OncoImmunology

ISSN: (Print) 2162-402X (Online) Journal homepage: http://www.tandfonline.com/loi/koni20

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To cite this article: Il-Kyu Kim, Yeonseok Chung & Chang-Yuil Kang (2015): GITR drives T_H9-mediated antitumor immunity, OncoImmunology, DOI: 10.1080/2162402X.2015.1122862

To link to this article: http://dx.doi.org/10.1080/2162402X.2015.1122862

Accepted author version posted online: 29 Dec 2015.
Published online: 29 Dec 2015.

ONCOIMMUNOLOGY
2016, VOL. 0, NO. 0, e1122862 (3 pages)
http://dx.doi.org/10.1080/2162402X.2015.1122862

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AUTHOR'S VIEW

GITR drives T_H9-mediated antitumor immunity

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ABSTRACT

T_H9 cells have been implicated in triggering antitumor immunity. We have identified that GITR co-stimulation inhibits iT_{reg} cell generation but drives T_H9 cell differentiation, thereby suppressing tumor growth via enhancing the function of DCs and CTLs *in vivo*. Our findings provide novel mechanisms by which GITR agonists exert antitumor activity.

ARTICLE HISTORY
Received 16 November 2015
Accepted 16 November 2015

KEYWORDS
Antitumor immunity; GITR; iT_{reg} cell; IL-9; T_H9 cell

Increasing evidence has indicated the advantages of $CD4^+$ T helper (T_H) cells in the treatment of cancer in experimental animals as well as in humans.[1,2] The IL-9-producing $CD4^+$ T (T_H9) cell population is a newly identified T_H subset and has been shown to play roles in mediating parasite expulsion, allergic inflammation and antitumor immunity.[3] Notably, T_H9 cells have been shown to exert the greatest antitumor activity among the T_H subsets in melanoma rejection.[4] Hence, the induction of T_H9 cell immunity might provide an efficacious strategy for the treatment of tumors in humans.

Glucocorticoid-induced tumor necrosis factor receptor (TNFR)—related protein (GITR) is one of the molecules in the TNFR family that co-stimulates T cells. Treatment with GITR agonists showed strong antitumor effects in various tumor models.[5] However, it has been unclear how GITR co-stimulation on T cells generates antitumor activity. In a recent study, we have found that GITR signaling profoundly enhances T_H9 cell differentiation and that IL-9 production is required for the antitumor activity mediated by GITR agonists.[6]

First, we examined whether IL-4Ra signaling plays any role in tumor rejection mediated by the anti-GITR agonistic antibody DTA-1 in a CT26 colon cancer model because DTA-1 treatment increased the expression of IL-4 and IL-13 in tumor-bearing hosts. Although T_H2 cell immunity has been suggested as protumorigenic, it is also known to mediate antitumor immunity.[7] We found that IL-4Ra signaling was essential for GITR agonist-induced tumor regression by showing that the tumor growth in IL-4Ra knockout (*Il4ra*$^{-/-}$) mice was not affected by DTA-1. Notably, we observed that treatment with DTA-1 substantially enhanced IL-9 expression in WT tumor-bearing hosts, while it failed to do so in *Il4ra*$^{-/-}$ recipients. This result prompted us to analyze the effect of IL-9 on antitumor immunity induced by GITR stimulation. GITR ligation *in vivo* upregulated IL-9 expression in $CD4^+$ T cells as early as 2 d after DTA-1 treatment. More importantly, the inhibition of tumor growth induced by DTA-1 was markedly reversed by a neutralizing antibody to IL-9. In addition, we employed a mouse model of B16 melanoma expressing ovalbumin (OVA) and compared the antitumor activity of adoptively transferred OVA-specific T_H9 cells generated with or without GITR co-stimulation. In this experiment, we observed that GITR engagement endowed donor T_H9 cells with superior antitumor efficacy in an IL-9-dependent manner. Based on this finding, we concluded that $CD4^+$ T cells are the main cell type that responds to GITR co-stimulation to induce IL-9-dependent tumor regression.

Due to the lack of IL-9 receptor expression on CT26 and B16 tumor cells, we thought IL-9 acted on the intermediary cells rather than the tumors to exert antitumor activity. When we measured $CD8^+$ cytotoxic T lymphocyte (CTL) responses, we found that tumor-specific cytolytic activity and cytokine and cytolytic marker expression (granzyme B, IFN-γ, TNF-α and CD107a) were all enhanced in CTLs treated with DTA-1 in an IL-9-dependent manner. Interestingly, although IL-9 expression was rapidly upregulated upon GITR ligation as early as day 2, we hardly detected tumor-specific CTL responses and the expression of related effector molecules in tumor-specific CTLs at the early time points. However, these were detected approximately a week after DTA-1 treatment in our experimental setting. In addition, our *in vitro* study revealed that IL-9 did not directly affect $CD8^+$ T cell cytotoxicity. These observations led us to hypothesize that there must be other mediator(s) that responds to IL-9 and stimulates CTL responses *in vivo*. In a recent paper, Lu *et al.* have demonstrated that T cell-derived IL-9 chemoattracted dendritic cells (DCs) into the tumor site, thereby mediating T_H9 antitumor immunity through the activation of tumor-specific CTLs.[8] We observed that DCs accumulated in the tumor tissue of DTA-1-treated mice, expressed higher levels of CD80, CD86 and MHC class II, and cross-presented tumor antigen (Ag) more efficiently than those of control IgG-treated mice, which were also affected by IL-9.

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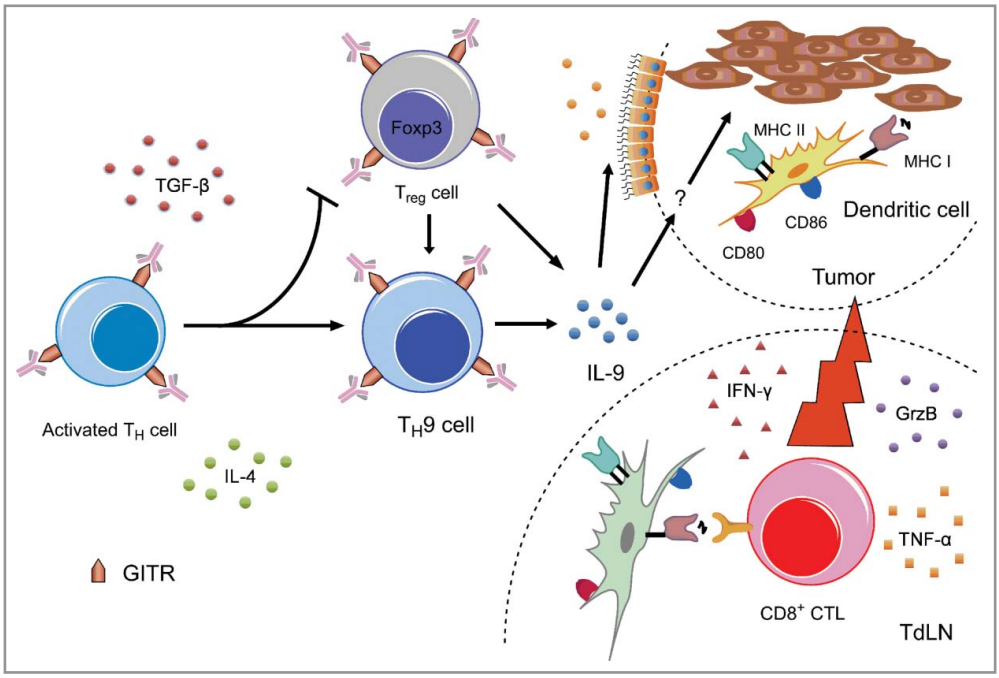

Figure 1. A schematic illustration of T_H9 cell-mediated antitumor immunity induced by GITR co-stimulation. GITR triggering inhibits iT_{reg} cell generation and promotes T_H9 cell differentiation. IL-9 production triggers epithelial cells to chemoattract DCs into the tumor and enhances the cross-presentation and costimulatory capacity of the tumor-infiltrating DCs. These tumor-Ag-crosspresenting DCs then potentiate tumor-specific $CD8^+$ CTL responses, thereby facilitating tumor regression.

Collectively, we propose that GITR-triggered IL-9 production from $CD4^+$ T cells promotes tumor-specific CTL responses by activating tumor-infiltrating DCs *in vivo*, which in turn eradicates tumors (Fig. 1).

Next, by using an *in vitro* culture system, we demonstrated that GITR co-stimulation preferentially enhanced mouse T_H9 differentiation in a cell-intrinsic manner. Additionally, we observed an increase in human T_H9 differentiation when human GITR was triggered by a stimulatory antibody, suggesting that GITR stimulation might be capable of inducing T_H9 responses in humans. Notably, under induced T_{reg} (iT_{reg}) cell-polarizing conditions, GITR co-stimulation inhibited iT_{reg} cell generation and diverted the differentiation of $CD4^+$ T cells toward T_H9 effector cells (Fig. 1). Hence, GITR co-stimulation might have two advantages: (i) eliminating potential immune suppressors by inhibiting iT_{reg} cell generation in tumor sites and (ii) promoting antitumor T_H9 effectors that repress tumor growth. A recent study by Xiao et al. supports this notion by showing that this phenomenon could take place *in vivo* in lymphopenic tumor-bearing hosts.[9] Moreover, we demonstrated that reprogramming established T_{reg} cells into IL-9-producing cells is possible when they are simulated with a GITR agonist in the presence of IL-4.

In summary, our study demonstrated a role for GITR co-stimulation in T_H9 cell development and its functional cascade in the tumor microenvironment. However, it remains unanswered which cell type is the direct target of IL-9 to facilitate tumor-Ag cross-presentation by DCs. We speculate that cytolytic innate cells such as eosinophils,[10] macrophages, NK cells and other cell types might be involved in this process. It will be important to investigate whether the antitumor activity of the GITR-agonistic antibody in human cancer patients is also IL-9-dependent. A combination of IL-9-inducing GITR agonists with immune checkpoint blockers likely further improves antitumor immunity *in vivo*.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This study was supported by the National R&D Program for Cancer Control, Ministry of Health and Welfare (No. 0720500, C.-Y. Kang) and the Mid-career Researcher Program (No. 2015R1A2A1A10055844, C.-Y. Kang) of the National Research Foundation of Korea (NRF).

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