The Effect of a Pro-inflammatory Milieu on Tregalizumab (BT-061)-Induced Regulatory T-cell Activity

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Abstract

Background

Regulatory T cells (Tregs) are essential for maintaining normal immune homeostasis. We have previously reported that tregalizumab is a humanized, non-depleting, CD4 agonistic antibody that selectively activates Tregs. The specific functionality of tregalizumab may originate from the recognition of a unique epitope on domain 2 of CD4 that is not recognized by other anti-CD4 monoclonal antibodies. Tregalizumab is in clinical development for the treatment of rheumatoid arthritis (RA). Currently, a Phase IIb trial -*TREAT 2b*- is in progress to further evaluate the efficacy and safety of tregalizumab and define the optimal dose in combination with methotrexate (MTX) in adults with RA and an inadequate response to MTX.

Recent data have shown that pro-inflammatory cytokines may have a profound negative effect on the suppressive properties of Tregs or on the responsiveness of effector cells to suppression. Serum cytokine levels for RA patients have been reported in the range of: IL-1ß: 0-0.269 ng/mL; IL-6: 0-1.078 ng/mL; TNFα: 0.001-2.952 ng/mL [1]. Therefore, we performed *in vitro* studies to investigate the effects of pro-inflammatory cytokines on the ability of tregalizumab to activate Treg suppressive activity.

Methods

Allogenic effector T cells (Teffs) isolated from healthy volunteers were co-cultured with freshly isolated Tregs and APCs from a different blood donor (mixed lymphocyte reaction, MLR) in presence of different concentrations of cytokines as previously described by Trinschek *et al.* [2]. Cell proliferation was measured by incorporation of radioactive thymidine. Percent of suppression is derived by the ratio of the radioactive count obtained in the co-culture in presence of Treg versus no Treg. At least 3 independent experiments were performed at different days using cells isolated from different blood donors.

Results

In the absence of cytokine, activation of Tregs with tregalizumab resulted in strong suppression of Teff proliferation, on average at least 50% reduction of cell proliferation was measured with tregalizumab at 1 µg/mL. In presence of pro-inflammatory cytokines, little effects were observed. At the concentrations tested, corresponding to levels rarely measured in plasma from RA patients (up to 2000 ng/mL of IL-1ß and 500 ng/mL of IL-6), neither IL-1ß nor IL-6 inhibited tregalizumab-induced suppression of Teff proliferation. In case of TNFα, only the highest concentrations tested (50 and 100 ng/mL) had a marginal effect on tregalizumab-induced suppression.

Conclusion

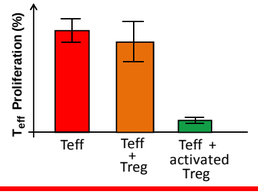
In this *in vitro* study, activation of Tregs by tregalizumab and the suppression of Teffs was not notably inhibited by pro-inflammatory cytokines, only moderately by TNFα at very high concentration. This result gives further insights into the potential of tregalizumab to activate Tregs in the presence of systemic levels of pro-inflammatory cytokines that are elevated in autoimmune diseases such as RA. Further *in vitro* investigations are in progress to determine if the observed moderate effect of TNFα is the result of a reduction of Treg suppressive activity or an increased Teff-resistance to Treg suppression. In similar experimental conditions, effects of MTX or prednisolone will also be assessed.

Background Treg suppression

Naturally-occurring Tregs are key regulators of immune responses to self-tissues and infectious agents. Tregs can suppress activation and proliferation of Teffs.

However, effectiveness of Treg-mediated suppression dependent on their preliminary activation [3, 4]. This can be achieved in vitro for example by using a stimulation with anti-CD3 antibody (Fig. 1).

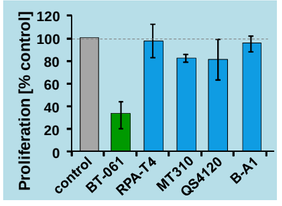
Figure 1: Schematic effect of Treg activation on Teff proliferation



Background BT-061

BT-061 is a humanized, IgG1 agonistic antibody to CD4. It binds a specific epitope on the CD4 molecule at a site distinct from the binding region of other known ligands. We demonstrated that only BT-061 was able to induce suppression of CD8+ T cell proliferation in contrast to other commercially available anti-CD4 antibodies (Fig. 2). Similar results were obtained using CD4+ effector cells. BT-061 differentiates also from anti-CD3 as a non-depleting antibody, and by activating only Tregs and not Teffs.

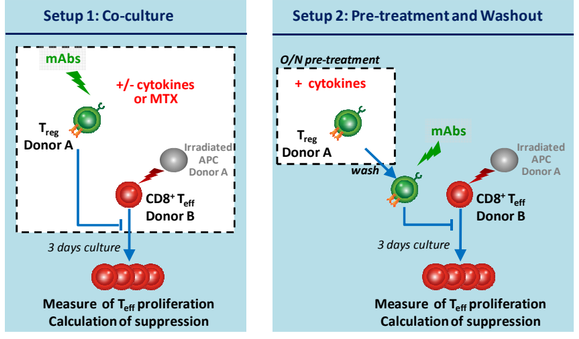
Figure 2: BT-061 increases functional activity of Tregs [3]



Experimental design

Functional assay was used to assess the influence of soluble factors on the BT-061-induced suppression following 2 assay principles (Fig. 3).

Figure 3: Two assay setups



Human T cells were isolated by standard protocols from buffy coats of healthy volunteers (purity >90%). Tregs are defined as $CD4^+CD25^+$ T cells, Teff as $CD8^+CD4^-CD25^-$ T cells. CD3-depleted PBMC were used as stimulatory cells (APCs). Tregs from a donor A were transferred to a mixed lymphocyte reaction in which $CD8^+$ T cells from a donor B were stimulated with irradiated allogeneic APCs from donor A, while the Tregs and APCs were syngeneic.

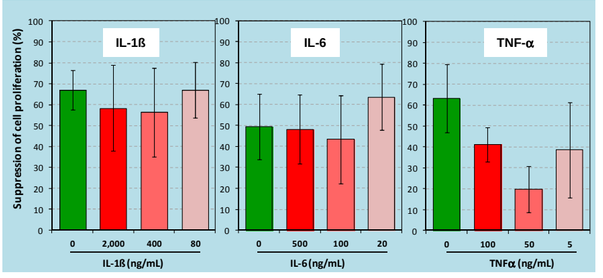
Tregs were either added untreated into co-culture containing cytokines or MTX or buffer, or first pre-incubated overnight with cytokines or buffer (washout setup). Final co-cultures were completed with 1 µg/mL BT-061 or anti-CD3 (used as positive control). Cell proliferation was measured after few days, and percent suppression was calculated in comparison to culture without Tregs (0% suppression) and without APCs (no proliferation).

Effect of cytokines in co-culture

The selected cytokine concentrations to be examined were higher than classically reported plasma concentrations in RA [1], but selected to challenge the test system.

As shown in Figure 4, IL-1β and IL-6 had no effect on the suppression level as measured in absence of cytokines. In contrast, the presence of TNFα seems to impair moderately the suppressive function of BT-061-activated Tregs. However, this effect was not dose-dependent. It is speculated that these TNFα effects are the sum of cytotoxic effect at 100 ng/mL, and additional Teff proliferation following stimulation with TNF-activated APCs (data not shown) .

Figure 4: BT-061-induced Suppression in presence of cytokines (co-culture setup, BT-061 at 1 µg/mL)



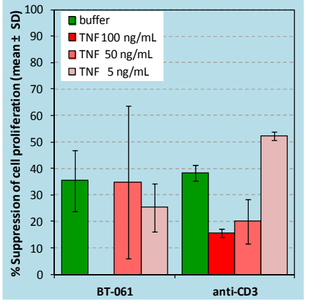
Treg pre-treatment with cytokines

In the next experiments, we investigated whether the reduced suppression observed in co-culture in presence of TNFα was due to reduced activity of the Treg. For that, the washout setting was used.

Data showed that pre-treatment of Tregs with TNFα at 100 ng/mL lead to strong effects (Fig. 5), which are speculated related to a reduced viability of the cells at this concentration (as observed during cell preparation). No remarkable effect were otherwise detected for the BT-061-induced Treg suppression.

For conditions using anti-CD3 as activator, an apparent dose-response seems detected across tested concentration range.

Figure 5: Suppression using TNFα pre-treated Tregs



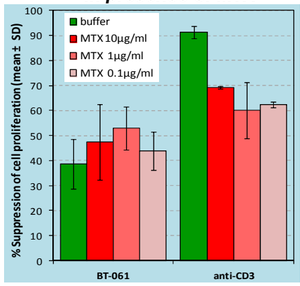
Effect of MTX in co-culture

As BT-061 is currently investigated in the clinic in combination with methotrexate (MTX), we wanted to assess the effect of MTX on the BT-061-induced Treg suppression.

Similarly to the concentrations of cytokines, the top MTX concentration of 10 µg/mL is above expected clinical concentrations for RA [4].

Figure 6 shows no significant effect of MTX on the BT-061-induced suppression. In contrast, a moderate decrease was measured when using the anti-CD3 antibody to activate the Tregs. Further experiments are required to fully understand this effect.

Figure 6: Treg Suppression in presence of MTX



Conclusion

Recent data have shown that pro-inflammatory cytokines might have profound inhibitory effect on Treg suppressive functionality. It is also described that biological therapy neutralizing cytokines can enhance Treg functionality [5]. The objective of the presented in-vitro experiments was to further investigate the mechanism of action of BT-061 in conditions closer to the RA situation, although selected concentrations of cytokines were higher than those described in the RA population in order to challenge the test system.

We demonstrated that even high level of IL-1ß and IL-6 have no impact on the BT-061-induced suppression of cell proliferation. In contrast, it was shown a moderate effect of TNFα at concentration significantly above the plasma concentration measured in RA patients . Further experiments are required to fully understand this effect. Finally, the data supported that the direct stimulation of Treg by BT-061 is not impaired by MTX, even at high concentrations.

References

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Disclosure

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