T Helper 17 Cells Promote Cytotoxic T Cell Activation in Tumor Immunity

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SUMMARY

Although T helper 17 (Th17) cells have been found in tumor tissues, their function in cancer immunity is unclear. We found that interleukin-17A (IL-17A)-deficient mice were more susceptible to developing lung melanoma. Conversely, adoptive T cell therapy with tumor-specific Th17 cells prevented tumor development. Importantly, the Th17 cells retained their cytokine signature and exhibited stronger therapeutic efficacy than Th1 cells. Unexpectedly, therapy using Th17 cells elicited a remarkable activation of tumor-specific CD8+ T cells, which were necessary for the antitumor effect. Th17 cells promoted dendritic cell recruitment into the tumor tissues and in draining lymph nodes increased CD8α+ dendritic cells containing tumor material. Moreover, Th17 cells promoted CCL20 chemokine production by tumor tissues, and tumor-bearing CCR6-deficient mice did not respond to Th17 cell therapy. Thus, Th17 cells elicited a protective inflammation that promotes the activation of tumor-specific CD8+ T cells. These findings have important implications in antitumor immunotherapies.

INTRODUCTION

CD4+ T cells, upon activation by antigen-presenting cells (APCs), differentiate into cytokine-expressing effector helper T (Th) cells, which are classified as Th1, Th2, Th17, and T follicular helper (Tfh) cell subsets on the basis of their cytokine secretion and immune regulatory function. Th17 cells produce the proinflammatory cytokines IL-17A, IL-17F, and IL-22 (Dong, 2008). As the signature cytokine of Th17 cells, IL-17A induces the expression of several chemokines (CCL2, CCL7, CXCL1, and CCL20) and matrix metalloproteinases (MMP3 and MMP13); transgenic overexpression of IL-17A in the lung provokes the induction of proinflammatory gene expression and tissue infiltration by leukocytes (Park et al., 2005). Conversely, inhibition of IL-17A signaling leads to impaired host defense against bacterial infection (Ye et al., 2001) and resistance to autoimmune diseases (Langrish et al., 2005; Nakae et al., 2003; Park et al., 2005; Yang et al., 2008).

Th17 cells and IL-17A expression have been found in various human tumors (Kryczek et al., 2007; Langowski et al., 2006; Miyahara et al., 2008; Sfanos et al., 2008; Zhang et al., 2008); however, their function in cancer immunity is unclear. IL-17A overexpression in tumor cell lines promotes angiogenesis and tumor growth when the tumors are implanted in immunodeficient mice, therefore suggesting a protumor activity (Numasaki et al., 2003). In contrast, the expression of IL-17A in a hematopoietically derived tumor was reported to promote tumor protection in immunocompetent hosts (Benchetrit et al., 2002). The basis for this discrepancy has not been understood, and the presence or absence of the adaptive immune system has been suggested to account for it (Martin-Orozco and Dong, 2009). Th17 cells highly express IL-23R; IL-23 is required for the late stage of Th17 cell development and also functions to expand Th17 cells and promote their function (Langrish et al., 2005; McGeachy et al., 2009). IL-23a (p19) mRNA expression has been found in several human carcinomas (Langowski et al., 2006). Moreover, IL-23-deficient mice (Il23a−/− and Il12b−/−) have been reported to be resistant to chemically induced tumors (Langowski et al., 2006). Paradoxically, the expression of IL-23 at the tumor site or therapy with dendritic cells expressing IL-23 can induce potent tumor-specific immunity against melanoma and glioma (Hu et al., 2006; Overwijk et al., 2006). More recently, it was shown that Th17 cells could protect against skin melanoma in a lymphopenic environment (Muranski et al., 2008); however, because the protection was dependent on IFN-γ, presumably because of conversion of Th17 to Th1 cells, the exact function of Th17 cells remains unclear.

In the current study, we first analyzed tumor development in IL-17-deficient mice by using a poorly immunogenic B16-F10 melanoma that colonizes the lung. Additionally, we used adoptive transfer of Th17 cells in several tumor prevention and treatment models. Our results indicate that IL-17A and Th17 cells play a protective role against tumors. Unexpectedly, tumor-specific Th17 cells triggered a strong CD8+ T cell response against the tumor. Th17 cell therapy promoted dendritic cell (DC) infiltration into tumor tissues and presentation of tumor antigens in the tumor-draining lymph nodes. Compared to Th1 cells, Th17 cells strongly induced CCL20 expression in the tumor tissues and CCR6 deficiency abrogated the antitumor effects of Th17 cells.
T Helper 17 Cells Promote Antitumor Immunity

Enhanced Tumor Growth in the Absence of IL-17

To investigate the role of IL-17 in tumor development in vivo, we challenged IL-17A-deficient mice (Yang et al., 2008) and wild-type (WT) age-matched controls on 129xB6 mixed background with B16-F10 melanoma injected intravenously. On days 14 and 16 after the challenge, Il17a−/− mice exhibited increased numbers of tumor foci and larger tumors in size when compared to WT mice (Figure 1A and Figure S1A available online). Consistently, Il17a−/− mice that had been backcrossed to the C57BL/6 background also exhibit increased tumor burdens when compared to WT C57BL/6 mice (Figure S1B).

Figure 1. IL-17-Deficient Mice Are More Susceptible to B16-F10 Melanoma Development in the Lung

Il17a−/− (KO) and wild-type (WT) mice were challenged i.v. with 1 × 10^5 B16-F10 melanoma cells and lungs were analyzed on days 14 and day 16.

(A) Graphs show the total number of tumor colonies present in the lung lobes (n = 4, average ± SD).

(B–E) At day 16, lung leukocyte and lung cell fractions were isolated and processed for FACS analysis or RNA extraction.

(B) Total leukocyte cell numbers from lungs. The numbers were calculated from the percentages of total live cells that were gated on CD45+ cells (n = 4, average ± SD).

(C) Expression of CD44 on CD45+CD4+ T cells from the leukocyte fraction.

(D) Myeloid populations (n = 4, average ± SD).

(E) Chemokine and chemokine receptor gene expression analyses from lung fractions, which were free of leukocytes, was performed by RT-PCR. Data shown were normalized to the reference gene Actb. The lower expression of each gene was referred as 1. Shown are the averages of four mice after duplicate analysis per sample (n = 4, average ± SD). Results shown are from a representative experiment of three, each using 4–5 mice per group. (* = p < 0.05, ** = p < 0.01).

Our results thus reveal a protective function of Th17 cells in tumor immunity by eliciting cytotoxic T cell activation.

RESULTS

Enhanced Tumor Growth in the Absence of IL-17

To further understand the function of Th17 cells in tumor protection, possibly by regulating chemokine-mediated leukocyte migration into tissues.

Antitumor Th17 Cells Reduce Tumor Growth in Prevention Models

To further understand the function of Th17 cells in tumor immunity, we used a B16-F10 line that expresses chicken ovalbumin (B16-OVA) and performed adopted transfer experiments with OVA-specific Th17 cells. CD4+ T cells purified from CD45.1 OT-II transgenic mice were differentiated into Th17 effector cells in vitro (Chung et al., 2009; Nurieva et al., 2009). In each experiment, the differentiated Th17 cells typically contained >35% IL-17A- and/or IL-17F-expressing T cells, with <2% IFN-γ-producing cells as evaluated by intracellular cytokine staining (Figure 2A). First, we transferred Th17 cells on the same day as B16-OVA tumor challenge. Mice treated with Th17 cells contained significantly (p = 0.014) reduced numbers of tumor colonies in the lung on day 16 compared to control mice that had not received any T cells (Figure 2B and Figure S2A). The donor cells were detected in substantial percentages in the lung (average 15% ± 3% SD) and secondary lymphoid organs at the end point of the experiment, suggesting
that Th17 cells did not suffer deletion and were circulating in the mice harboring tumors (Figure S2C). To substantiate the above finding, we used the BW TRP-1 TCR transgenic mouse, in which CD4+ T cells recognize the tyrosinase-related protein 1 (TRP-1), a melanocyte differentiation antigen present in normal melanocytes and in melanomas (Muranski et al., 2008). Transfer of TRP-1 Th17 cells at the time of the B16 tumor implantation completely inhibited the tumor growth in the lung (Figure 2C and Figure S2B). From these results, we conclude that tumor-specific Th17 cells, regardless of their antigenic specificities, can protect mice from developing B16-F10 lung melanoma.

To understand how Th17 cells mediate tumor protection, we analyzed the cellular composition of the lungs from the mice described above. OVA-specific Th17 cell-treated tumor-bearing mice had higher numbers of CD45+ leukocytes as well as CD4+ and CD8+ T cells in the lungs compared to the control mice (Figure 2D and Figure S2C) and mice receiving TRP-1-specific Th17 cells had five times more CD8+ T cells over the control animals (Figures S3A and S3B). Interestingly, CD4+ and CD8+ T cells from the mice receiving Th17 cells showed higher CD44 expression than control mice (Figure 2E and Figures S2E and S3C). When we analyzed the antigen-presenting cells from leukocyte fractions, we found that Th17 cell-recipient mice also had increased numbers of CD11c+CD11b+ and CD11c+CD8α+ DCs and granulocytes, whereas the numbers of macrophages were not altered (Figure 2F and Figure S3D). Therefore, transferred Th17 cells induced the recruitment of DC as well as activated CD4+ and CD8+ T cells to the lung. To understand the inflammatory regulation by Th17 cells, we further analyzed the expression of several chemokines and chemokine receptor genes by lung cells using real-time PCR. We analyzed nonfractionated total lung cells (total lung), leukocytes, and leukocyte-depleted lung cells. We found that the expression of Ccl20 and Ccl2 was greatly increased in lungs from Th17 cell-treated mice (Figure 2G), whereas Ccl7 and Cxcl1 (Grov) expression was not increased. Further analysis after cellular fractionation revealed that the leukocyte fraction from both Th17 cell-treated and control mice expressed similar levels of chemokines and chemokine receptors (Figure 2G); however, the leukocyte-free lung cells of Th17 cell-treated mice showed greatly increased expression of Ccl20 and Ccl2 compared to those from control animals (Figure 2G). These results suggest that Th17 cells in the lung might activate lung and/or tumor cells to produce chemokines CCL20 and CCL2 that promoted the recruitment of DCs and activated T cells.

To determine whether tumor-specific Th17 cells protect against tumors in different tissues other than lung and also to test a different tumor model, we applied a subcutaneous melanoma model and a subcutaneous fibrosarcoma model. Equal numbers of either Th1 or Th1 OT-II cells were transferred into C57BL/6 mice on the same day when B16-OVA or MCA205-OVA cells were implanted subcutaneously. Th17 cells greatly reduced growth of both B16 and MCA 205 in the skin, but Th1
cells had very minimal effects when compared with mice receiving no T cells (Figures S4A and S4C). In the MCA model, there was no survival advantage of Th17 over Th1 cells because similar numbers of CD45.1 donor cells were recovered from draining lymph nodes on day 24 (Figures S4D–S4F). Taken together, these results indicate that Th17 cells can induce effective antitumor responses for several tumors types and organ locations in preventive settings. Th17 cells induce lung cells to produce CCL2 and CCL20, resulting in DC and activated T cell recruitment.

**Therapeutic Effects of Th17 Cells in Mice with Established Tumors**

We then tested whether the transfer of Th17 cells could also help eliminate established tumors. We compared Th17 and Th1 cells in their protection against 5 day established pulmonary melanomas. Both Th17 and Th1 cell cytokine production were confirmed by ICS (Figure S5) before transfer, and equal numbers of cells were injected in C57BL/6 mice harboring B16-OVA. Compared with untreated control mice, those treated with Th1 cells had 40% fewer tumor foci, whereas Th17 cell-treated ones showed 75% reduction (Figure 3A). Therefore, both Th1 and Th17 cell therapies helped control tumor growth, with Th17 cells showing greater potency. When we analyzed cytokine production by ICS after activation with OVA323-339 peptide, we found that both Th1 and Th17 cells maintained their cytokine profiles throughout the experiment (data not shown).

To compare the inflammatory responses caused by Th1 and Th17 cell therapy, we analyzed the lung infiltrates of all experimental mice. There was increased recruitment of CD4+ T cells in the lungs of mice receiving Th1 or Th17 cells (Figure 3B). These mice also had increased numbers of CD4+ T cells in the lung lymph nodes (LLNs). Both Th1 and Th17 cell-treated mice had activated CD4+ and CD8+ T cells in lung and LLNs with increased expression of CD44 (Figure 3C). However, only Th17 cell-treated mice showed 75% reduction (Figure 3A). Therefore, both Th1 and Th17 cell therapies helped control tumor growth, with Th17 cells showing greater...
populations showed that Th17 cell treatment elicited the greatest infiltration of granulocytes, macrophages, and DC, whereas Th1 cell treatment showed slightly increased DC but reduced macrophage numbers when compared to the control mice (Figure 3D). In addition, mice receiving Th17 cells had increased numbers of leukocytes in LLNs, particularly CD8⁺ DCs (data not shown and Figure S6B).

Our data suggested that Th17 and Th1 cells induced different antitumor inflammatory responses in the lung. We thus analyzed the chemokine and chemokine receptor expression of the lungs and leukocytes from lungs of mice treated with Th17 or Th1 cells. Elevated expression of Ccl20 and Ccl2 mRNA by the lungs was present only in Th17 cell-treated mice but not in Th1 cell- or control-treated mice (Figure 3E). Ccl20 and Ccl2 expression was predominant in lung cells depleted of leukocytes, which indicated a direct effect of Th17 cells on lung cell production of chemokines. Th1 cell-treated lungs showed a reduced expression of Ccl7. Ccl20 was highly expressed in the leukocyte fractions from both Th17 and Th1 cell-treated mice, but it was dominant in Th17 cell-treated mice (Figure 3E). Ccr6 expression was slightly higher in leukocytes from Th1 cell-treated mice compared with control mice but was highly increased in Th17 cell-treated mice. Taken together, these results indicate that Th17 cells can诱导 an effective antitumor responses for established tumors. Also the antitumor Th17 cell-induced response is different and more effective than the one induced by Th1 cells in the lung.

**Th17 Cells Maintain Their Phenotypes In Vivo**

We recently demonstrated that adoptive cell therapy with Th17 cells together with total body irradiation in mice was protective against subcutaneous melanoma; this effect of Th17 cells was dependent on IFN-γ but independent of IL-17 and IL-23 for their protective effects (Muranski et al., 2008). This study suggests a possible conversion of Th17 to Th1 cells upon transfer into the irradiated hosts. Our above data on Th17 versus Th1 cell function in nonirradiated tumor-bearing mice suggested that Th17 cells might not require conversion to Th1 cells to exert their antitumor function in a normal, nonlymphopenic environment. To determine whether Th17 cells were the direct cause of the tumor protection, we further purified Th17 cells by using our IL-17F-RFP reporter mice (Yang et al., 2008) and tested their capacity to protect mice with established B16 lung melanoma. CD4⁺ T cells from OT-II⁺ IL-17F-RFP mice were cultured in Th17 cell-polarizing conditions and on day 4, live CD4⁺RFP⁺ T cells were sorted (Figure 4A) and transferred into mice bearing lung tumors. We found that both total and sorted RFP⁺ Th17 cells significantly (p = 0.00309 and p = 0.018) reduced the number of tumor foci on day 1 and every other day until day 14 after tumor challenge. Shown are the tumor colonies present in the lung lobes of each group of mice (n = 4, average ± SD) (** p < 0.01).
seems IFN-γ promotes the activation or recruitment of tumor antigen-specific CD8+ T cells in the lung, suggesting that Th17 cells may promote the activation or recruitment of tumor antigen-specific CD8+ T cells. In the B16-OVA tumor model, we searched for endogenous CD8+ T cells reactive against the SIINFEKL peptide derived from OVA protein by using a K b tetramer (OVA-tet). In the lungs of mice that received OT-II-Th17 cells, there was a distinct population of CD8+ OVA-tet+ T cells, ~10% and 5% of total CD8+ T cells in the prevention or therapeutic tumor model, respectively. Such OVA-tet+ T cells were absent in untreated control mice (Figures 5A and 5B). In contrast, Th1 cells elicited a small population of OVA-tet+ T cells in the lung (1.17% ± SD 0.07%) (Figure 5B). We also detected an increased number of OVA-tet+ T cells in mice with flank B16-OVA tumors that were also treated with Th17 cells (Figure S4B). Altogether, these results suggest that Th17 cells “help” the activation of endogenous antitumor CD8+ cells and also promote their subsequent localization to the tumor sites.

To analyze whether CD8+ T cells mediated the observed tumor protection by Th17 cells, we depleted CD8+ T cells in tumor-bearing mice before they were treated with Th17 cells. Anti-CD8 was injected on day 4 after B16-OVA tumor challenge and every 4 days until the end point. The CD8+ T cell depletion efficiency was confirmed in blood- and lung-derived cells and reached 95% and 86%, respectively. Depletion of CD8+ T cells reduced tumor protection mediated by Th17 (from 75% to 40% reduction of tumor foci), resulting in a degree of protection similar to that of Th1 cells without CD8 depletion (Figure 5C). Therefore, CD8+ T cells in large part mediate the tumor protection conferred by Th17 cells.

To further assess CD8+ T cell regulation by Th17 cells, we labeled CD8+ OT-I T cells with CFDA-SE and transferred them alone or together with Th17 OT-II cells into mice bearing lung tumors for 5 days. OT-I T cells, when cotransferred with Th17 cells, proliferated more extensively in LLNs with at least three more cell divisions than those transferred alone (Figure 6A). However, OT-I cells proliferated similarly with or without Th17 cell partners in lung or spleen, suggesting that Th17 cell transfer enhances CD8+ T cell priming only in LLNs. In addition, the LLNs from mice receiving Th17 cells, there were increased numbers of OT-I T cells producing IFN-γ accompanying their cell division, with ~30% cells in the fifth division producing IFN-γ. OT-I T cells transferred alone lost their cytokine expression during their minimal divisions with only 10% IFN-γ+ T cells in the third division (Figure 6B). In contrast, cotransfer of Th1 cells did not influence OT-I cell division or cytokine production of OT-I cells in LLN (Figure S7A). These data demonstrate that Th17 cells promote CD8+ T cell proliferation while sustaining their cytokine expression capacities.

Moreover, we injected IFN-γ neutralizing antibodies 1 day before the transfer of Th17 cells and every other subsequent day until the endpoint. The same IFN-γ antibody regime has been tested and reported by us to inhibit Th1 cell-mediated type I diabetes (Martin-Orozco et al., 2009). We found that blockade of IFN-γ did not alter the tumor-protective effect of Th17 cells (Figure 4D). Moreover, the anti-IFN-γ treatment did not reduce the numbers of total CD45+ leukocytes or CD4+ or CD8+ T cells in the lung (Figure 4E). There was also no change in the DC populations in the lungs of Th17 cell-treated mice as a result of the IFN-γ blocking antibody (Figure 4F). Therefore, it seems IFN-γ is not involved in the recruitment of leukocytes to the lung nor does it play a role in the tumor-protective effect elicited by Th17 cells. Our results indicate that the Th17 cell tumor-protection effect was not dependent on these cells converting to a Th1 cell phenotype.

### Th17 Cells Enhance the Activation of Tumor-Specific CD8+ T Cells

Although Th17 cells have potent protective effect against B16 melanoma in vivo, we did not find any proapoptotic effect of IL-17 on B16 cells cultured in vitro (data not shown), suggesting that Th17 cells do not directly kill melanoma cells. In Th17 but not Th1 cell-treated tumor-bearing mice, we observed increased numbers of CD8+ T cells in the lung, suggesting that Th17 cells may promote the activation or recruitment of tumor antigen-specific CD8+ T cells. In the B16-OVA tumor model, we searched for endogenous CD8+ T cells reactive against the SIINFEKL peptide derived from OVA protein by using a K b tetramer (OVA-tet). In the lungs of mice that received OT-II-Th17 cells, there was a distinct population of CD8+ OVA-tet+ T cells, ~10% and 5% of total CD8+ T cells in the prevention or therapeutic tumor model, respectively. Such OVA-tet+ T cells were absent in untreated control mice (Figures 5A and 5B). In contrast, Th1 cells elicited a small population of OVA-tet+ T cells in the lung (1.17% ± SD 0.07%) (Figure 5B). We also detected an increased number of OVA-tet+ T cells in mice with flank B16-OVA tumors that were also treated with Th17 cells (Figure S4B). Altogether, these results suggest that Th17 cells “help” the activation of endogenous antitumor CD8+ cells and also promote their subsequent localization to the tumor sites.

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To confirm that IL-17 can influence CD8+ T cell priming, we transferred OT-I cells labeled with CFDA-SE into WT or IL-17A/C0/C0 mice that had been inoculated with B16-OVA tumors. We found that on day 4 after transfer, Il17a/C0/C0 mice had reduced numbers of CFSE-labeled OT-I cells in LLNs (Figure 6C), with lower production of IFN-γ when compared to WT mice (Figure 6D). This result confirms that IL-17 influences the priming of CD8+ T cells to generate IFN-γ-producing effector cells. Taken together, these results indicate that Th17 cells induce a strong antitumor CD8 response that participates in the elimination of the tumor by favoring priming of tumor antigens in tumor-draining lymph nodes.

**Th17 Cells Regulate DC Function in a CCR6-Dependent Manner**

We further investigated the basis of Th17 cell “help” to CD8+ T cells. Th17 cell cytokines could possibly act on CD8+ T cells directly during priming. However, we did not observe an effect on CD8+ T cell proliferation and cytokine production when they were activated in vitro in the presence of IL-17A, IL-17F, or Th17 cell supernatants (data not shown). Alternatively, Th17 cells could indirectly influence CD8+ T cell priming by improving presentation of tumor antigens by DCs. Previous studies have reported that therapy with DCs loaded with class I peptides or dead tumor cells protected mice against established B16 solid tumors (Goldszmid et al., 2003; Lou et al., 2004). Consistent with this idea, we have found that CD8α+ DC doubled in percentages in the lung as early as 3 days after Th17 cell transfer, and such a result did not occur with Th1 cells (Figure S7). Therefore, we decide to assess this hypothesis by using B16 melanoma that coexpresses OVA and GFP and identify DC uptake of tumor materials and migration to LLNs. Mice were injected with B16-OVA-GFP and Th17 or Th1 cells, and 72 hr later, LLN were harvested for flow cytometry analysis of DC uptake of GFP. High levels of GFP were found in CD8α+DCs from mice that received Th17 cells, and this signal was higher than Th1 cell-treated mice or controls (Figure 7A). GFP in CD11b+ DCs from mice treated with either Th1 or Th17 cells was also higher than in control mice (Figure 7A). Moreover, given that the total numbers of DCs present in LLNs were similar, the total number of CD8α+ DCs...
containing GFP was ten times more in mice treated with Th17 cells than in control or Th1 cell-treated mice (Figure 7B). Therefore, Th17 cells provoked inflammation in the lung that resulted in increased numbers of DCs in the lung and DCs containing tumor materials in LLNs, resulting in improved antitumor CD8+ T cell priming.

Interestingly, we also found that both CD11b+ and CD8α+ DCs expressed CCR6 on their surface; however, there was only increased CCR6 expression on CD8α+ but not CD11b+ DCs from mice treated with Th17 cells compared to those from the control mice (Figure 7C). Given that Ccl20 expression in tumor tissues was enhanced by Th17 cells, we implanted B16-OVA tumor in CCR6-deficient (Ccr6−/−) mice and treated them with OT-II Th17 cells. Tumor colonies in the lung of Ccr6−/− mice grew similarly to those in WT mice, whereas Ccr6−/− mice did not respond to treatment with Th17 cells and developed similar numbers of tumor colonies as the untreated mice (Figure 7D). Furthermore, CD8α+ DC numbers were only increased in the LLNs of WT mice treated with Th17 cells but not in CCR6-deficient mice (Figure 7D). The total CD4+ and CD8+ T cells and CD8α+ DCs in the lung of Ccr6−/− mice were not increased by Th17 cell treatment (Figures S8A and S8B). However, there was an increased number of GR1+, CD11b+ DCs and macrophages in Ccr6−/− mice treated with Th17 cells. Therefore, we conclude that CCR6 is required for the response to Th17 cell therapy; the signaling through CCL20 may allow CD8α+ DCs loaded with tumor antigens to prime antitumor CD8+ T cells in LLNs.

**DISCUSSION**

Although Th17 cells have been found in human tumors, their physiological function in tumor development has been poorly defined. By using IL-17-deficient mice in a model of lung melanoma, we have provided direct evidence for a protective role of IL-17 in antitumor responses. Moreover, we show that the adoptive transfer of tumor-specific Th17 cells protect against various tumors and, in the lung melanoma model, promote tumor-specific cytotoxic T cell responses.

The function of IL-17 in tumor immunity has been a controversial subject. The effects of IL-17 on tumor development are directly influenced by the existence of an adaptive immune system—in the presence of lymphocytes, IL-17 promotes tumor rejection, whereas in the absence of them, IL-17 favors tumor...
growth and angiogenesis (Martin-Orozco and Dong, 2009). This notion is consistent with our current data on Il17a−/− mice and adoptive transfer of Th17 cells. Our current data also indicate an active role of IL-17 in tumor immunosurveillance; IL-17 deficiency resulted in reduced leukocyte infiltration into the target tissue, and these mice were more susceptible to the tumor development. Also, antitumor CD8+ effector T cell differentiation was compromised in IL-17-deficient mice. In contrast to our results, IL-23, important in Th17 cell regulation, has been shown to promote tumor growth and prevent immunosurveillance (Langowski et al., 2006). There are two possibilities to account for these discrepancies. First, other cells or factors regulated by IL-23 may have a distinct function in cancer immunity. It has been recently described that IL-23 produced by macrophages activates STAT-3 in the macrophages and Treg cells and promotes tumor growth (Kortylewski et al., 2009). Second, it is also possible that the IL-23-IL-17 axis of inflammatory responses may have differential functions in different settings; its regulation of chronic inflammation may provide a supportive role for certain types of tumors. Short acute inflammation associated with anti-tumor response might help the fight against the tumor (Overwijk et al., 2006). Further studies on different tumor models may reveal the complex relationships between IL-17-IL-23, inflammation, and cancer.

In our current study, we found that Th17 cells provide better protection to tumors than Th1 cells, and this difference was largely due to their unique ability to promote CD8+ T cell priming. Because anti-IFN-γ did not influence the protective immunity mediated by Th17 cells against tumors, CD8+ T cells may kill tumors independent of this cytokine, possibly by utilizing the cytolytic enzymes. It was also recently shown in subcutaneous B16 melanoma that transfer of antitumor CD4+ Th17 cells together with whole-body irradiation, vaccination, and IL-2 treatment could control tumor growth. However, the effect of Th17 cell vaccination was dependent on IFN-γ and independent of IL-17 and IL-23 (Muranski et al., 2008). We believe that in a lymphopenic environment, conversion of Th17 to Th1 cells occurs; however, Th17 cells maintain their phenotypes in normal hosts in the presence or absence of inflammation (Martin-Orozco et al., 2009; Nurieva et al., 2009). Although large numbers of Th1 cells did provide some degree of tumor protection in our model as well, our Th1 donor cells were more effective in several tumor models tested while maintaining their Th17 cell cytokine expression profiles. Additionally, when Th17 cells were purified with the RFP reporter, they efficiently provided tumor protection comparable to the total Th17 cells. Thus, our experimental settings including a normal, nonlymphopenic environment allow us to more directly address the physiological function of Th17 cells in cancer.

The protective function of Th17 cells against tumors is probably due to their ability to enhance inflammatory responses, and such enhancement results in increased antigen presentation by DCs. Leukocyte homing to tumors, usually inhibited by the tumor cells (Gajewski, 2007), was reduced in Il17a−/− mice and promoted by transfer of tumor-specific Th17 cells. Increased numbers of DCs infiltrating the lung after Th17 cell transfer may lead to increased capture of tumor antigens, which are then presented to tumor-reactive CD8+ T cells in the draining lymph nodes. It has been reported that the presence of increased numbers of mature DCs within solid tumor masses induces more effective antitumor immune responses in animal models (Furumoto et al., 2004) and is associated with improved prognosis in clinical patients (Lotze, 1997). Moreover, patients with advanced melanoma that have been treated with GM-CSF showed a general increase of mature DCs, which has been associated with disease remission and delayed tumor recurrence (Daud et al., 2008). At this point, our report is the first to show that Th17 cells influence the generation of CD8+ effector T cells in vivo. Given that CD4+ T cell help is essential for the generation of CD8+ antitumor effector-memory T cells. Th17 cells, on one hand, promote proliferation of tumor-specific CD8+ T cells. On the other, they also allow for sustained IFN-γ expression by dividing CD8+ T cells, thus preventing them from becoming “exhausted.”

We did not found a direct effect of Th17 cells on CD8+ T cells effector differentiation; however, we observed increased numbers of DCs carrying tumor-derived material in the lymph nodes of Th17 cell-treated mice. The predominant DCs recruited were CD8α+ which are indispensable for cross-presentation of self-antigens in several tissues and in the lung (den Haan and Bevan, 2002; Dudziak et al., 2007; Schnorrer et al., 2006). In comparison to Th1 cell-mediated effects, we found that Th17 cells selectively induced the expression of CCL20 and that CCR6 deficiency selectively impairs the recruitment of CD8α+ DC, and such results may account for the difference of Th1 and Th17 cells in activation of tumor-specific CD8+ T cells and in their potency of tumor immunity. Previously, Furumoto et al. reported that overexpression of CCL20 by B16 cells, although it increased intratumor DC numbers, did not promote tumor regression but helped the regression of CT26 colon adenocarcinomas (Furumoto et al., 2004), suggesting that CCL20-CCR6 might be necessary but not sufficient in the induction of tumor immunity.

Our data altogether suggest the following model: Th17 cells go to the tumor site and, by secreting IL-17, activates residential cells to produce CCL2 and CCL20, which provokes the mobilization of DCs and other leukocytes to the tumor site. DCs uptake tumor antigens in the lung and migrate to the lymph nodes where they activate CD8+ T cells against the tumor. The new wave of effector CD8+ T cells migrates back to the lung and kills established tumors (Figure S9).

Our data suggest that tumor immunosurveillance in the lung in the steady state is dependent, in part, on IL-17 but more importantly that tumor-specific Th17 cells may be used in adoptive T cell therapy. To date, adoptive cell therapy for cancer with in vitro-expanded, tumor-infiltrating CD8+ lymphocytes has achieved some degree of success in cancer therapy (Dudley and Rosenberg, 2007; Rosenberg and Dudley, 2004). Together with the current literature on the crucial function of Th17 cells in autoimmunity, our results suggest that Th17 cells directed against tumor antigens may be employed in cancer patients. In addition, Th17 and CD8+ T cells derived from the tumors may be expanded ex vivo and employed together to enhance cancer immunity. Therefore, our data demonstrating that Th17 cells and IL-17 participate in antitumor immunity by facilitating T cell
recruitment to the tumor site and CD8+ T cell priming and effector differentiation suggests a new avenue for developing Th17 cell-based therapy for tumors or chronic viral infections and as an adjunct for vaccinations.

**EXPERIMENTAL PROCEDURES**

**Mice**

C57BL/6 mice were purchased from the NCI and OT-II (C57BL/6-Tg(TcraTcrb)425Cbn/J), OT-I (C57BL/6-Tg(TcraTcrb)11000Mj/J), CD45.1 (B6.SJL-Ptprca Pepcb/BoyJ), and Ccr6 KO (B6.129P2-Crrevtm11J) mice were purchased from the Jackson Laboratory (Yamazaki et al., 2008). OT-II RAG2 (C57BL/6-Rag2m1 canceled/OT-II) were mice were bred at Taconic Farms. IL-17A-deficient mice and IL-17F-RFP mice were generated in our lab (Yang et al., 2008). Homozygous KO and WT animals on the same 129 x C57BL/6 F1 mixed background were bred and used in some experiments. For some experiments, IL-17AKO mice that had been backcrossed to C57BL/6 mice for six generations were used. IL-17F-RFP mice and CD45.1 mice were crossed to OT-II mice for OT-II-IL-17F-RFP and OT-II-CD45.1 mice generation, respectively. Mice were maintained in the MD Anderson Animal Facility and the MD Anderson Cancer Center Institutional Animal Care and Use Committee approved all animal studies. Rag1−/− B6 TR-1 TCR transgenic mice were bred at the National Institutes of Health (NIH).

**Induction and Assessment of B16-F10 Lung Melanoma**

Six-week-old mice were injected intravenously (i.v.) with 1 x 10⁶ wild-type B16-F10 cells or with B16/F10 transfected with OVA (B16-OVA). For adoptive transfer experiments, the mice were injected i.v. on the same day with 1 x 10⁵ in vitro differentiated Th17 OT-II cells in the prevention model or 5 days after the tumor injection in the therapeutic model. At day 14 or 16 after tumor introduction, mice were sacrificed for enumeration of metastatic lung foci. All after the tumor injection in the therapeutic model. At day 14 or 16 after tumor introduction, mice were sacrificed for enumeration of metastatic lung foci. All mice were sacrificed, lung, LLNs, and spleen were harvested on day 3 after transfer and restimulated with 5 μg/ml of OVA323-339 peptide in the presence of Golgi-plug for 6 hr. ICS for IL-17, IL-17F, and IFN-γ was performed with the BD Cytoflex/cytoperm kit.

**CD8+ T cells from OT-I mice were purified with anti-CD8 beads (Miltenyi) and the AutoMacs system. After purification, the cells were labeled with CFDA-SE and 5 x 10⁶ cells were injected i.v. into mice bearing 5 day established B16-OVA lung tumors. On the same day, the mice received i.v. injections of 3 to 5 million Th17 cells or PBS. Lung, LLNs, and spleen were harvested on day 3 after transfer and restimulated with 5 μg/ml of OVA257-264 peptide in the presence of Golgi-plug for 6 hr. ICS staining for IFN-γ and IL-2 was performed with the BD Cytoflex/cytoperm kit.**

**Dendritic Cell Analysis**

Mice were injected i.v. with 1 x 10⁵ B16-F10 expressing OVA-GFP and 1 x 10⁶ of either Th1 or Th17 polarized OT-II cells. LLN were harvested at 72 hr and were digested with 1 mg/ml of collagenase and with 0.01 EDTA for preventing DC-T cell aggregates. Cells were counted and were incubated with anti-CD16 plus anti-CD32 (FcBlock) for 20 min, additionally labeled with mAb against CD11b, CD11c, GR1, CD8+, or Isotype Rat IgG, and analyzed by flow cytometry.

Leukocytes from lung fractionation were analyzed similarly for determining DC, macrophage, and granulocyte populations.

**ELISPOT**

On day 16 after tumor injection, inguinal lymph node cells from mice that were treated with TR-1 Th17 cells were counted and plated in HA plates (Millipore) previously coated with anti-IFN-γ or IL-17 (BD Biosciences). Cells were pulsed with TRP-1 (106-130) peptide and cultured for 18 hr. Plates were blotted with biontinated anti-IFN-γ or IL-17 and developed with avidin-alkaline phosphatase and 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium chloride (BCIP/NBT). ELISPOT dots were counted on a C.T.L. ImmunoSpot with Image Acquisition 4.4 software for image capture and Immunospot 3 for analysis.

**SUPPLEMENTAL DATA**

Supplemental Data include nine figures and can be found with this article online at http://www.cell.com/immunity/supplemental/S1074-7613(09)00451-8.

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Blood 17 inhibits tumor cell growth by means of a T-cell-dependent mechanism.


