In vivo expansion of regulatory T cells by GITR triggering

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Regulatory T cells (Tregs) are specialized cells that control immune responses to pathogens and mediate immunological self-tolerance and homeostasis.[1] *Glucocorticoid-induced TNFR-related* (GITR, also know as TNFRSF18) plays a crucial role in the maturation and expansion of Tregs.[2,3,4] Indeed, functional studies demonstrated that $GITR^+$-depleted T cells injected in nude mice caused death in 90% of them due to autoimmune disease[5], whereas injection of $CD25^+$-depleted T cells did not cause death.

Cells with suppressive activity express high levels of GITR in FoxP3 transgenic mice[6,7] and GITR expression is observed independently of CD25, demonstrating that $CD4^+CD25^-GITR^+$ cells (spGITR cells) exist and form a subset of peripheral Tregs (pTregs) that express molecules associated with regulatory activity, such as CTLA-4 and IL-10.[6] GITR triggering costimulates Tregs, favouring their expansion. However, GITR triggering favors expansion/activation of CD4+ and CD8+ effector T cells too.

The aim of our in vivo study was to expand $GITR^+$ Treg subsets with low doses of solely anti-mouse GITR antibody (an IgM produced by the rat hybridoma G3C), which has different properties from the most used anti-GITR Ab (DTA1)[8], in long term treatment experimental conditions.

First we demonstrated by flow cytometry that in $CD4^+$ T cells GITR expression is higher in the $FoxP3^+CTLA4^+$ double positive cell population (witch is the sub-population with the higher regulatory activity) compared to that in $FoxP3^+$ or $CTLA4^+$ single positive cells. The lowest levels of GITR expression were found in $FoxP3^-CTLA4^-$ double negative cells, further suggesting that GITR expression correlates with a regulatory phenotype.

Then, we encapsulated the rat G3C hybridoma cells within the alginate-based microcapsule through an already described technique.[9] By this method is possible to implant microencapsulated syngenic or allogeneic live cells into the body without the rejection of the xenografts; the capsules are designed so that IgM produced by the hybridoma can be released.

We implanted microencapsulated G3C hybridoma cells (mecG3C) in the peritoneum of SV129 mice deleted of GITR ($GITR^{-/-}$) or wild type (WT). As a further control, empty microcapsules (mec) were implanted. After 3 weeks mice were sacrificed and studied. We observed that: 1) G3C-derived IgMs were detectable in the serum of the mecG3C-treated mice (as demonstrated by a home-made ELISA assay), demonstrating that G3C cells produce the anti-GITR IgMs and release them; 2) spGITR cell subset was expanded in the spleen and lymph nodes of mecG3C-treated WT mice as compared to mec-treated WT mice, but the expansion was not observed in mecG3C-treated $GITR^{-/-}$mice, demonstrating that anti-GITR IgMs expand spGITR subset by triggering GITR; 3) CD4+C25+ cells were decreased in mecG3C-treated WT mice as compared to mec-treated WT mice, suggesting that spGITR expansion decreases the number of activated T cells; 4) CD8+ cells were decreased in mecG3C-treated WT mice as compared to mec-treated WT mice but not in mecG3C-treated $GITR^{-/-}$ mice, suggesting that spGITR expansion decreases the number of cytotoxic T cells.

These results indicate that long term treatment with low doses of an anti-mouse GITR antibody can expand Treg subsets and these treatment may be useful to cure autoimmunity diseases.

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