

# Soluble hexameric CD40 ligand activates human dendritic cells and boosts memory T cell response



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## Background

Despite the advances in developing vaccine candidates for treatment of cancer and infectious diseases, the quality of vaccine-specific immunity has still to be improved and one axis of research is the identification of efficient immune potentiators and delivery system. In particular, inclusion of adjuvant able to trigger early innate immunity would help in the generation of robust and long-lasting adoptive immunity. The CD40 triggering is essential in the induction of an efficient response. It induces activation of cells of the innate immunity system such as dendritic cells (DC) and also contributes to direct activation of CD8 cells.

## Objective

To evaluate the potency of a soluble hexameric CD40 ligand (sCD40L) as adjuvant of the memory T cell response directed against viral antigens. We investigated the impact of sCD40L on DC maturation and on the magnitude of anti-viral memory T cell response.

## Methods

We used a soluble hexameric CD40L made of the hemagglutinin-derived signal peptide, the 8-residue-long M2 flag tag, the ACRP30 fragment (aa18-111) and the extracellular CD40L region (aa115-262) (*French et al 2005, Blood 105:219*).

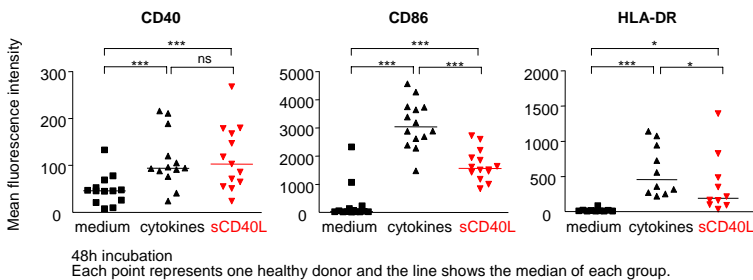
Whole PBMC and monocyte-derived DC (GM-CSF/IL4) from healthy donors were stimulated with sCD40L (400ng/ml) or with a cocktail of cytokines (IL1 $\beta$  at 10ng/ml, IL6 at 10ng/ml, TNF $\alpha$  at 10ng/ml and PGE2 at 1mg/ml) for 12h to 48h. To monitor the activation of DC upon stimulation, the mean fluorescence intensity of cell surface CD40, CD86, HLA-DR was analyzed in Lin-1<sup>-</sup> CD11c<sup>+</sup> cells. The statistical analysis was performed using a paired t test: \*, p<0.05, \*\*, p<0.01, \*\*\*, p<0.001.

To evaluate the effect of sCD40L on anti-viral memory response, immature DC (iDC) and sCD40L-mature DC (sCD40L-mDC) were loaded with lysates of CMV- or EBV-infected cells and used as stimulators of CFSE-labeled CD4- or CD8-enriched PBMC. sCD40L (400ng/ml) was added or not to the cultures. The frequency of CD4 and CD8 cells that have proliferated was monitored by flow cytometry after 5-days of culture.

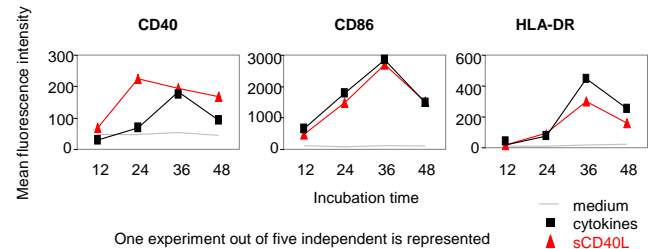
## Results

### 1. Effect of sCD40L on DC

#### 1.1. Monocyte-derived DC

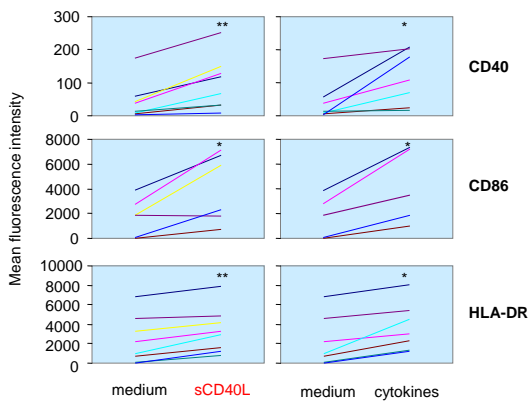


### 2. Kinetics of monocyte-derived DC activation by sCD40L



sCD40L activates monocyte-derived DC with the same kinetics as the cocktail of cytokines. The peak of DC response is obtained between 24 and 48h depending on donors.

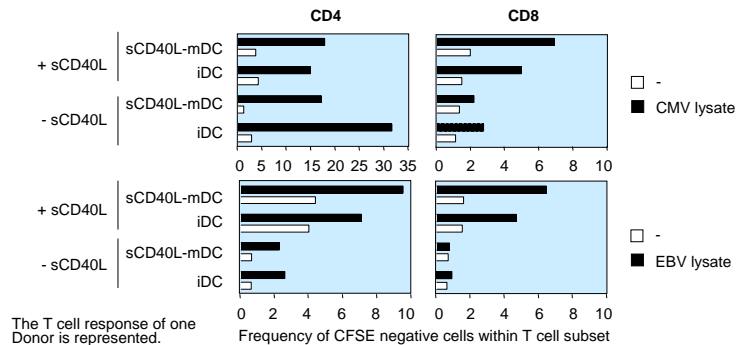
#### 1.2. CD11c<sup>+</sup> myeloid DC ex vivo



sCD40L up-regulates CD40, CD86 and HLA-DR molecules at the cell surface of:

- monocyte-derived DC obtained *in vitro*
- *ex vivo* CD11c<sup>+</sup> myeloid DC

### 3. Effect of sCD40L on anti-viral memory T cell response



- Both iDC and sCD40L-mDC loaded with CMV- or EBV-lysates induce the proliferation of **CD4 T cells** suggesting that Ag uptake and exogenous class II presentation are maintained in sCD40L-mDC.
- The frequency of proliferating **CD4 T cells** upon stimulation with iDC or sCD40L-mDC in presence or absence of sCD40L is variable between individuals.
- The addition of sCD40L in the proliferation assay leads to an increased frequency of **CD8** responding to either EBV lysate (6/6 donors) or CMV lysate (5/5 donors).
- In the presence of sCD40L, sCD40L-mDC stimulates a higher frequency of EBV- (3/5 donors) and CMV- (4/5 donors) specific **CD8 T cells** than iDC.

These results suggest that in addition to their ability to exogenously present class II viral peptides, sCD40L-mDC are able to cross-present class I viral peptides.

## Conclusions

### Soluble hexameric CD40L

- Activates dendritic cells
- Maintains the Ag uptake and exogenous class II presentation ability of activated dendritic cells
- Enhances the EBV- and CMV-specific CD8 memory response
- Is able to function as an adjuvant for boosting T cell memory response