Interleukin (IL)-9 was originally described more than 20 years ago as a T-cell growth factor.1 IL-9 has recently attracted renewed interest owing to the identification of the most consistent IL-9-producing helper T (T<sub>H</sub>9) cells, which can be generated with IL-4 and transforming growth factor β1 (TGFβ1) in combination.2 IL-9 is a pleiotropic cytokine that has direct or indirect effects on multiple cell types, and hence can function as a positive or negative regulator of immune responses.3 IL-9 production was first linked to T<sub>H</sub>2 responses and mast cell biology, and IL-9 appears to have a detrimental role in allergy and autoimmunity.4 Recent studies suggest that IL-9 promotes the immunosuppressive functions of regulatory T cells (Tregs) and mast cells.5 Nevertheless, the role of IL-9 and T<sub>H</sub>9 cells in antitumor immunity remains still unclear.

We have recently analyzed the effects of endogenous IL-9 in a B16 melanoma lung tumor model.6 Enhanced tumor growth was observed in mice depleted of IL-9, coinciding with decreased leukocyte infiltration and reduced activation of intratumoral CD8<sup>+</sup> T cells. These results suggested that endogenous IL-9 exerts antitumor effects and prompted us to examine the role of T<sub>H</sub>9 cells in melanoma-elicited immune responses. We generated T<sub>H</sub>9 cells specific for B16 melanoma cells expressing the model antigen ovalbumin (OVA), and adoptively transferred these T<sub>H</sub>9 cells into melanoma-bearing mice. Our results demonstrate that the transfer of tumor-specific T<sub>H</sub>9 cells induces potent antitumor responses in both prophylactic and therapeutic settings. The antitumor effects observed in T<sub>H</sub>9 cell-receiving mice were associated with a significantly increased number or tumor-infiltrating CD4<sup>+</sup> T, CD8<sup>+</sup> T, and CD8α<sup>+</sup> dendritic cells (DCs), as well as with T-cell activation. In addition, the transfer of T<sub>H</sub>9 cells substantially reduced the growth of OVA-expressing melanoma implanted under the skin. A comparison of the antitumor effects mediated by T<sub>H</sub>1 and T<sub>H</sub>9 cells revealed that the transfer of the latter promoted greater tumor clearance than that of the former, suggesting that T<sub>H</sub>9 cells may be manipulated to develop novel T-cell-based immunotherapies.

To gain mechanistic insights into how tumor-specific T<sub>H</sub>9 cells may inhibit tumor growth, we first confirmed that T<sub>H</sub>9 cells retain their cytokine expression profile in vivo and do not directly lyse tumor cells in vitro. The study of the inflammatory status of pulmonary melanoma lesions revealed that the transfer of T<sub>H</sub>9 cells not only induces the activation of immune effector cells in tumor draining lymph nodes (TDLNs), but also recruits interferon γ (IFN γ)- and IL-17-producing effector cells at the tumor site, where they exert prominent tumor killing functions. In contrast, in our model T<sub>H</sub>1 cells preferentially homed to TDLNs and both T<sub>H</sub>1 and cells and host effector T cells largely failed to migrate into the tumor lesions. This unique inflammatory response induced by T<sub>H</sub>9 cells prompted us to investigate whether IL-9 regulates the expression of chemokines at the tumor site. By screening the expression levels of candidate chemokines and their receptors, we found that CCL20 as well as its receptor CCR6 are substantially upregulated in tumor tissues of T<sub>H</sub>9 cell-transferred mice, while sharply downregulated in IL-9-depleted animals. Such an IL-9- and T<sub>H</sub>9 cell-induced production of CCL20 in pulmonary melanoma lesions might be responsible for the recruitment of DCs and activated effector cells into the tumor, driving antigen presentation and the destruction of tumor cells, respectively.

We next evaluated cytotoxic CD8<sup>+</sup> T lymphocyte (CTL) responses, as CD8<sup>+</sup> T cells represented a major cell population recruited by the transfer of T<sub>H</sub>9 cells. Our results indicate that T<sub>H</sub>9 cells elicit a strong activation of CTLs, as demonstrated by the large population of OVA-tetramer<sup>+</sup> CTLs that could be detected in mice receiving T<sub>H</sub>9 cells but not in PBS or T<sub>H</sub>1 cells. As the deletion of CD8<sup>+</sup> T cells almost completely abrogated tumor rejection as promoted by T<sub>H</sub>9 cells, we further

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T<sub>H</sub>9 cells join the ever-growing list of CD4<sup>+</sup> T helper subsets and primarily mediate anti-parasite immune responses. We have recently demonstrated that tumor-specific T<sub>H</sub>9 cells induce a CCL20-CCR6-dependent regulation of DCs while stimulating CD8<sup>+</sup> T cell-mediated antitumor immunity. These findings offer a novel immunotherapeutic strategy against cancer.

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as they favor T H2 immune responses and induce the generation/expansion of Tregs, hence suppressing T H1 antitumor immunity.7,8 An interesting finding of our study was that the transfer of T H9 cells specifically recruited and activated CD8 α+ DCs, which are specialized in antigen cross-presentation to CD8+ T cells on MHC Class I molecules, thus eliciting significant antitumor responses.9 In some cases, CCL20 may only contribute to the intratumoral accumulation of immature DCs and hence promote tolerance phenomena.10 Future work is needed to determine how CCL20 as induced by T H9 cells specifically promotes the activation of CD8α+ DCs.

Our study demonstrated that tumor-infiltrating DCs recruited by T H9-cell transfer are responsible for the delivery of tumor antigens and for the priming of CTLs. We detected highly increased numbers of GFP+ DCs, especially GFP+CD8α+ DCs, in TDLNs of T H9 cell-transferred mice bearing GFP- and OVA-expressing B16 melanomas. The presence of GFP+ DCs in TDLNs indicates that the transfer of T H9 cells results in a substantially increased infiltration of DCs into tumor tissues. These DCs take up tumor material for antigen presentation, migrate into TDLNs, and activate tumor-specific CTLs. The regulation of DC functions by T H9 cells depends on CCL20-CCR6 chemotaxis. Indeed, Ccr6 deficiency electively impaired the recruitment of CD8α+ DCs, abrogated the infiltration of activated CTLs and compromised antitumor responses mediated by T H9 cells (Fig. 1). CD11b+CD8α+ DCs were the major subset of DCs found in pulmonary melanoma lesions of PBS- and T H1 cell-receiving mice. These DCs have been defined as “natural occurring regulatory DCs,” as they favor T H2 immune responses and induce the generation/expansion of Tregs, hence suppressing T H1 antitumor immunity.7,8 An interesting finding of our study was that the transfer of T H9 cells specifically recruited and activated CD8α+ DCs, which are specialized in antigen cross-presentation to CD8α+ T cells on MHC Class I molecules, thus eliciting significant antitumor responses.9 In some cases, CCL20 may only contribute to the intratumoral accumulation of immature DCs and hence promote tolerance phenomena.10 Future work is needed to determine how CCL20 as induced by T H9 cells specifically promotes the activation of CD8α+ DCs.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.
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