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Utilizing T_H9 cells as a novel therapeutic strategy for malignancies

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T_H9 cells join the ever-growing list of $CD4^+$ T helper subsets and primarily mediate anti-parasite immune responses. We have recently demonstrated that tumor-specific T_H9 cells induce a CCL20-CCR6-dependent regulation of DCs while stimulating $CD8^+$ T cell-mediated antitumor immunity. These findings offer a novel immunotherapeutic strategy against cancer.

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_H9) 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 that hence can function as a positive of negative regulator of immune responses.[3] IL-9 production was first linked to T_H2 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_H9 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 $CD8^+$ T cells. These results suggested that endogenous IL-9 exert antitumor effects and prompted us to examine the role of T_H9 cells in melanoma-elicited immune responses. We generated T_H9 cells specific for B16 melanoma cells expressing the model antigen ovalbumin (OVA), and adoptively transferred these T_H9 cells into melanoma-bearing mice. Our results demonstrate that the transfer of tumor-specific T_H9 cells induces potent antitumor responses in both prophylactic and therapeutic settings. The antitumor effects observed in T_H9 cell-receiving mice were associated with a significantly increased number or tumor-infiltrating $CD4^+$ T, $CD8^+$ T, and $CD8\alpha^+$ dendritic cells (DCs), as well as with T-cell activation. In addition, the transfer of T_H9 cells substantially reduced the growth of OVA-expressing melanoma implanted under the skin. A comparison of the antitumor effects mediated by T_H1 and T_H9 cells revealed that the transfer of the latter promoted greater tumor clearance than that of the former, suggesting that T_H9 cells may be manipulated to develop novel T cell-based immunotherapies.

To gain mechanistic insights into how tumor-specific T_H9 cells may inhibit tumor growth, we first confirmed that T_H9 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_H9 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_H1 cells preferentially homed to TDLNs and both T_H1 cells and host effector T cells largely failed to migrate into the tumor lesions. This unique inflammatory response induced by T_H9 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_H9 cell-transferred mice, while sharply downregulated in IL-9-depleted animals. Such an IL-9- and T_H9 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 $CD8^+$ T lymphocyte (CTL) responses, as $CD8^+$ T cells represented a major cell population recruited by the transfer of T_H9 cells. Our results indicate that T_H9 cells elicit a strong activation of CTLs, as demonstrated by the large population of OVA-tetramer$^+$ CTLs that could be detected in mice receiving T_H9 cells but not in PBS or T_H1 cells. As the deletion of $CD8^+$ T cells almost completely abrogated tumor rejection as promoted by T_H9 cells, we further

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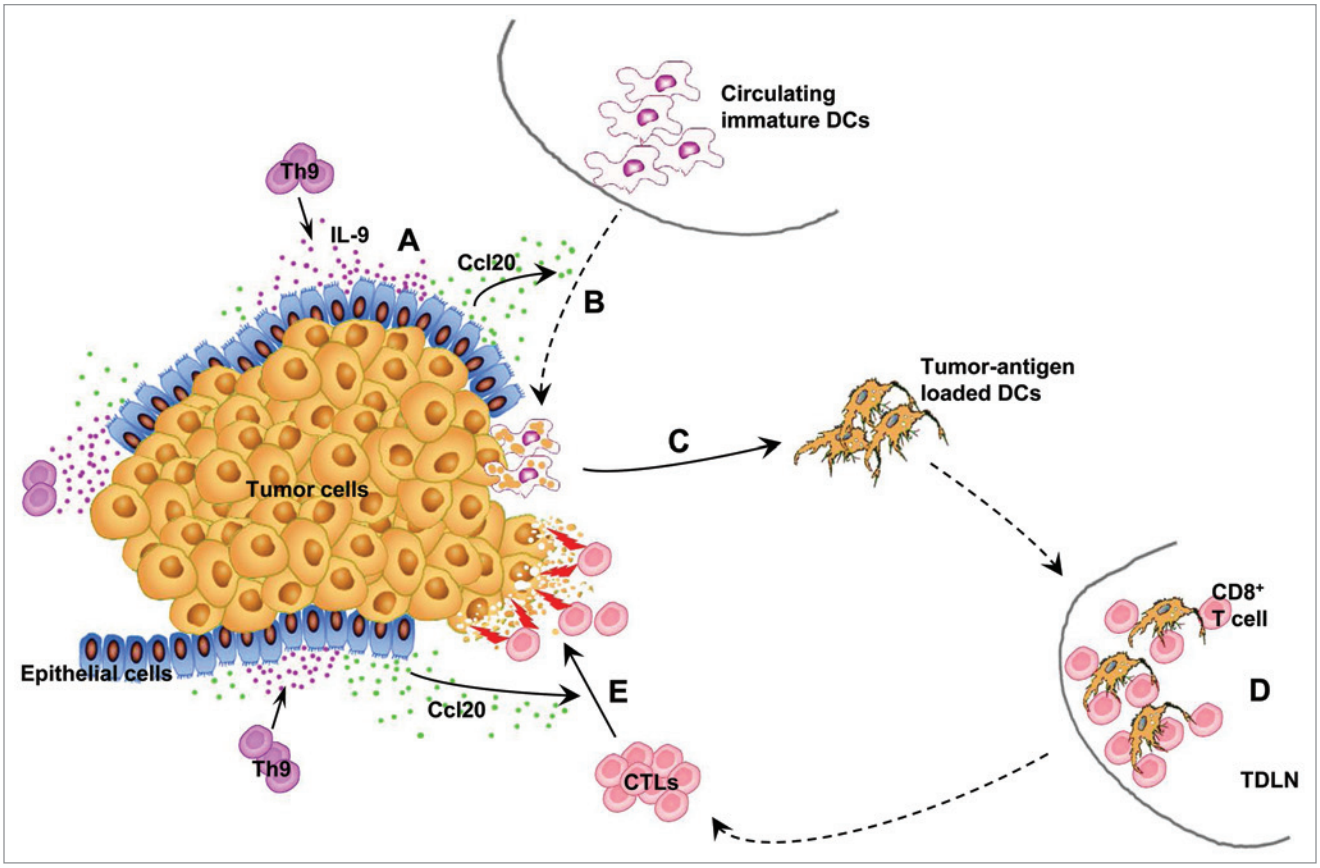

Figure 1. T_H9 cell-mediated antitumor immune response. (**A**) Tumor-infiltrating T_H9 cells produce interleukin (IL-9) in the tumor microenvironment, hence inducing tumor tissues, and especially epithelial cells, to produce CCL20. (**B**) CCL20 recruits dendritic cells (DCs), especially CD8α$^+$ DCs, into the tumor, where they take tumor material. (**C**) DCs become activated and migrate to tumor-draining lymph nodes (TDLNs). (**D**) Tumor antigen-loaded DCs promote the activation of host effect cells in TDLNs. (**E**) Activated host effector cells, especially cytotoxic CD8$^+$ T lymphocytes (CTLs) migrate into tumor lesions guided by CCL20 to kill tumor cells.

confirmed that CCR6$^+$ tumor-infiltrating CTLs are the major cell population that contributes to tumor rejection. In addition, we demonstrated that the adoptive transfer of T_H9 cells can promote naïve OT-I cells to differentiate into IFNγ- and granzyme B-producing effector cells in TDLNs, and sustain their relocation to pulmonary neoplastic lesions. These results suggest that T_H9 cells might provide indirect "help" to tumor-specific CTLs in vivo, possibly by delivering tumor antigens for the priming of CTLs in TDLNs and/or by promoting the subsequent infiltration of these cells into the tumor.

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 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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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