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AUTHOR'S VIEW

Targeting immunosuppression by Tregs with monoclonal antibodies against GARP

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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.

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Regulatory T cells (Tregs) are immunosuppressive lymphocytes that are essential to maintain immunological tolerance, but detrimental in cancer or chronic infections.[1] 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.[1] 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.[1,2] 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.[2] 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.[2] 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.[3]

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.[4,5] 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 $GARP_{137-139}$ 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 immunodeficient 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

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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-ß1 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.[6]

The notion that active TGF-ß1 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*.[7] However, several reports do support a role for Treg-derived TGF-ß1 in suppressing autoimmunity in mice *in vivo*,[8,9] 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[10] 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-ß1 by non-Treg cells. This may prove advantageous by comparison to global inhibition with anti-TGF-ß1 mAbs or TGF-ß receptor kinase inhibitors, which inhibit the activity of TGF-ß1 produced by all cell types. Global inhibition brings forth the risk of severe side effects, including stimulating the growth of pre-neoplasic lesions, because TGF-ß1 exerts a potent cytostatic effect on pre-malignant cells. Anti-GARP mAbs may allow for specific inhibition of TGF-ß1 activity in immune cells suppressed by Tregs.

Active TGF-ß1 released from GARP/TGF-ß1 complexes at the surface of activated Tregs inhibits T lymphocytes nearby. Anti-GARP mAbs can block active TGF-ß1 production by Tregs, and thus relieve Treg immunosuppression *in vivo*.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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