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Commentary

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Th9 and Th17 cells: the controversial twins in cancer immunity

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Th17 cells (producing IL-17) and Th9 cells (producing IL-9) exhibit functional plasticity, and their role in tumorigenicity is controversial. Th17/IL-17 and Th9/IL-9 exhibit critical, but often opposing, roles in tumor progression. In this issue of the *JCI*, Salazar et al. show that while IL-17 and IL-9 induced distinct but complementary molecular pathways, both cytokines also induced epithelial-mesenchymal transition (EMT) in lung cancer cells and promoted metastatic spreading. A key question before us now is whether IL-9 and IL-17 contribute to tumor progression in a sequential and stage-specific manner within the tumor microenvironment.

Th cell subsets play various roles in cancer

T lymphocytes of the adaptive immune system have multifaceted roles in cancer. Following activation, CD4⁺ Th cells can differentiate into various Th subsets, from the long-studied antitumoral Th1 cells and protumoral Th2 cells to the relatively recently added members, including Th17, Th9, and T follicular helper cells or immunosuppressive Tregs. However, other Th lineages may exist (1). Th17 cell differentiation requires costimulation with the cytokines TGF- β , IL-6, and IL-23 as well as IL-21 in mice (2) or IL-1 β in humans (3). These cells express the IL-23 receptor (IL-23R) and secrete high levels of the proinflammatory cytokines IL-17 (or IL-17A) and IL-17F as well as IL-21, IL-22, and GM-CSF (4–6). While Th17 cells play critical roles in autoimmunity and during immune responses against extracellular bacteria and fungi (7), the role of Th17 cells and their signature cytokine IL-17 in cancer is highly controversial (8).

In 2008, Th9 cells were first described as a CD4⁺ T cell subset (9, 10). These cells secrete the pleiotropic cytokine IL-9 (11) and are the most studied IL-9–producing

cells in tumor immunity. While almost all reports show that Th9 cells play a broad antitumor role, initial studies extensively focused on melanoma and breast cancers. However, with the exception of human hepatocellular carcinoma (12) and lung cancer (13), in which Th9 cell–derived IL-9 can promote tumorigenesis by activating the STAT3 signaling pathway, little information on the protumoral role of Th9 cells has been reported.

The tumor-immune interplay

In this issue of the *JCI*, Salazar and colleagues add another twist to the story by studying Th17/IL-17 and Th9/IL-9 axes in lung cancer metastasis (Figure 1 and ref. 14). Salazar et al. established a coculture system of lymphocytes and the human lung cancer cell line (A549), or primary human non–small cell lung carcinoma (NSCLC) cells (PTCs), to mimic the tumor-immune interplay (14). Induction of epithelial-mesenchymal transition (EMT) in lung cancer cells was identified as a consistent feature, both upon direct coculturing of tumor cells with either Th9 or Th17 cells and upon exposure of primary human lung cancer cells or cell lines to

the respective signatory cytokines IL-9 and IL-17. Salazar et al. (14) showed that Th17/IL-17 and Th9/IL-9 induced a gene expression profile associated with EMT, metastasis, angiogenesis, and cancer cell migration. As a result, tumor cells transitioned to a highly migratory mesenchymal phenotype characterized by loss of epithelial markers (E-cadherin, ZO2) and gain of mesenchymal markers (vimentin, N-cadherin). This EMT was associated with enhanced expression of several MMPs, EMT- and migration-related proteins, and transcription factors that regulate the expression of genes that crosstalk directly with a specific Th subtype. Interestingly, even though Th9 and Th17 and their signature cytokines had similar effects on lung cancer cells, Th9 and Th17 tumor cell cocultures elicited distinct and complementary molecular pathways in modulating tumor progression and metastasis. Throughout the time that the level of Th9/IL-9 predominantly increased during chemokine-mediated inflammation and angiogenesis, the Th17/IL-17 axis modulated the oxidative stress response and Ras signaling pathways (14).

In addition to the *in vitro* models, these EMT/tumor progression features were reproduced with *in vivo* studies via the adoptive transfer of Th9 and/or Th17 cells in WT and immunodeficient mouse lung cancer models (14). Notably, increased lung metastasis was observed after coinjection of Th9 and Th17 cells in *Rag1*^{−/−} mice. In the *Il9r*^{−/−} and *Il17ra*^{−/−} mouse models, coinjection of LLC1 cells with Th9 or Th17 in cells *Il9r*^{−/−} or *Il17ra*^{−/−} mice caused increased EMT and metastasis in a discordant transfer setting (Th9 in *Il17ra*^{−/−} mice and Th17 in *Il9r*^{−/−} mice), with only limited efficacy in the concordant constellation (Th9 in *Il9r*^{−/−} mice and Th17 in *Il17ra*^{−/−} mice). Further use of neutralizing antibodies decreased EMT and slowed lung cancer progression and metastasis, which again reinforces a specific metastatic-promoting role for effector cytokines IL-9 and IL-17.

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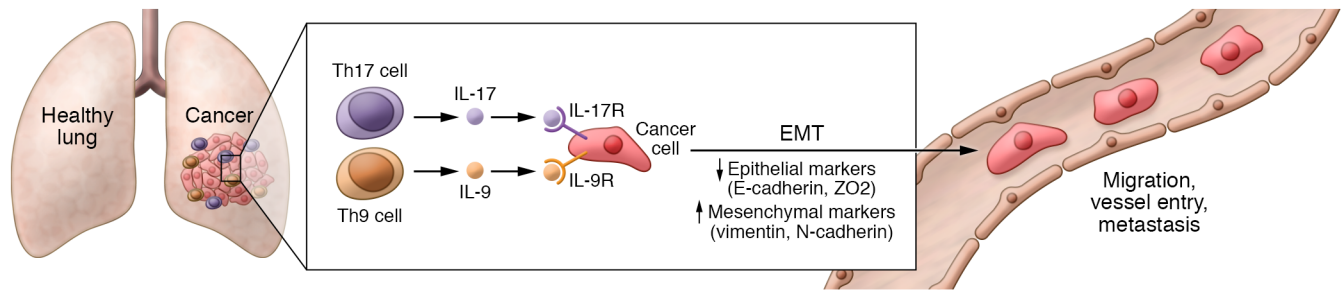


Figure 1. Th9/IL-9 and Th17/IL-17 induce lung cancer cell EMT and thereby promote metastatic spreading. Salazar et al. showed that Th9 and Th17 cells were enriched in human lung tumor tissues compared with nontumor samples (14). The study suggests that the release of the signature cytokines IL-9 and IL-17 by tumor-infiltrating Th9/Th17 lymphocytes alters the gene regulatory profile in lung cancer cells to induce EMT and promote tumor cell migration, vessel entrance, and metastasis.

Correspondingly, enhanced numbers of Th9 and Th17 cells in tissue from 66 patients with NSCLC negatively correlated with survival (14). Thus, with the detection of the receptors for IL-9 and IL-17 on the tumor cells, the authors conclude that the crosstalk between Th9/Th17 lymphocytes and cancer cells likely promotes lung cancer progression and in particular metastasis (14). These observations potentially highlight therapeutic strategies for lung cancer, such as anti-IL-9/IL-17 treatment.

Considerations and remaining questions

Salazar and colleagues performed a series of experiments *in vitro* and *in vivo* using mouse models and human lung cancer tissue. The naive T cells were polarized under Th9 conditions by adding TGF- β and IL-4 or Th17 conditions also with TGF- β and IL-6. Compared with traditional Th9 differentiation conditions (IL-4 combined with TGF- β), Th9 cells exposed to IL-4 and IL-1 β produce cells with stronger cytotoxic effects and tumor-killing abilities that exert a powerful antitumor effect in melanoma (15). It would be interesting to see if an IL-1 β -enriched tumor microenvironment would polarize Th9 cells and impart a higher potential to stimulate lung cancer cell metastasis. Moreover, it is intriguing that Th9/IL-9 and Th17/IL-17 play roles in other stages of lung cancer development, for example, influencing the mesenchymal-epithelial transition and forming new colonies at metastatic loci (16).

It is worth noting that in human cancer tissues various immune cells, including Th2 cells, type 2 innate lymphoid cells, Tc9 cells, Tregs, and mast cells may also produce IL-9. Notably, Th9 cells may not

always be the major source of IL-9-dependent responses in the tumor microenvironment (17). Thus, the unique marker(s) for Th9 cells in patients with cancer remain unidentified. The association of IL-9 with patient prognosis may, in fact, reflect the role of other IL-9-producing cells in human cancers. Therefore, identifying of more specific Th9 biomarkers, in addition to IL-9, will facilitate our understanding of Th9 cells in human cancers.

Th17 cell gene expression is regulated by the transcription factor retinoid orphan nuclear receptor (ROR γ t in mice or RORC in humans) (18). Unexpectedly, IL-9 production is increased in Rorc-deficient CD4⁺ T cells and is associated with inhibited B16F10 melanoma growth (17). These observations suggest that the ROR γ t/IL-17 axis may serve as a negative regulator of IL-9 expression. It is essential to unravel the mechanisms used by Th17/IL-17 and Th9/IL-9 in human cancer progression to develop effective therapeutic strategies that reeducate the immune cells. What would be the effect of anti-IL-9 or anti-IL-17 treatment approaches on the immune cells in the tumor microenvironment? Would interference with IL-9 and/or IL-17 signaling synergize with immune checkpoint blockade treatment of lung cancer? Currently, the effect of IL-9 and/or IL-17 neutralization in humans is still unknown. In addition, it is unclear whether blockade of IL-9 and/or IL-17 in other mouse models of cancer would give similar results.

In general, the role of IL-9 and IL-17 in propagating cancer is complex. Therefore, due to contradictory observations using various mouse cancer models, determining the actual role that these Th9 and Th17 mixed-blessing twins play in human can-

cers remains an unmet need. The effect of Th9 and Th17 lymphocytes on carcinogenesis may largely depend on the context of the tumor type and cancer stage, cytokine availability, receptor distribution, and crosstalk among different stimuli, cell types, and signaling pathways. For example, the effect of IL-17 in cancers may differ under homeostatic versus inflammatory conditions (19). IL-17-mediated effects may also differ depending on the type of cancer, because the IL-17R generates diverse intracellular signals in different forms of cancer (20). In addition, Th9 and Th17 cell plasticity and reprogramming may allow these cells to converge toward a phenotype like that of many of other T cell subsets. This plasticity/reprogramming can occur simultaneously or sequentially in response to specific microenvironmental cues to ultimately fuel complex immune interactions that participate in tumor progression (21, 22). Therefore, we need to pinpoint tumor/stage- and/or patient-specific responses in cancers to advance our understanding of Th9 and Th17 cell biology and guide new approaches in precision medicine. For these reasons, the study by Salazar et al. (14) represents an exciting step forward in the field and provides an excellent example of meticulous experimental design that unravels new Th cell functions. These studies point to the need to develop therapeutics targeting Th9/IL-9 and Th17/IL-17 to elucidate their effect on cancer progression.

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