

AUTHOR'S VIEW

GITR drives T_H9-mediated antitumor immunity

Il-Kyu Kim^{a,b}, Yeonseok Chung^{c,d}, and Chang-Yuil Kang^{a,b}

^aLaboratory of Immunology, Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul, Republic of Korea; ^bDepartment of Molecular Medicine and Biopharmaceutical Science, Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Republic of Korea; ^cLaboratory of Immune Regulation, Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul, Republic of Korea; ^dCenter for Immunology and Autoimmune Diseases, Institute of Molecular Medicine, The University of Texas Medical School at Houston, Houston, TX, USA

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.

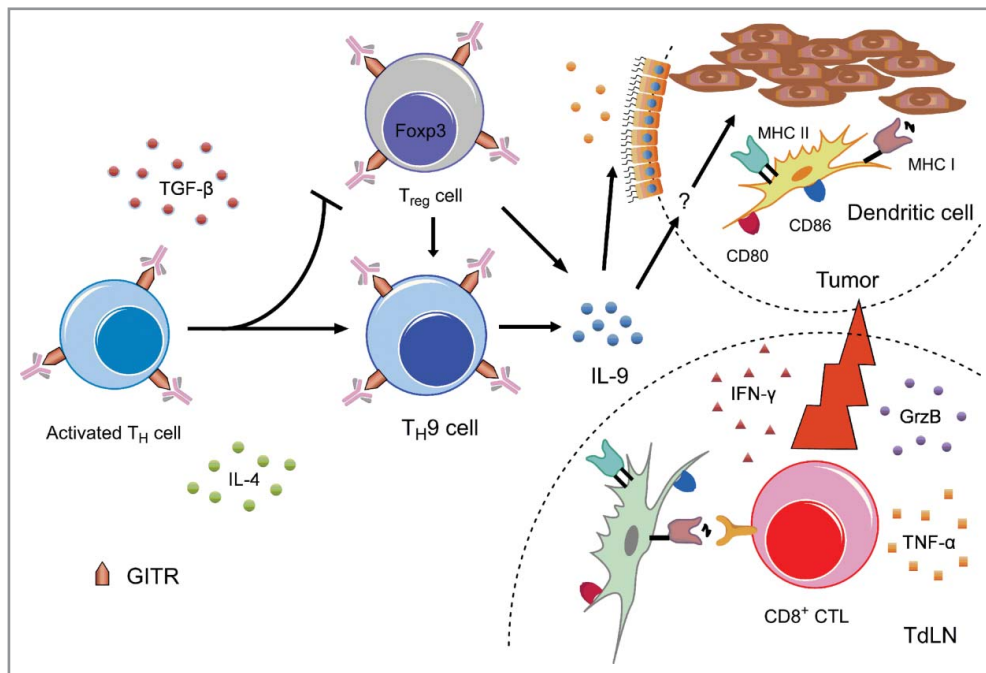


Figure 1. A schematic illustration of T_H9 cell-mediated antitumor immunity induced by GITR co-stimulation. GITR triggering inhibits iT_{reg} cell generation and promotes T_H9 cell differentiation. IL-9 production triggers epithelial cells to chemoattract DCs into the tumor and enhances the cross-presentation 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 anti-tumor 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).

References

1. Wang RF. The role of MHC class II-restricted tumor antigens and $CD4^+$ T cells in antitumor immunity. *Trends Immunol* 2001; 22:269-76; PMID:11323286; [http://dx.doi.org/10.1016/S1471-4906\(01\)01896-8](http://dx.doi.org/10.1016/S1471-4906(01)01896-8)
2. Muranski P, Restifo NP. Adoptive immunotherapy of cancer using $CD4^+$ T cells. *Curr Opin Immunol* 2009; 21:200-8; PMID:19285848; <http://dx.doi.org/10.1016/j.coi.2009.02.004>
3. Schmitt E, Klein M, Bopp T. Th9 cells, new players in adaptive immunity. *Trends Immunol* 2014; 35:61-8; PMID:24215739; <http://dx.doi.org/10.1016/j.it.2013.10.004>
4. Purwar R, Schlapbach C, Xiao S, Kang HS, Elyaman W, Jiang X, Jetten AM, Khoury SJ, Fuhlbrigge RC, Kuchroo VK et al. Robust tumor immunity to melanoma mediated by interleukin-9-producing T cells. *Nat Med* 2012; 18:1248-53; PMID:22772464; <http://dx.doi.org/10.1038/nm.2856>
5. Schaer DA, Murphy JT, Wolchok JD. Modulation of GITR for cancer immunotherapy. *Curr Opin Immunol* 2012; 24:217-24; PMID:22245556; <http://dx.doi.org/10.1016/j.coi.2011.12.011>
6. Kim IK, Kim BS, Koh CH, Seok JW, Park JS, Shin KS, Bae EA, Lee GE, Jeon H, Cho J et al. Glucocorticoid-induced tumor necrosis factor receptor-related protein co-stimulation facilitates tumor regression by

- inducing IL-9-producing helper T cells. *Nat Med* 2015; 21:1010-7; PMID:26280119; <http://dx.doi.org/10.1038/nm.3922>
7. Ellyard JI, Simson L, Parish CR. Th2-mediated anti-tumour immunity: friend or foe? *Tissue Antigens* 2007; 70:1-11; PMID:17559575; <http://dx.doi.org/10.1111/j.1399-0039.2007.00869.x>
 8. Lu Y, Hong S, Li H, Park J, Hong B, Wang L, Zheng Y, Liu Z, Xu J, He J et al. Th9 cells promote antitumor immune responses in vivo. *J Clin Invest* 2012; 122:4160-71; PMID:23064366; <http://dx.doi.org/10.1172/JCI65459>
 9. Xiao X, Shi X, Fan Y, Zhang X, Wu M, Lan P, Minze L, Fu YX, Ghobrial RM, Liu W et al. GITR subverts Foxp3(+) Tregs to boost Th9 immunity through regulation of histone acetylation. *Nat Commun* 2015; 6:8266; PMID:26365427; <http://dx.doi.org/10.1038/ncomms9266>
 10. Carretero R, Sektioglu IM, Garbi N, Salgado OC, Beckhove P, Hammerling G. Eosinophils orchestrate cancer rejection by normalizing tumor vessels and enhancing infiltration of CD8(+) T cells. *Nat Immunol* 2015; 16:609-17; PMID:25915731; <http://dx.doi.org/10.1038/ni.3159>