ONCOIMMUNOLOGY
2016, VOL. 5, NO. 2, e1082707 (2 pages)
http://dx.doi.org/10.1080/2162402X.2015.1082707

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AUTHOR'S VIEW

MAF drives CD8$^+$ T-cell exhaustion

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ABSTRACT
The molecular regulation of tumor induced T-cell exhaustion remains poorly characterized. Recently, we compared the transcriptome of "exhausted" CD8$^+$ T cells infiltrating melanomas to those of naive and acutely stimulated CD8$^+$ T cells. We demonstrated that *MAF* is over-expressed and plays a key role in driving the transcriptional program of T-cell exhaustion.

ARTICLE HISTORY
Received 6 August 2015
Accepted 8 August 2015

KEYWORDS
CD8$^+$ T cell; exhaustion; *maf*; melanoma

Numerous studies in humans and in mice clearly established that T cells can recognize tumor cells and control tumor progression. However, despite evidence for spontaneous immune activation in a limited number of cancer patients, the immune system most frequently fails to protect patients with metastatic cancers as tumors develop strategies to escape T-cell-mediated immunity. We know that multiple mechanisms diminish antitumor T-cell responses. These include, for instance, the concomitant accumulation of high numbers of regulatory T cells and myeloid-derived suppressor cells. Further cells and factors contribute to the immunosuppressive tumor micro-environment which inhibits anticancer effector T cells.

A major limitation is the T-cell intrinsic attenuation of tumor-antigen-specific T cells to exert effector functions against tumors. So far, the prevailing explanation for this failure was that tumor-targeting T cells become tolerized or anergized over time. More recently, another T-cell differentiation pathway has been suggested, and different lines argue in favor of the fact that tumor-reactive T cells undergo similar differentiation as antigen-specific T cells in chronic infection, i.e. they undergo "exhaustion" and become functionally attenuated. This state has been thoroughly studied during chronic viral infection, both in mouse and human. It is characterized by the expression of multiple inhibitory receptors (PD-1, CTLA-4, TIM-3…) found to be also expressed on functionally impaired tumor-infiltrated lymphocytes (TILs) (reviewed in[1,2]). These phenotypical and functional similarities led to the extension of the term exhaustion to TILs. However, scarce details are available on the transcriptional program of TILs and on the transcription factors regulating this state.

In our recent study,[3] we took advantage of a mouse model of induced melanoma based on conditional deletion of tumor suppressor genes with concomitant expression of a natural mouse tumor antigen (TiRP mice). In this model, tumor-intrinsic factors control the development of aggressive tumors and their expression of an inflammatory/immunosuppressive program.[4] Intra-tumor T cells expressed high levels of inhibitory receptors such as PD-1 and had poor capacity to produce IFNγ upon restimulation, suggesting that they are exhausted. Using this model, we have established the gene expression signature associated with CD8$^+$ T-cell exhaustion during melanoma development. We showed that tumor- and virus-induced exhaustion share many features, with expression of genes encoding molecules such as inhibitory receptors or particular transcription factors. Among the latter, we focused our study on the two transcription factors with the highest fold increase in TILs compared to effector T cells. *Nr4a2*, encoding an orphan nuclear receptor, was highly expressed in both virus- and tumor-induced exhaustion, whereas *maf* was highly overexpressed in tumor-exhausted CD8$^+$ T cells and only very weakly during chronic viral infection. We confirmed the overexpression for both genes in Melan-A/MART-1 specific CD8$^+$ T cells isolated from tumor-infiltrated lymph nodes (TILN) from melanoma patients.

Importantly, overexpression of *maf* by retroviral transduction of tumor-specific CD8$^+$ T cells dampened their intratumor accumulation and antitumor activity, while overexpression of *nr4a2* did not affect CD8$^+$ T-cell properties. We went on to show that *maf* expression in antitumor CD8$^+$ T cells contributes to their polarization toward an exhausted phenotype, with high expression of exhaustion associated genes such as *il10*, *bcl6*, *pdcd1* and others (Fig. 1). We demonstrated *in vitro* that *maf* expression could be regulated by IL-6 and/or TGFβ when CD8$^+$ T cells are stimulated. These cytokines are produced at high levels in our TiRP melanoma and appear to be important regulators of exhaustion in this model. *In vitro*, these cytokines could partially mimic the transcriptional program associated with exhaustion, inducing higher levels of *bcl6* or *il10* transcripts and dampening the expression of transcripts encoding for *ifng* or *gzmb*. However, the expression of some other genes were not affected (*nr4a2*, *nr4a3*, *rgs1*or *rgs16*) showing that the action of the microenvironment on T cells is much more complex than our *in vitro* system. Using *maf* knockout mice in similar experiments, we established that *maf*$^{-/-}$ CD8$^+$ T cells were much more resistant to the presence of these inhibitory cytokines in terms of proliferation, survival

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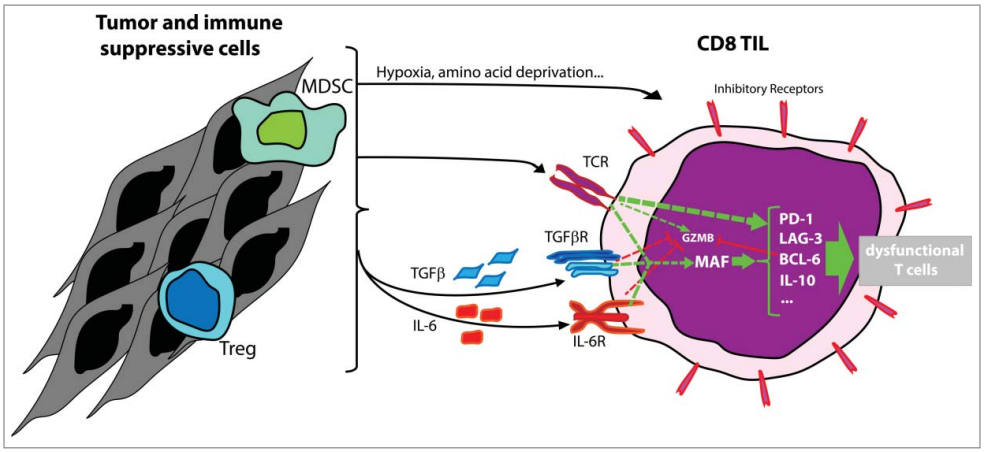

Figure 1. *In vivo*, tumor infiltrating lymphocytes (TIL) found inside melanoma express high level of *MAF*. *In vitro*, overexpression of *MAF* can be induced by TCR triggering together with IL-6, TGFβ or both. Inside the tumor microenvironment, *maf* expression could also be promoted by the production of other factors such as hypoxia, amino acid deprivation, other cytokines produced by tumor cells or immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs) or regulatory T cells (Treg). In $CD8^+$ TILs, *MAF* enhances the expression of *pdcd1*, *lag3, bcl6, il10* and further genes associated with exhaustion, leading to the impairment of T-cell function.

and production of cytokines. *In vivo*, we also demonstrated that $maf^{-/-}$ tumor specific $CD8^+$ T cells had heightened capacity to restrain tumor growth correlated with a better accumulation inside the tumor, a higher capacity to produce IFNγ upon restimulation and lower surface expression of PD-1 and LAG-3.

Maf plays central role in several subtypes of $CD4^+$ T cells such as Th2 cells (IL-4 production[5]), Th17 cells (IL-10 and IL-22 production[6,7]) or Tfh.[8] To our knowledge, our study was the first to show high level of *maf* in $CD8^+$ T cells *in vivo*. How general this high expression is in other tumor types still needs to be determined. Nevertheless, it is clear that high level of *maf* leads to a biased polarization toward an exhausted phenotype, is deleterious to cytotoxic functions of $CD8^+$ T cells, and prevents Th1 differentiation in $CD4^+$ T cells. Therefore, *maf* represents an interesting target to improve immunotherapy of cancer.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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