

Th9 Cells: A Novel CD4 T-cell Subset in the Immune War against Cancer

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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. *Cancer Res*; 75(3); 475–9. ©2014 AACR.

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 particular cocktail of cytokines. The original classification of CD4 T lymphocytes by Mosmann and Colleagues (2) described two populations of effector CD4 T cells called Th1 and Th2 subsets. Th1 are IFN γ -producing cells and were known as the classical antitumor cells because of the capacity of IFN γ to activate the killer functions of macrophages, natural killer (NK) cells, and CD8 CTLs. Th2 cells are characterized by their production of IL4, and play a central role in the development of asthma and atopic dermatitis. The role of Th2 cells in cancer is mainly deleterious as IL4 directly favors tumor growth. In addition, Th2 cells could induce M2 polarization of tumor-infiltrating macrophages, which drive tumor immune tolerance and neoangiogenesis (3). CD4 T cells are now known to differentiate into additional new effector T-cell subsets like Th17 cells and follicular helper T cells as well as immunosuppressive cells like Foxp3 regulatory T cells. Foxp3 regulatory T cells are well-known immunosuppressive cells. These cells inhibit the anticancer immune response and induce immunosuppression in many cancer types (4). In contrast, the infiltration of

tumors by follicular helper T cells seems to be associated with a coordinated antitumoral immune response and a better clinical outcome (5). The role of Th17 cells in cancer remains a matter of debate. IL17 could promote neoangiogenesis and the expression of prosurvival genes in cancer cells (6). In addition, ectonucleotidase-expressing Th17 cells compromise anticancer immunity through adenosine (7), whereas adoptively transferred Th17 cells demonstrated dramatic anticancer functions *in vivo* notably via their ability to differentiate into IFN γ -producing cells (8).

In 2008, IL9-producing CD4 T helper cells (Th9) were identified as a new subset of CD4 T helper cells with proinflammatory functions (9, 10). Th9 cells arise from reprogrammed Th2 cells upon stimulation with TGF β (9, 10). Mouse and human Th9 cells secrete IL9 and IL21 and were initially proposed to contribute to the development of autoimmune and allergic diseases (11, 12). We and others, however, recently found that Th9 cells also featured potent anticancer properties (13–15). In this review, we discuss the potential of using Th9 cells for anticancer immunotherapy.

The Biology of Th9 Cells

Generation of Th9 cells

IL9 was initially categorized as a Th2 cytokine. In 2008, a new Th subset that preferentially produces IL9 and appears to be distinct from Th2 cells was reported. These cells are generated from mouse naïve T cells after stimulation with TGF β and IL4 in the presence of TCR signaling and costimulation (9, 10). However, IL9 is not specific to Th9 cells and could be secreted in smaller amounts by Treg, Th17, or Th2 cells. During Th9 differentiation, these cells discontinue expressing the Th2 cytokines, IL4, IL5, and IL13, while initiating transcription of IL9 (13). Many cytokines are known to affect Th9 differentiation. Clearly Th1-related cytokines like IFN γ and IL27 inhibit IL9 production and Th9 differentiation (16). In contrast, some Th17-related cytokines like IL21 and IL23 inhibit Th9 cell polarization probably via their capacity to induce STAT3 activation (13, 17). Similarly, some cytokines related to type 2 immunity like IL2, IL10, and IL25 promote IL9 production

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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 indispensable 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, TGF β 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. TGF β 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 conformation 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 IL1 β . 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 inflamma-

tion 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).

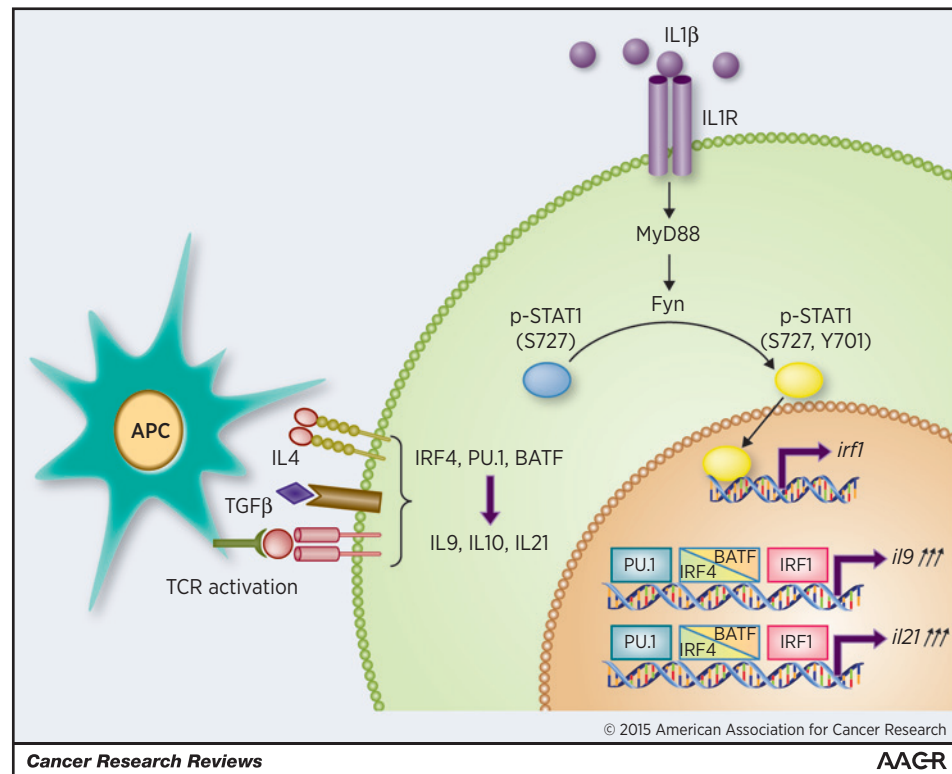
The Mechanism of the Antitumor Effects of Th9

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 ROR γ t-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 ROR γ t-deficient mice. To test the role of IL9 in this model, they treated melanoma-bearing ROR γ t-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 anticancer 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 IL1 β 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

Figure 1.

IL1 β enhances Th9 differentiation in an IRF1-dependent manner. Classical Th9 differentiation in the presence of TGF β and IL4 induces IRF4, PU.1, and BATF transcription factors that interact with *il9* and *il21* promoters to activate their expression. In an IL1 β context, such as cancer, the engagement of IL1 receptor induces the activation of the tyrosine kinase Fyn via MyD88 adaptor. Fyn drives the phosphorylation of the transcription factor STAT1 on the tyrosine 701 residue and its binding to the promoter of the gene encoding IRF1. IRF1 binds to the *il9* and *il21* promoters, thus increasing the secretion of both IL9 and IL21.



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 IL1 β . IL1 β 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).

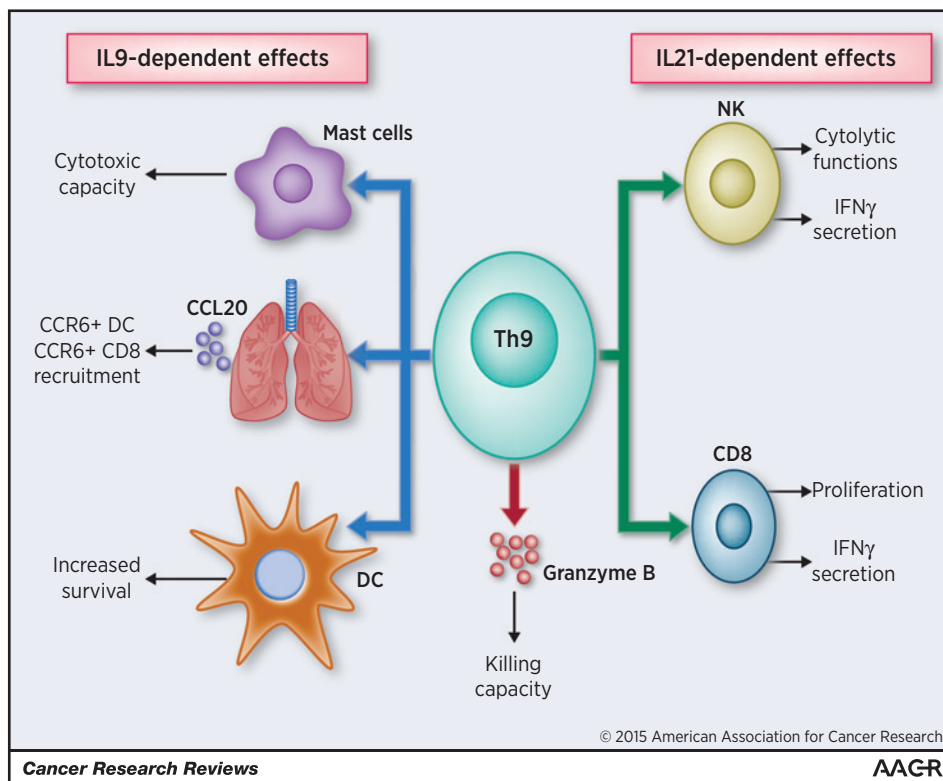
Interestingly, IL1 β stimulation boosted the antitumor activity of Th9 cells in different models of adoptive cellular therapy (15). Th9 cells differentiated with IL1 β exert antitumor effects in the ovalbumin-B16F10 model, the ovalbumin-LLC model and in the B16F10 model using TCR transgenic mice, which recognize tyrosinase-related protein 1 (TRP-1), a melanocyte differentiation antigen expressed by B16F10. More importantly, IL1 β -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 IL1 β -induced Th9 cells had an antitumor effect on primary ocular tumors, but only IL1 β -induced Th9 cells inhibited the onset of metastasis.

Conventional Th9 cells produce large amounts of IL9 and small amounts of IL21, and their anticancer effects are dependent on IL9. In contrast, for Th9 cells differentiated in the presence of IL1 β , IL9 blockade has a minor effect on their anticancer 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 IL1 β 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 IFN γ 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 IFN γ production by NK and CD8 cells. This IFN γ produced by host NK and CD8 T cells was required for the anticancer 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 IL1 β -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

**Figure 2.**

Antitumor activity of Th9 cells. Th9 cells have anticancer properties via granzyme B release. On the other hand, IL9 can increase the cytotoxic capacity of mast cells and DC survival. IL9 also activates epithelial lung cells to produce CCL20, which attracts CCR6⁺ DC and CCR6⁺ CD8 into the tumor bed. IL21-dependent effects occur through CD8 proliferation and increases in NK cytolytic functions. IL21 derived from Th9 cells induces IFN γ production by NK and CD8 cells.

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 IL1 β 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 IFN γ -secreting Th1 cells. However, Th17 cells convert into IFN γ -producing cells after adoptive transfer, and

tumor rejection is mainly dependent on the secretion of IFN γ (36, 37). This transdifferentiation is not observed in the case of Th9 cells differentiated in presence of IL1 β . These cells conserved their ability to produce IL9 and IL21 days after *in vivo* injection. In addition, Th9 cells differentiated in presence of IL1 β antitumor activity are strictly dependent on IL21, and IFN γ -deficient Th9 conserved their anticancer function. In contrast, Th9 cells differentiated without IL1 β 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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