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ORIGINAL ARTICLE

A critical role for regulatory T cells in driving cytokine profiles of Th17 cells and their modulation of glioma microenvironment

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Abstract IL-17A, produced by Th17 cells, may play a dual role in antitumor immunity. Using the GL261-glioma model, we investigated the effects of Th17 cells on tumor growth and microenvironment. Th17 cells infiltrate mouse gliomas, increase significantly in a time-dependent manner similarly to Treg and do not express Foxp3. To characterize the direct effects of Th17 cells on GL261 murine gliomas and on tumor microenvironment, we isolated IL-17-producing cells enriched from splenocytes derived from naïve (nTh17) or glioma-bearing mice (gTh17) and pre-stimulated in vitro with or without TGF-β. Spleen-derived Th17 cells co-expressing IL-17, IFN-γ and IL-10, but not Treg marker Foxp3, were co-injected intracranially with GL261 in immune-competent mice. Mice co-injected with GL261 and nTh17 survived significantly longer than gTh17 ($P < 0.006$) and gliomas expressed high level of IFN-γ and TNF-α, low levels of IL-10 and TGF-β. In vitro IL-17 per se did not exert effects on GL261 proliferation; in vivo gliomas grew equally well intracranially in IL-17 deficient and wild-type mice. We further analyzed relationship between Th17 cells and Treg. Treg were significantly higher in splenocytes from glioma-bearing than naïve mice ($P = 0.01$) and gTh17 produced more IL-10 than IFN-γ ($P = 0.002$). In vitro depletion of Treg using PC61 in splenocytes from glioma-bearing mice causes increased IL-17/IFN-γ cells ($P = 0.007$) and decreased IL-17/IL-10 cells ($P = 0.03$). These results suggest that Th17 polarization may be induced by Treg and that Th17 cells in gliomas modulate tumor growth depending on locally produced cytokines.

Keywords Th17 cells · Regulatory T cells · Glioma microenvironment · IL-17A

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Introduction

Th17 cells are a subset of T helper cells producing IL-17. They play a role in inflammation and tissue injury [1] and their dysregulation may cause autoimmune diseases [2, 3]. Simultaneous presence of TGF-β and IL-6 may drive the differentiation of murine Th17 cells [4]. Mechanisms causing the formation of human Th17 cells are not well defined. Recent observations suggest that IL-1 in combination with IL-6 and IL-23 provide an optimal cytokine cocktail for Th17 differentiation [5–7]. Other studies suggest that TGF-β is also important for human Th17 generation [8–10]. The involvement and function of Th17 cells in cancer must be elucidated. Th17 cells have been investigated in several human cancer such as ovarian

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characterized by the co-expression of cytokines IFN-γ and IL-17 and the transcription factors T-bet and RORγt [27]. In this regard, our results show that ex vivo but not in vitro nTh17 cells express T-bet, while TGF-nTh17 cells show decreased T-bet expression in agreement with the observation that TGF-β blocks T-bet induction and IFN-γ expression [28] (Supplemental Fig. 4e–f). In vivo Th17 cells could undergo an evolution in their ability to secrete cytokines; in addition their antitumor or tumor-promoting effect might not be mutually exclusive, but dependent on timing and tumor microenvironment. These conditions are supported by opposite results obtained using intracranial co-injection of GL261 and nTh17 or gTh17 cells. nTh17 cells produce higher quantities of IFN-γ than the other fractions inducing antitumor effect in early stages of glioma; on the contrary, gTh17 cells might be generated at later stages under the effect of immunosuppressive microenvironment and the interaction with Treg.

The other key issue that our experiments have addressed is represented by the controversial role of IL-17 in tumors. In particular, IL-17 can have a pro-tumorigenic activity in immune-deficient mice [29, 30] or an antitumorigenic activity in immune-competent mice [31]. Recently, opposite results were obtained from two groups on the same experimental tumor, MC38 [32, 33]. When we injected GL261 in IL-17 KO or wild-type mice, we did not find differences in tumor initiation and growth, suggesting that IL-17 per se has no significant effect on tumor progression.

It has been reported that, in experimental murine tumors, peripheral Treg can be converted into Th17 under the influence of inflammation [23, 34, 35]. In addition, IL-17 has been shown to exert pro-inflammatory effects on astrocytes and microglial cells into the CNS through direct interaction with their constitutively expressed IL-17 receptor (IL-17R). Above all that, the response of glial cells to IL-17 depends on the interaction between IL-17 and other cytokine signaling systems. Only one report showed that few established glioma cell lines express IL-17R and that IL-17 can stimulate the secretion of IL-6 and IL-18 [36]. GL261 cells express IL-17R, but exogenous IL-17 does not provide a direct effect on GL261-cell proliferation, supporting the idea that Th17 cells may contrast or favor tumor development depending on the immunological context, as demonstrated by the presence of IFN-γ or IL-10.

The tumor inhibitory activity of Th17 cells depends on the production of IFN-γ that in this setting seems to function as an anti-proliferative factor rather than as an immune stimulatory factor. We previously suggested that high concentrations of IFN-γ are able to reduce GL261 proliferation [26], and now, we found that the simultaneous injection of GL261 with IFN-γ-producing Th17 cells could favor such anti-proliferative effect. Interestingly, Muranski et al. [17] reported that the therapeutic effect of Th17 cells on a murine model of melanoma critically depends on IFN-γ production and that IL-17 depletion has a little influence on this effect. Th17 cells also seem to indirectly mediate an antitumor activity, by promoting the recruitment of other effector cells [37, 38] or by eliciting pro-inflammatory conditions that can promote CD8+ effector cells [16]. On the contrary, Th17 might favor tumor development by inhibiting CD8+ cell infiltration and enhancing myeloid-derived suppressor cells at tumor site [39].

In summary, Th17-mediated effects in vivo might depend on the accumulation of Treg during glioma development. The ability of these cells to secrete IFN-γ as previously demonstrated [40] might result in a change of plasticity or in the ability to secrete IL-10 or IFN-γ, due to the presence of Treg. These results should be considered for further evaluations of the Th17 cell involvement in human antitumor activity.

A question that remains open is whether tumor-infiltrating Th17 cells are recruited from the periphery or induced in tumor microenvironment. Many factors released by tumor cells, such as TGF-β, IL-6, PGE2 and TNF-α, play a role in the induction of Th17 differentiation [41]. Moreover, recent observations showed that dendritic cells, derived from monocytes and migrating across the blood–brain barrier, secrete IL-12, TGF-β and IL-6, thus promoting the proliferation and expansion of IL-17-secreting Th17 CD4+ T lymphocytes [42].

Th17 cell regulation could provide useful insights in immunotherapy protocols. For example, in prostate cancer, a higher frequency of Th17 cells before immunotherapy is correlated with a shorter time to metastatic progression [43]. In gliomas, the role of Th17 cells could vary during tumor development: IFN-γ-producing Th17 cells could be inversely related to Treg and thus considered as predictors of stronger antitumor reactivity.

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Conflict of interest The authors declare that they have no competing interests.

References

1. Steinman L (2007) A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. Nat Med 13:139–145
2. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT (2005) Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 6:1123–1132