Targeting immunosuppression by Tregs with monoclonal antibodies against GARP

Stéphanie Liénart, Julie Stockis, Olivier Dedobbeleer & Sophie Lucas

To cite this article: Stéphanie Liénart, Julie Stockis, Olivier Dedobbeleer & Sophie Lucas (2016) Targeting immunosuppression by Tregs with monoclonal antibodies against GARP, OncoImmunology, 5:3, e1074379, DOI: 10.1080/2162402X.2015.1074379

To link to this article: http://dx.doi.org/10.1080/2162402X.2015.1074379

Accepted author version posted online: 23 Feb 2016.

Submit your article to this journal

Article views: 30

View related articles

View Crossmark data
Targeting immunosuppression by Tregs with monoclonal antibodies against GARP

Stéphanie Liénart, Julie Stockis, Olivier Dedobbeleer and Sophie Lucas

de de Duve Institute, Université catholique de Louvain, Brussels, Belgium

ABSTRACT

Reducing Treg function in cancer patients should augment antitumor immune responses. We recently uncovered a mechanism of immunosuppression by human Tregs that implies transmembrane protein GARP and production of active TGF-ß1. We obtained monoclonal antibodies that block this process and could thus serve as a novel approach for cancer immunotherapy.

Regulatory T cells (Tregs) are immunosuppressive lymphocytes that are essential to maintain immunological tolerance, but detrimental in cancer or chronic infections. Transiently reducing Treg numbers or function is thus a promising immunotherapeutic approach for oncology. It has faced very limited success thus far, owing to two major hurdles in the field.

First, we lack a Treg-specific protein marker to study and target these cells in humans. Whereas transcription factor FOXP3 is restricted to Tregs in mice, it is also found in many activated non-Treg human T cells. In addition, surface proteins present at high levels on Tregs and sometimes used as Treg markers are also found on activated non-Treg T cells. This is the case for CD25, CTLA-4, GITR or OX40. Monoclonal antibodies (mAbs) against these proteins target not only Tregs but also activated T cells, confounding interpretation of their overall effects on immune responses.

Second, a variety of Treg suppressive mechanisms have been identified in mice and include production of soluble immunosuppressive cytokines, reduction of the T cell-stimulatory capacity of antigen-presenting cells, transfer of cAMP to effector T cells through GAP junctions, or production of adenosine. The importance of any one mechanism may depend on the type of immune response to suppress. Which, if any, plays a major role in humans is not known.

To study immunosuppression by human Tregs, we derived clones of these cells to circumvent the difficulty of repeatedly isolating rare and difficult-to-identify Treg populations with poorly reproducible suppressive functions. Clones were defined as Tregs if they carried a stable epigenetic mark only found in fully differentiated Tregs in both mice and humans, namely demethylation of a regulatory region of the FOXP3 gene. Our Treg clones expressed FOXP3, were suppressive in vitro and turned out to represent uniquely pure and stable cell populations available to study human Treg function. Analyzing their transcriptional profiles, we demonstrated that activated human Tregs, but not other T cells, produced active TGF-ß1. A possible contribution of soluble TGF-ß1 to immunosuppression by Tregs was in line with the fatal autoimmune phenotype of Tgfb1–/– mice, but not with the contact dependency of immunosuppression by Tregs. But then we observed that TGF-ß1-signaling in T cells co-cultured with Tregs was also contact-dependent, suggesting that active TGF-ß1 was produced close to the Treg surface. This prompted us to study the mechanisms of TGF-ß1 activation by Tregs. Indeed, most cells produce inactive forms of TGF-ß1 but very few activate the cytokine, via tightly regulated mechanisms that are cell-type specific. Virtually all immune cells produce the proTGF-ß1 precursor, cleaved to yield latent TGF-ß1 in which the C-ter fragment, or mature TGF-ß1, remains non-covalently bound to the N-ter fragment, or Latency Associated Peptide (LAP). Latent TGF-ß1 is inactive because LAP prevents TGF-ß1 binding to its receptor. Further processing is required to release mature TGF-ß1 from LAP. We and others showed that activated Tregs, but not other T cells, display on their surface latent TGF-ß1 bound to membrane protein GARP. We hypothesized and could recently demonstrate that GARP contributed to TGF-ß1 activation at the Treg surface. Out of 31 newly derived anti-GARP mAbs, two proved capable of blocking active TGF-ß1 production by human Tregs. These two anti-GARP mAbs recognize a conformational epitope that requires amino-acids GARP137–139 within GARP/TGF-ß1 complexes. The other mAbs bound other GARP epitopes and did not block TGF-ß1 activation. We assessed the activity of the blocking anti-GARP mAbs in immunodecient NSG mice grafted with human PBMCs. These mice develop graft-versus-host disease (GVHD) due to the activity of human T cells against murine tissues. Co-transfer of human Tregs attenuates GVHD, and blocking anti-GARP mAbs abrogated this protection. They did not act by depleting human Tregs in NSG mice: human Treg numbers were not
decreased, and a blocking anti-hGARP mAb carrying a mutation which precludes binding to Fc receptors retained full activity. Our results indicate that (i) GARP-mediated production of active TGF-β1 by human Tregs contributes to their immunosuppressive function in vivo and (ii) anti-GARP mAbs can inhibit Treg-mediated immunosuppression in vivo without depleting the Tregs.

The notion that active TGF-β1 even only partly contributes to suppression by Tregs is far from accepted in the field, notably because murine Tgfb1−/− Tregs show no defect in their suppressive activity in vitro. However, several reports do support a role for Treg-derived TGF-β1 in suppressing autoimmunity in mice in vivo, and it must also be considered that human Tregs may suppress through mechanisms different from those of murine Tregs.

What renders anti-GARP mAbs attractive for cancer immunotherapy?

For one thing, none of the immunostimulatory antibodies currently in clinical use act by inhibiting Treg function. Anti-CTLA-4 antibodies may act in part by depleting the Tregs inside tumors. This was demonstrated in murine models and may also hold true in patients, in whom their use is associated with severe immune-related adverse effects. It is therefore tempting to speculate that anti-GARP mAbs, which transiently inhibit Treg function without depleting them, may show less toxicity than anti-CTLA-4-based immunotherapies.

For another, anti-GARP antibodies should not affect the production of active TGF-β1 by non-Treg cells. This may prove advantageous by comparison to global inhibition with anti-TGF-β1 mAbs or TGF-β receptor kinase inhibitors, which inhibit the activity of TGF-β1 produced by all cell types. Global inhibition brings forth the risk of severe side effects, including stimulating the growth of pre-neoplastic or pre-malignant cells. Anti-GARP mAbs may allow for specific inhibition of TGF-β1 activity in immune cells suppressed by Tregs.

Active TGF-β1 released from GARP/TGF-β1 complexes at the surface of activated Tregs inhibits T lymphocytes nearby. Anti-GARP mAbs can block active TGF-β1 production by Tregs, and thus relieve Treg immunosuppression in vivo.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

9. Li MO, Wan YY, Flavell RA. T cell-produced transforming growth factor-beta controls T cell tolerance and regulates Th1- and Th17-cell differentiation. Immunity 2007; 26:579-91; PMID:17481928; http://dx.doi.org/10.1016/j.immuni.2007.03.014