Assessment of factors that contribute to inflammatory toxicities during CAR-T cell therapy

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Abstract

Inflammatory toxicities such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) are commonly associated with chimeric antigen receptor T cell (CAR-T cell) therapy. Although the mechanisms responsible for development of inflammatory toxicities are not completely understood, activation of bystander myeloid cells (MCA) and release of pro-inflammatory cytokines such as IL-6 and IL-1β following CAR-T cell therapy contribute to this. It is unclear how myeloid cells are activated during CAR-T cell therapy. GM-CSF produced by activated CAR-T cells has been suggested to activate myeloid cells; however, GM-CSF protein neutralization by antibodies or gene knock-out (KO) in T cells using CRISPR/Cas does not completely prevent MCA. This suggests that other T cell factors contribute to MCA. Fractionation studies of activated T cell supernatant identified a fraction of size range 100-300 kDa, which significantly activated myeloid cells compared to <100kDa and >300kDa fractions. Mass spectrometry analysis of 100-300kDa fraction identified several candidate proteins that may contribute to MCA. Protein function analysis and literature review identified a novel protein, GP1BA as one of the candidates. Neutralization of GP1BA in T cell supernatant using antibodies from 2 different sources significantly reduced MCA. GP1BA gene KO in T cells using CRISPR/Cas9 reduced myeloid cell activation by 40% suggesting presence of other inflammatory factors. Current studies are underway to identify additional inflammatory factor(s) that contribute to MCA. Our long-term goals are to identify soluble factors released by CAR-T cells that contribute to inflammatory toxicities, and rationally engineer CAR-T cells that are safer and effective.

Methods

T cells from healthy donors were isolated using anti-CD3 beads. T cells were either activated with anti-CD3/CD28 or PMA/Ionomycin. Activated T cells were transduced with lentiviral vector to express CD19 CAR. Transduced cells were expanded in culture for 2 weeks, and harvested. T cell or CAR-T cell supernatant was subjected to size-exclusion columns. Cell-free supernatant or various fractions obtained from size-exclusion columns were used for activation of myeloid cells (THP-1 or primary monocytes). IL-6 and IL-1beta released by THP-1 cells were measured after 24 hours.

Results

Soluble factors other than GM-CSF released by CAR-T cells contribute to myeloid cell activation

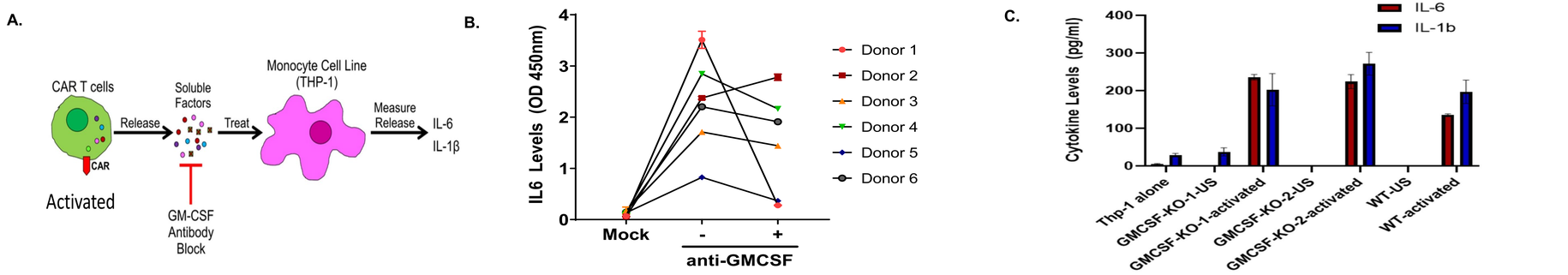

Figure 1. (A) Schematic of in vitro myeloid cell activation assay. **(B)** CD19 CAR-T cells were activated, and cell-free supernatant was subjected to GM-CSF neutralization. Monocytic cell line (THP-1) was co-cultured with GM-CSF neutralized supernatant. IL-6 released by THP-1 was measured after 24 hours. **(C)** GM-CSF knockout (KO) Jurkat cell clones (KO-1 and KO-2) were activated, and cell-free supernatant was co-cultured with THP-1 cells. IL-6 and IL-1beta (IL-1b) released by THP-1 was measured after 24 hours. Mock= Supernatant from unstimulated CAR-T cells. US= Unstimulated.

Identification of a novel inflammatory factor released by CAR-T cells

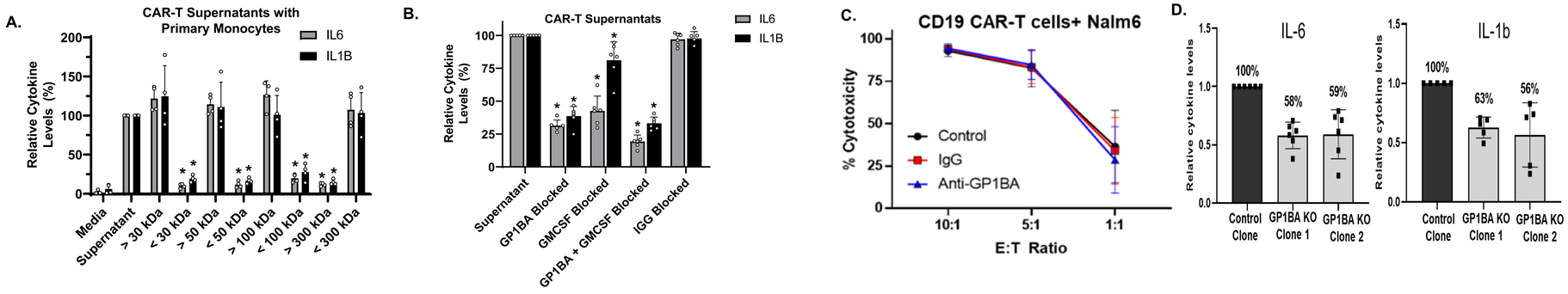

Figure 2. (A) CD19 CAR-T cells were activated, and cell-free supernatant was subjected to size-exclusion columns. Each fraction was co-cultured with THP-1 cells, and IL-6 and IL-1b was measured after 24 hours. **(B)** Fraction (100-300) was subjected to Mass Spectrometry, GP1BA was identified as one of the inflammatory proteins. Activated CAR-T cell supernatant (N=6) was treated with GP1BA, or GM-CSF or both antibodies. THP-1 was co-cultured with anti-body treated supernatants. IL-6 and IL-1b released by THP-1 was measured after 24 hours. **(C)** CD19 CAR-T cells were co-cultured with Nalm6 in the presence of GP1BA antibodies, and target cell lysis was measured at 24 hours. **(D)** GP1BA knockout Jurkat cell clones were generated using CRISPR/Cas9. Supernatant from activated GP1BA KO clones or control clone (edited with non-specific gRNA) was co-cultured with THP-1 cells. IL-6 and IL-1b released by THP-1 was measured after 24 hours. Each experiment was independently performed at least 5 times, and average is shown.

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Conclusions

- Our studies have demonstrated factors other than GM-CSF are released by CAR-T cells that contribute to myeloid cell activation.
- We identified GP1BA as an inflammatory factor released by CAR-T cells following activation.
- Neutralization of GP1BA protein or knockout of GP1BA gene in human T cells significantly reduces myeloid cell activation *in vitro*.
- Current studies are underway to study effect of GP1BA and GM-CSF knockout in CAR-T cells, and to identify additional inflammatory factors released by CAR-T cells.