Abatacept (CTLA-4Ig) treatment reduces T cell apoptosis and regulatory T cell suppression in patients with rheumatoid arthritis (RA)

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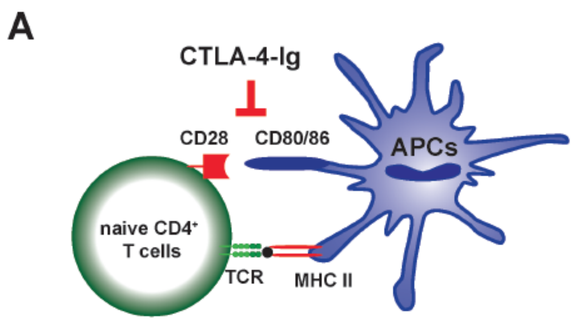
OBJECTIVE:

Abatacept (CTLA-4Ig) blocks CD28-mediated T cells activation by binding to the costimulatory B7 ligands CD80/CD86 on antigen presenting cells (APC). Costimulatory molecules, however, can also be expressed on T cells upon activation. We therefore analysed whether CTLA-4Ig directly affects distinct T cell subsets in RA patients.

METHODS:

Phenotypic and functional analyses of $CD4^+$ T cells, including $CD4^+FoxP3^+CD25^+$ regulatory T cells (Treg), from RA patients were performed before and during CTLA-4Ig therapy. In addition T cells from HC were analysed upon in vitro culture with CTLA-4Ig or anti-CD80 and anti-CD86 antibodies. Apoptotic DNA fragmentation in $CD4^+$ and $CD4^+FoxP3^+$ T cells was measured by TUNEL staining.



CTLA-4Ig therapy blocks CD28-mediated T cells activation

Figure 1

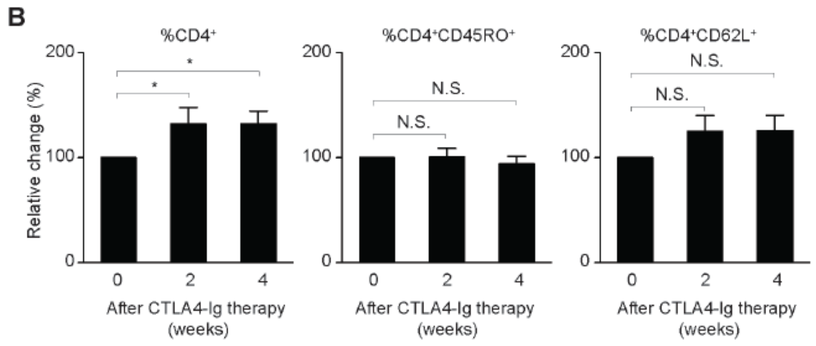


Figure 1. Increased proportions of $CD4^+$ T cells during CTLA-4Ig therapy
A) Heparinized whole blood samples were taken before the patients received an i.v. infusion with CTLA-4Ig at week 0, 2 and 4. **B**) T cells were analysed in RA patients (n=15) by flow cytometry before and at different time point during CTLA-4Ig therapy. Proportions of $CD4^+$, $CD4^+CD62L^+$ naïve and $CD4^+CD45RO^+$ memory T cells were determined. Asterisks indicate significant p values ($^*p < 0.05$).

Figure 2

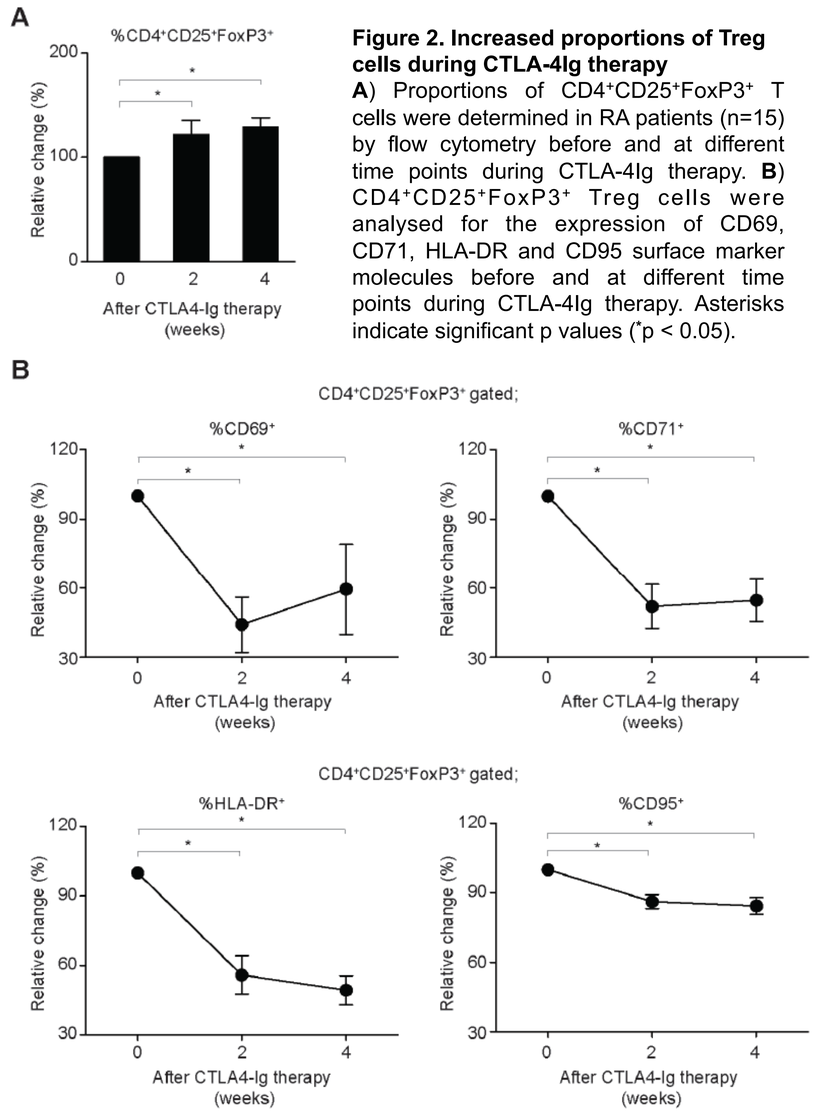


Figure 2. Increased proportions of Treg cells during CTLA-4Ig therapy
A) Proportions of $CD4^+CD25^+FoxP3^+$ T cells were determined in RA patients (n=15) by flow cytometry before and at different time points during CTLA-4Ig therapy. **B**) $CD4^+CD25^+FoxP3^+$ Treg cells were analysed for the expression of CD69, CD71, HLA-DR and CD95 surface marker molecules before and at different time points during CTLA-4Ig therapy. Asterisks indicate significant p values ($^*p < 0.05$).

Figure 3

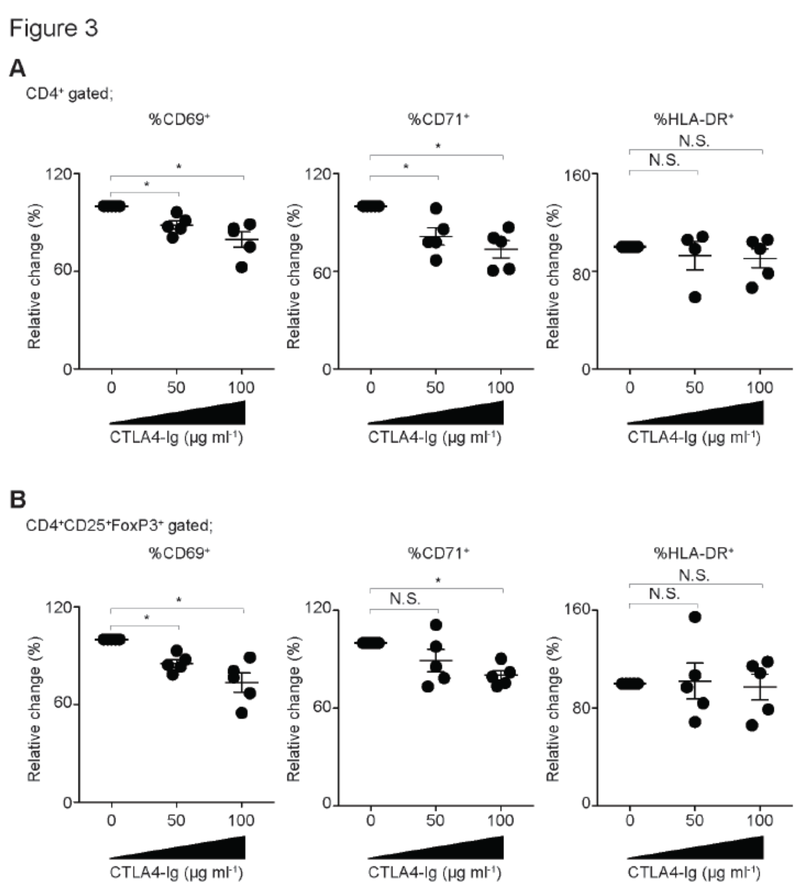


Figure 3. CTLA-4Ig inhibits the activation of $CD4^+$ and $CD4^+CD25^+FoxP3^+$ T cells in vitro.
PBMCs from HC (n=5) were incubated without or with 50 µg/ml or 100 µg/ml CTLA-4Ig for 3 hours and afterwards stimulated with anti-CD3 over night. **A)** $CD4^+$ T cells and **B)** $CD4^+CD25^+FoxP3^+$ Treg cells were analysed for the expression of CD69, CD71, HLA-DR and CD95 surface marker molecules. The relative change (mean % in-, or decrease±SEM) in positive cells is shown for cells that were incubated with 50 µg/ml and with 100 µg/ml CTLA-4Ig. Asterisks indicate significant p values ($^*p < 0.05$).

Figure 4

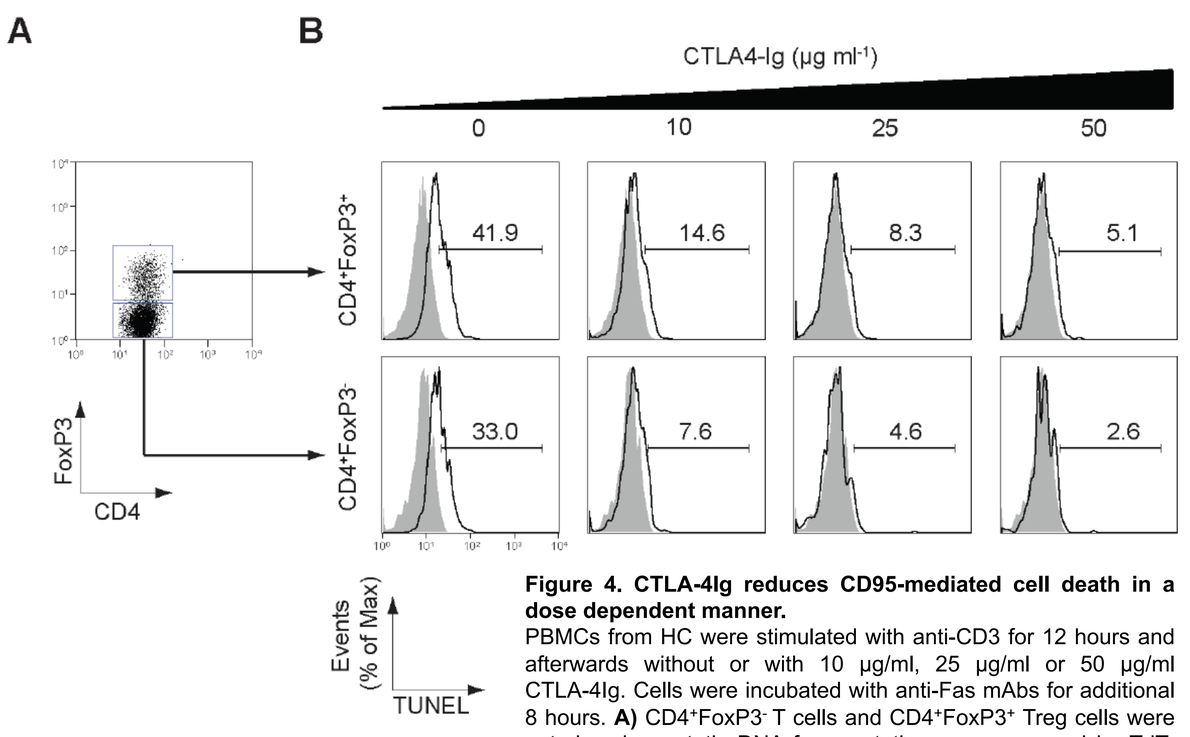


Figure 4. CTLA-4Ig reduces CD95-mediated cell death in a dose dependent manner.
PBMCs from HC were stimulated with anti-CD3 for 12 hours and afterwards without or with 10 µg/ml, 25 µg/ml or 50 µg/ml CTLA-4Ig. Cells were incubated with anti-Fas mAbs for additional 8 hours. **A)** $CD4^+FoxP3^-$ T cells and $CD4^+FoxP3^+$ Treg cells were gated and apoptotic DNA fragmentation was measured by TdT-mediated dUTP nick-end labeling (TUNEL) staining using the FLOWTACS kit. One representative dot plot out of five is shown. **B)** Unfilled Histograms show the precentage of $TUNEL^+$ cells among $CD4^+FoxP3^-$ T cells and $CD4^+FoxP3^+$ Treg cells and the appropriate isotype controls (filled histograms). One representative example out of 5 is shown.

Figure 5

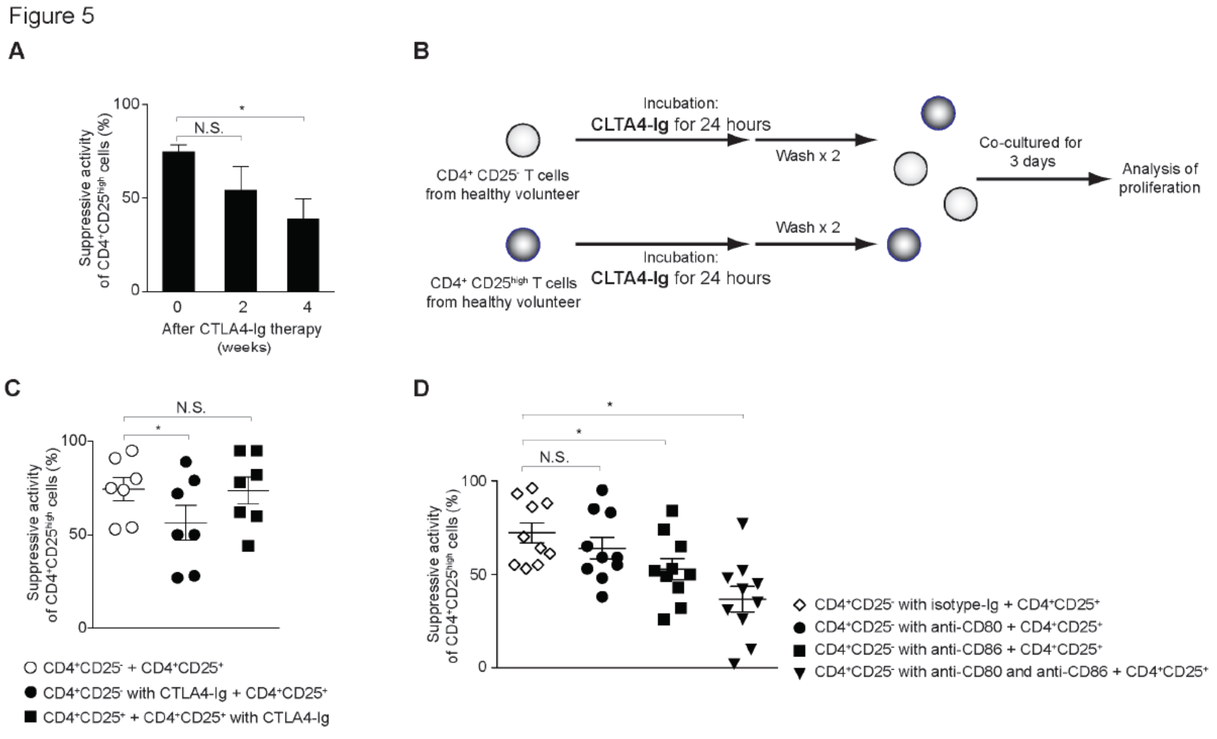


Figure 5. CTLA-4Ig affects Treg cell mediated suppression through interaction with responder T cells.
A) $CD4^+CD25^{high}$ and $CD4^+CD25^-$ T cells were isolated by FACS from PBMC from RA patients before and at different time points during CTLA-4Ig therapy. $CD4^+CD25^{high}$ Treg cells were cocultured with $CD4^+CD25^-$ T responder cells (ratio: 1:1) and stimulated with soluble anti-CD3 mAb and irradiated PBMC. Proliferation of T cells was monitored by measuring [methyl-3H] thymidine incorporation on day 4 of culture. Asterisks indicate significant p values (n=7). **B)** $CD4^+CD25^{high}$ Treg cells and $CD4^+CD25^-$ responder T cells were isolated by FACS from HC and were pre-incubated with 100 µg/ml CTLA-4Ig over night before the start of the suppression assay (n=7). **C)** Pre-incubation of $CD4^+CD25^-$ responder T cells but not of $CD4^+CD25^{high}$ Treg cells led to a significant reduction in the extent of T cell suppression. **D)** $CD4^+CD25^-$ T cells were isolated by FACS from HC and were pre-incubated with anti-CD80, anti-CD86, or anti-CD80 + anti-CD86 Abs or the appropriate isotype-matched negative control Abs over night. Pre-incubation of $CD4^+CD25^-$ responder T cells with anti-CD86 mAbs and anti-CD80+anti-CD86 mAbs led to a significant reduction of Treg cell mediated T cell suppression. Asterisks indicate a significant p value (n=10).

CONCLUSION:

CTLA-4Ig therapy in RA patients exerts effects beyond the suppression of T cell activation, which has to be taken into account as an additional mechanism of CTLA-4Ig treatment.