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Tregs in gliomas – the jury is still out

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See the article by Thomas et al., on pages 801-809.

Regulatory T cells (*Tregs*) promote tumor growth chiefly by suppressing tumor-specific T cell responses in the tumor tissue. This is the common notion that provides the rational for targeting *Tregs* in cancer with the aim at enhancing antitumor immune responses. If this rational is true and if we think that there is a natural – albeit insufficient - antitumor immune response, then tumor-infiltrating and/or circulating *Tregs* should influence the natural course of tumor disease with high numbers predicting poor outcome. Conversely, the number of intratumoral T cells with antitumor properties, particularly CD8+ cytotoxic T lymphocytes, ought to predict a favorable outcome. While most studies analysing tumor-infiltrating T cells in gliomas support the latter, there has been ambiguous data on the relevance of tumor-infiltrating *Tregs* in human gliomas.[1–6]

Tregs are classically viewed synonymous with FoxP3 + CD25 + CD4+ T cells. These cells are usually identified by flow cytometry from peripheral blood or preparations of mononuclear cell suspensions from fresh tumor specimens. In the current study by Thomas and colleagues (*this issue*) tumor-infiltrating *Tregs* and CD3+ T cells were measured not by flow cytometry but by epigenetic qPCR and by immunohistochemistry. This approach offers the significant advantage of performing analyses on archival tissue but at a risk of losing specificity. Although FoxP3 transcripts and methylation patterns are generally considered to be specific for *Tregs*, expression has been detected in CD8+ T cells[7] and non-lymphoid CNS tissue.[8] Hence, *Treg* quantifications based on FoxP3 expression without single-cell resolution have to be viewed with caution. With their methods Thomas and colleagues did not find peripheral or tumor-infiltrating *Tregs* to be predictive of outcome in their small study cohort.

Does this and the negative results of most previous studies mean that *Tregs* are not important in glioma biology? FoxP3 + CD4+ *Tregs* in humans comprise several subsets, which are heterogeneous both with respect to phenotype and function. For instance, FoxP3^{lo}CD45RA + CD25^{lo} cells are naive or resting *Treg* (r*Treg*) cells, which differentiate into highly suppressive FoxP3^{hi}CD45RA-CD25^{hi} effector *Treg* (e*Treg*) cells upon antigenic stimulation. In contrast, FoxP3^{lo}CD45RA-CD25^{lo} non-*Treg* cells do not possess suppressive activity but can secrete pro-inflammatory cytokines.[9] In addition, there is an increasing arsenal of additional markers subdividing the *Treg* family including CD127, Helios, CTLA-4 and CD39. Furthermore, the diversity of T cells extends well into the CD8+ compartment including FoxP3 expression.[7] Future studies ought to incorporate the complexity of *Treg* development and differentiation including antigen-specificity to answer this question.

Is this a worthwhile effort or just *l'art pour l'art*? With the increasing arsenal of checkpoint inhibitors spilling over into the glioma arena we need to provide answers to the central questions: Is there a meaningful natural (antigen-specific) anti-glioma T cell immunity and if so is this immunity suppressed by cellular mediators, such as *Tregs*, resulting in a net poorly immunogenic tumor phenotype? Because only the presence of a meaningful natural anti-glioma T cell immunity would then provide the rational to release the break on this anti-glioma T cell immunity by introducing checkpoint inhibitors or other strategies to inhibit tumor-associated immune suppression (e.g. *Tregs*) as single agents. This has been demonstrated for melanoma,[10] but solid evidence for gliomas is lacking. Consequently, clinical studies testing checkpoint inhibitors in gliomas clearly should be backed by an in-depth analysis of pre- and post-treatment tumor tissue including analyses of tumor-infiltrating *Tregs* and the repertoire of antigen-specific effector T cells. In addition to the relevance of *Tregs* for suppressing the natural anti-glioma immunity, this immune cell population may constitute a significant barrier for antigen-specific peptide vaccines, which are on the verge of entering the clinical arena.[11–15] If tumor-infiltrating *Tregs* (or even circulating *Tregs*) counteracted this induced antigen-specific anti-glioma T- and B cell immunity, then this would again constitute a strong rationale for combining peptide vaccines with checkpoint inhibitors, agents depleting *Tregs* or simply temozolomide chemotherapy upfront. Also here, clinical trials evaluating the efficacy of antigen-specific vaccines ought to incorporate pre- and post-treatment analyses of mechanisms preventing the induction of an effective anti-glioma immune response including *Tregs*. Importantly, these analyses need to take into account

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the heterogeneity and tissue-specificity of *Tregs*, which extends well beyond the classic CD4 + CD25 + FoxP3+ phenotype, which most studies including the study by Thomas and colleagues are restricted to.

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