Prevention of diabetes in NOD mice by increasing GITR positive Treg cells by low doses of anti-GITR antibody

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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, both thymus-derived (tTreg) and peripherally derived (pTreg).2-4 In particular, CD4+CD25-/lowGITRint/high (CD4+GITRsp) cells are pTregs with a memory phenotype, described both in humans and in mice.5-6 It is known that tTregs and CD4+GITRsp pTregs are expanded in transgenic mice that overexpress the ligand of GITR (GITRL).7 Thus, we investigated whether GITR triggering by an antibody (Ab) had a similar effect and whether treatment may be of help in autoimmune diseases.

To this aim, we first verified whether anti-mouse GITR Ab (an IgM produced by the rat hybridoma G3C) expanded Tregs in mice following long-term treatment. In particular, we transferred G3C-releasing hybridoma cells incorporated within specially formulated ultrapure alginate-based microcapsules8 (HY/cps) in the peritoneum and evaluated Treg number in thymus, spleen and lymph nodes after 3 weeks as compared to mice treated with empty microcapsules (E/cps) and to HY/cps-treated GITR knock-out mice. In HY/cps treated mice we detected a plasma concentration of G3C Ab (0.9 □g/ml) and observed expansion of CD4+FoxP3+GITRhi Tregs (1.5 folds), CD4+GITRsp Tregs (1.7 folds), and CD8+GITRsp cells (possibly representing a new Treg subset)(2.2 folds). Moreover, we found decrease of CD4+CD25+GITR- cells (activated T cells), suggesting that GITR triggering by G3C can expand Treg subsets in vivo and that this treatment may be useful to cure/prevent autoimmune diseases.

To test this hypothesis, we studied NOD mice that develop diabetes spontaneously in a good percentage. We treated 7 NOD mice with HY/cps and 8 mice with E/cps (control) when 12 week old. All experimental animals were monitored, as far as not fasting blood glucose and body weight, on a weekly basis, and intra-peritoneal glucose tolerance test (IPGTT) when appropriate. Control animals progressively developed severe hyperglycemia, starting from week 14 to week 22, with 4/8 failing 77 days after treatment. At the same time, only 1/7 HY/cps NODs died due to hypoglycemia as possible consequence of an abscess of salivary glands. The remaining animals were euglycemic and had normal IPGTT when 27 week old (98 days after treatment). Morphological, immunocytochemical and immunophenotyping of T cells from thymus, spleen and lymph nodes are underway.

We hypothesize that the relevant increase of diabetes-free survival time in G3C-treated NOD mice is due to the expansion of islet B cell-specific Tregs promoted by G3C mAb and prevention of immune activation leading to the development of autoimmune reaction leading to diabetes. In

conclusion, GITR triggering appears to be an intriguing approach to expand Tregs and inhibit activation of effector T cells and development of autoimmune diseases such as diabetes.

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