCs isolated from B16-OVA tumors in IL-6−/− mice stimulated higher rates of proliferation, consistent with the lower frequency of Gr-1+cDCs in these tumors. It was possible that the higher proliferative response to tumor cDCs from IL-6−/− mice might be related to differences in the suppressive activity of tumor myeloid cells or lymphoid cells contained in the DC-depleted population (27). However, we obtained the same results when DC-depleted tumor-infiltrating cells from IL-6−/− mice and wild-type mice were combined together and stimulated with sorted tumor cDCs from wild-type or IL-6−/− mice.

**Discussion**

Our study has revealed that cancer-associated inflammation changes the composition and immunogenicity of tumor cDCs by inducing the differentiation of Gr-1+cDCs. We show that the development of this subpopulation is associated with defects in antitumor T cell immune responses and uncover the unanticipated importance of tumor cDCs in the stimulation of CTL proliferation, a key determinant of intratumor CTL frequency and efficacy.

Many embrace the view that all cDCs in tumors are functionally defective. Our study shows that the impairment resides mainly in the Gr-1+c cDC population. Gr-1+c cDCs are indistinguishable from cDCs in normal lymphoid tissues with respect to their ability to capture Ag, mature, and stimulate T cell responses in vitro and in vivo. Gr-1+c cDCs first appear in tumors at an early stage of tumor development and subsequently increase in frequency in draining lymph nodes and spleen with tumor growth. Gr-1+c cDCs in lymphoid tissues are similar to those in tumors and likely arise
through migration from the tumor, the local effects of tumor metastases, and the systemic effects of soluble molecules released from tumors. Several characteristics of Gr-1+ cDCs help explain their suppressive activity: low expression levels of MHC II and costimulatory molecules, resistance to maturation stimuli, and constitutive production of IL-10. Gr-1+ cDCs differ from Gr-1+ MDSCs, a heterogeneous population of neutrophils, macrophages, and primitive myeloid cells that accumulates in tumors, bone marrow, and lymphoid tissues of tumor-bearing mice. MDSCs lack expression of CD11c and MHC II and directly suppress MLRs through the release of NO, arginase, and other metabolites (28–30). By contrast, neither Gr-1+ cDCs nor Gr-1+ cDCs can inhibit the stimulatory activity of third-party APCs in vitro (data not shown). Gr-1+ cDCs are also distinct from “tip” DCs, a specialized population of inflammatory Gr-1+ DCs that arise from monocytes during some inflammatory processes (24).

**FIGURE 7.** Increased proliferation of tumor Ag-specific CTLs in tumors from IL-6−/− mice decreases tumor growth and prolongs survival. A and B, Frequency of OT-ICTLs in B16 and B16-OVA tumors 7 d after adoptive transfer of CD45.1 OT-I CTL into wild-type and IL-6−/− CD45.2 mice. Numbers in dot-plots indicate percentage OT-I CTL in gate. Error bars are mean ± SD. Data are representative of >6 experiments. C and D, Frequency of IFN-γ+ CD8+ T cells in B16 and B16-OVA tumors 7 d after adoptive transfer of CD45.1 OT-I CTLs into wild-type and IL-6−/− CD45.2 mice. E, B16-OVA tumor size in wild-type and IL-6−/− mice that received OT-I CTL or control T cells 5 d after tumor inoculation. Data are representative of three independent experiments (n = 5 mice per group). *p < 0.01 (paired Student t test). F, Tumor-free survival of mice from E, p = 0.012 (log-rank test).
Pre-cDCs are considered the main immediate precursor for cDCs in lymphoid tissues under steady-state conditions, and recently their role in DC development has been extended to various organs and tumors (14, 17, 18, 31–34). By monitoring the fate of pre-cDCs in tumors and in tumor-conditioned medium, we confirmed that pre-cDCs generate Gr-1+ cDCs, which were similar in all respects to endogenous Gr-1+ cDCs. Pre-cDCs upregulate MHC II expression rapidly when cultured in vitro, and at this stage these replication-competent cDCs are Gr-1^- (18). Because Gr-1+ cDCs were undetectable in normal mice, we believe they signify a pathologic response of pre-cDCs and their immediate Gr-1^- cDC progeny to the inflammatory milieu in tumors. Thus, Gr-1^+ cDC transformation represents a tumor-controlled process that decreases cDC immunogenicity. Whether pre-cDCs are the only source for Gr-1^- cDCs is unclear. We found that monocytes, the most likely alternative precursor, fail to generate cDCs in tumors after adoptive transfer, suggesting that the tumor microenvironment either inhibits, or lacks key supportive signals for, DC differentiation.

Although pre-cDCs and cDCs integrate numerous signals in the tumor microenvironment, our study suggests that altering the expression of a single cytokine—IL-6—upsets the balance and hinders differentiation of Gr-1^+ cDCs. Other studies have shown that IL-6 regulates MHC II assembly in DCs by activating the transcription factor STAT3 (35, 36), and inhibits monocyte differentiation into DCs (37). IL-6 has been documented to be a factor in tumorigenesis and tumor progression in many animal tumor models (4, 38). High serum concentrations of IL-6 occur in patients with various epithelial and lymphoid cancers and correlate with poor outcome and abnormal immune responses (39, 40). In patients with hepatocellular carcinoma, low expression of microRNA-26a in the tumor is associated with high IL-6 expression and poor survival (41). Many tumor-associated molecules and metabolites act as endogenous “danger signals,” which can initiate and enhance expression of inflammatory cytokines and chemokines in tumor cells and host-derived infiltrating cells (42). Versican, an extracellular matrix protein that is upregulated in LLC tumors and some human cancers, stimulates macrophages to produce IL-6 by signaling through TLR2 and its coreceptors TLR6 and CD14 (4). Whether this mechanism participates in Gr-1^+ cDC differentiation remains to be defined.

The success of CTL therapy for cancer relies on achieving sufficient numbers of functional CTLs in the tumor (43, 44). Our study indicates that transferred CTLs undergo multiple rounds of cell division in tumors, which was tumor Ag dependent. A small proportion of CTLs divided in lymphoid tissues; however, this also occurred in the absence of cognate Ag, consistent with autonomous proliferation. CTLs in tumors and lymphoid tissues expressed similar levels of IFN-γ, suggesting that CTLs in tumors maintained functional competence. The antitumor efficacy of CTL treatment in IL-6^-/- mice was better than that in wild-type mice; this response correlated directly with a 4-fold higher frequency of CTLs in their tumors. Greater intratumor CTL proliferation appeared to be the main cause for the difference. Tumor Ags can be presented to intratumor T cells by tumor cells directly or by APCs via cross-presentation pathways (45). The DC depletion experiments in CD11c-DTR chimeras and the in vitro Ag presentation studies with tumor cDCs demonstrated a link between tumor cDC and CTL proliferation. Further, the in vitro studies indicated that tumor cDCs could stimulate CTL proliferation even in the presence of other myeloid and lymphoid cells contained in
References


**Supplementary Figure 1.** Cytokine profile of LLC tumors. Cell lysates from lung tissue containing LLC tumor metastases and subcutaneous LLC tumors were prepared, and the expression level of various factors was measured by cytokine arrays and compared to levels detected in normal lung tissue. Results are representative of 2 independent experiments with 3 mice per group.
Supplementary Figure 2. Phenotype of spleen cDC in IL-6−/− and wild-type of mice. Flow cytometry of freshly isolated spleen cDC from IL-6−/− and wild-type mice for the indicated markers. Shaded histogram represents isotype control. Numbers indicate percent cells. Data are representative of three independent experiments with 3 mice per group.
Supplementary Figure 3. The frequency of OT-I CTL in lymph nodes of wild-type and IL-6^{-/-} mice is similar. (A) Frequency of OT-I CTL in lymph nodes 7 d after adoptive transfer of CD45.1/CD45.2 OT-CTL into wild-type and IL-6^{-/-} CD45.2 mice bearing B16 or B16-OVA tumors. Numbers in dot-plots indicate percent OT-I CTL in gate. Data are representative of > 6 experiments. (B) Frequency of IFN-γ^{+} CD8^{+} T cells in lymph nodes 7 days after adoptive transfer of CD45.1/CD45.2 OT-CTL into wild-type and IL-6^{-/-} CD45.2 mice bearing B16 or B16-OVA tumors.
Supplementary Figure 4. Depletion of DC in tumors by diphtheria toxin. CD11c-DTR bone marrow chimeras bearing B16 tumors were injected once intraperitoneally with PBS or diphtheria toxin at a dosage of 4 ng or 100 ng per g body weight. Tumors and spleens were recovered 24 h later for flow cytometric analysis. The gated area in the dot-plots indicates CD11c+ MHC II+ cDC.