Th9 Cells: A Novel CD4 T-cell Subset in the Immune War against Cancer
Frédérique Végran1,2, Lionel Apetoh1,2,3, and François Ghiringhelli1,2,3

Abstract
CD4 T cells are key components of the immune system that shape the anticancer immune response in animal models and in humans. The biology of CD4 T cells is complex because naïve T cells can differentiate into various subpopulations with various functions. Recently, a new population called Th9 cells was described. These cells are characterized by their ability to produce IL9 and IL21. They were first described in the context of parasite infections and allergic processes. However, some reports described their presence in the tumor bed in mice and humans. Their high secretion of IL9 and IL21 in the tumor bed contributes to their anticancer functions. Indeed, these cytokines trigger the activation of dendritic cells, mast cells, natural killer cells, and CD8 T cells to mount an antitumor immune response, thus explaining the remarkable ability of Th9 cells to control tumor growth. This review summarizes the latest advances in the Th9 field in cancer and focuses on their potential role as new tool for cell therapy.

The Role of CD4 T-cell Polarization in Cancer
Since the seminal observations of Dunn and colleagues (1) on cancer immunosurveillance, the role of the adaptive immune system in the development of cancer or tumor growth is clearly established. CD4 helper T cells are key elements of the adaptive immune response, and are known to differentiate from a naïve population into helper memory populations after stimulation by T-cell receptor (TCR) triggering by the cognate antigen and a population into helper memory populations after stimulation by immune response, and are known to differentiate from a naïve population into helper memory populations after stimulation by T-cell receptor (TCR) triggering by the cognate antigen and a population into helper memory populations after stimulation by T-cell receptor (TCR) triggering by the cognate antigen and a population into helper memory populations after stimulation by T-cell receptor (TCR) triggering by the cognate antigen...
and Th9 differentiation (12, 18). In addition, cytokine costimulation could play a role in Th9 differentiation. The Notch1/jagged2 pathway is required for optimal Th9 polarization (19). Finally, OX40 stimulation by OX40L expression on antigen-presenting cells seems to be a key costimulation involved in the specific induction of Th9 polarization (20).

Transcriptional program of Th9 cells

Though the transcriptional program of Th9 cells is not yet described completely, some transcription factors have been shown to be essential for Th9 polarization. These include STAT6, GATA3, PU.1, and IRF4. It is noteworthy that the transcription factors STAT6 and GATA3 are not expressed exclusively during Th9 cell differentiation, but are also expressed in Th2 cells. STAT6 is phosphorylated during Th9 differentiation because of the engagement of IL4R signaling, and is dispensable for Th9 differentiation. GATA3, whose expression is induced by phosphorylated STAT6, is also required for Th9 differentiation. Accordingly, the generation of Th9 cells was absent in STAT6-deficient and GATA3-deficient mice, which confirmed that STAT6 and GATA3 were essential in the generation of Th9 cells (9). However, some data demonstrate that GATA3 is not directly involved in the transcriptional regulation of the il9 gene, but acts rather as a molecule involved in the downregulation of Foxp3, a protein that could negatively affect Th9 development (21).

On the other hand, TGFβ is also required for Th9 differentiation, but only a small fraction of Th9 cells express Foxp3, suggesting that Foxp3 is not essential for Th9 lineage commitment. Moreover, the ectopic expression of Foxp3 reduces IL9 production by Th9 cells (21), thus demonstrating a negative effect of Foxp3 on Th9 differentiation. TGFβ induces the activation of the SMAD pathway and the expression of PU.1, which could restrain Th2 polarization (22). In the absence of PU.1, Th9 polarization was impaired, whereas PU.1 infection of Th2 cells decreased IL4 secretion and promoted Th9 polarization (22). PU.1 was shown to bind to the il9 promoter and to induce the recruitment of the histone acetyltransferases Gcn5 and PCAF, thus leading to permissive chromatin configuration of the il9 gene (23).

IRF4 is also required for Th9 differentiation, but this transcription factor is also essential for Th2 and Th17 cell differentiation. IRF4 heterodimerizes with PU.1 or the AP1 transcriptional factor BATF on DNA. Like in Th17 cells, IRF4 cooperates with BATF to induce the transcriptional program of Th9 cells (24). It remains to be determined whether IRF4–PU.1 heterodimers also have an impact on the Th9 transcription program. IRF1 is a Th1 transcription factor that is expressed in Th9 cells upon stimulation with IL1β. This factor binds directly on il9 and il21 promoters, and is essential to boost production of both cytokines (15). This factor is a powerful enhancer of the Th9 program. It remains to be determined whether IRF1 acts alone or in combination with other transcription factors involved in Th9 polarization.

In vivo presence of Th9 cells in physiopathologic contexts

Th9 cells have been observed in many inflammatory contexts in both humans and mouse models. However, the presence of Th9 cells is mainly associated with type 2 immunity-related processes. Th9 cells have been found in the peripheral blood of allergic and asthmatic patients. In a population of atopic patients, a greater production of IL9 was observed in CD4 T cells stimulated with house dust mite extract or cat allergens (25). Similar results were observed in murine models of an ovalbumin airway inflammation model in which Th9 cells could be detected in the draining lymph nodes and in lung tissues (26). In this context, Th9 cells seem to play a pathogenic role and induce mucus production and infiltration of the airspace by mast cells and eosinophils in goblet cell hyperplasia. In helminth parasite diseases, another type 2 immunity-related disease, Th9 are essential for parasite eradication (27).

In the context of cancer, the presence of Th9 cells has been described in lung metastatic pleural effusion and in tumor-infiltrating lymphocytes of human melanoma (14, 28).

The Mechanism of the Antitumor Effects of Th9

Three recent articles have shown the ability of Th9 cells to control tumor growth. The seminal observation was made by Purwar and colleagues (13) who inadvertently discovered their role. They observed that RORγt-deficient mice showed reduced tumor growth and presented a high number of IL9-producing CD4 T cells, suggesting that IL9 could play a role in the protective antitumor immunity observed in RORγt-deficient mice. To test the role of IL9 in this model, they treated melanoma-bearing RORγt-deficient mice with an IL9-neutralizing antibody, and noted that IL9 depletion promoted melanoma growth. In addition, they found that the antitumor effect of adoptive transfer of antigen-specific Th9 cells was greater than that of Th1 or Th17 cell transfer in the B16 melanoma model. These results were confirmed by Lu and colleagues (14), who found that the adoptive transfer of ovalbumin-specific Th9 cells had antitumor effects in the setting of subcutaneous lung metastasis of ovalbumin-B16F10. The underlying mechanism accounting for the anticancer functions of Th9 cells remains ambiguous. Purwar and colleagues (13) observed a peptide-specific and granzyme B–dependent killing capability of these cells. In these two reports, it was suggested that IL9 was involved in the antitumor effect of Th9 cells. IL9 could target the activation and proliferation of mast cells, which could have cytotoxic functions against tumor cells. However, the role of mast cells on cancer growth remains controversial and some report underline the proangiogenic and the immunosuppressive function of mast cells. In addition, mast-cell infiltration of human tumors is associated with a poor outcome in cancers (29). IL9 could induce an antitumor immune response through different mechanisms. Lu and colleagues (14) demonstrated that IL9 could activate epithelial lung cells to produce CCL20, the ligand of CCR6. This chemokine attracts CCR6+ dendritic cell (DC) into the tumor bed and favors tumor antigen uptake and presentation. In addition, this chemokine also attracts CCR6+ CD8 CTL into the tumor bed in which they could then eradicate cancer cells. IL9 was also shown to enhance DC survival and to enhance their ability to generate anticancer protective immunity. In lymphoma, these antitumor effects are imbalanced by the expression of IL9R on tumor cells. In this case, IL9 drives STAT3 and STAT5 activation in tumor cells and directly promotes survival and proliferation. As a consequence, high expression of IL9 is associated with a poor prognosis (30).

More recently, we observed that IL1β is a determinant factor in boosting Th9 polarization by enhancing IL9 and IL21 secretion without skewing Th9 cell polarization. We demonstrated that engagement of IL1 receptor induces the activation of the tyrosine kinase Fyn via MyD88 adaptor. Fyn drives the phosphorylation of the transcription factor STAT1, and its subsequent direct binding.
to the promoter of the gene encoding IRF1. IRF1 is, thus, essential for the transcription of both il9 and il21 by its capacity to bind to both promoters. IRF1 was detected only when Th9 cells were polarized in the presence of IL1β. IL1β does not globally affect the early differentiation of Th9 cells, but enhances their function through the IRF1-dependent increase in the production of IL9 and particularly IL21. Importantly, although IRF1 was identified as a specific Th1 cell–associated transcription factor (31), upregulation of IRF1 expression in Th9 cells does not skew Th9 polarization to IFN-γ-producing cells (Fig. 1; ref. 15).

Interestingly, IL1β stimulation boosted the antitumor activity of Th9 cells in different models of adoptive cellular therapy (15). Th9 cells differentiated with IL1β exert antitumor effects in the ovalbumin-B16F10 model, the ovalbumin-LLC model and in the B16F10 model using TCR transgenic mice, which recognize tyrosine-related protein 1 (TRP-1), a melanocyte differentiation antigen expressed by B16F10. More importantly, IL1β-induced Th9 cells are also effective in a model of spontaneous melanoma that developed in mice expressing the human RET oncogene under the control of the metallothionein promoter (MT/ret mice). RET transgenic mice develop an uveal melanoma associated with the rapid development of metastatic disease (32). In this model, both classical and IL1β-induced Th9 cells had an antitumor effect on primary ocular tumors, but only IL1β-induced Th9 cells inhibited the onset of metastasis.

Conventional Th9 cells produce large amounts of IL9 and small amounts of IL21, and their antitumor effects are dependent on IL9. In contrast, for Th9 cells differentiated in the presence of IL1β, IL9 blockade has a minor effect on their antitumor properties. In addition, in our model, Th9 cells did not feature expert killing functions and mast cells were not involved in their anticancer effect. Instead, Th9 cells differentiated in the presence of IL1β produced high levels of IL21 and exerted IL21-dependent anticancer effects. IL21 is a cytokine, which is classically produced by activated CD4 T cells. This cytokine is a well-known stimulator of IFNγ production and enhances the cytolytic activity of NK cells and CD8 T cells. In particular, IL21 boosts the ability of IL2 and IL15 to activate NK cells’ cytolytic and secreting function, augments IL15-induced proliferation of murine CD8 T cells, and promotes clonal expansion of antigen-stimulated human CD8 T cells (33, 34). In line with this, we observed that IL21 derived from Th9 cells induced IFNγ production by NK and CD8 cells. This IFNγ produced by host NK and CD8 T cells was required for the antitumor effects of Th9 cells (Fig. 2). In humans, recombinant IL21 was tested in a multicenter phase II study of patients with metastatic melanoma. This treatment gave interesting stigmata of efficacy with an overall response rate of about 25% in the first-line treatment of metastatic melanoma (35). In this respect, because IL1β-induced Th9 cells are able to secrete copious amounts of IL21, they could represent an attractive candidate for a clinical evaluation of anticancer cell therapy. The advantage of cell therapy rather than recombinant cytokine therapy could be the specific homing of transfer T cells to the tumor bed, which could induce a higher level of cytokines at the tumor site than is the case with systemic injections.

Future Direction and Concluding Remarks

Recent clinical trials using checkpoint inhibitors like anti-CTLA4 and anti-PD1/PDL1 underline the efficacy of redirecting endogenous anticancer immunity to fight cancer. In addition, cellular therapy with CD8 or CD4 T cells based on the transfer of
tumor-specific lymphocyte populations expanded in vitro also demonstrated some efficacy in many trials. This strategy, however, is currently based on the addition of empirical clinical trials, and will probably benefit from better understanding of the particular conditions needed to induce more potent and longer-lasting antitumor responses.

Recent studies on Th9 cells, which showed the ability of Th9 cells and especially Th9 cells differentiated in the presence of IL1β to induce major antitumor effects in different models, underscore the potential relevance of this subset for future clinical trials of adoptive cell therapies. These cells seem to be the ideal candidate because in contrast with other helper cells, their IL9 expression will induce the recruitment of bystander killer cells like NK cells and CD8 T cells with the CCL20/CCR6 axis. IL9 will also promote antigen presentation and CD8 priming (13, 14). Nevertheless, one of the major problems of adoptive transfer is the poor stability of helper T cell subsets once transferred in vivo. For example, it has been suggested that CD4 T cells differentiated into Th17 cells are better anticancer cells in the setting of established melanoma tumors than are IFNγ-secreting Th1 cells. However, Th17 cells convert into IFNγ-producing cells after adoptive transfer, and tumor rejection is mainly dependent on the secretion of IFNγ (36, 37). This transdifferentiation is not observed in the case of Th9 cells differentiated in presence of IL1β. These cells conserved their ability to produce IL9 and IL21 days after in vivo injection. In addition, Th9 cells differentiated in presence of IL1β antitumor activity are strictly dependent on IL21, and IFNγ-deficient Th9 conserved their anticancer function. In contrast, Th9 cells differentiated without IL1β antitumor activity are dependent on IL9. Together, these data provide a strong impetus to investigate the anticancer efficacy of adoptive transfer of Th9 cells in patients with melanoma cancer.

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

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