Ex vivo Expansion of T Lymphocytes Induces Substantial Alterations in Cell Size and Actin-Binding Cytoskeletal Proteins

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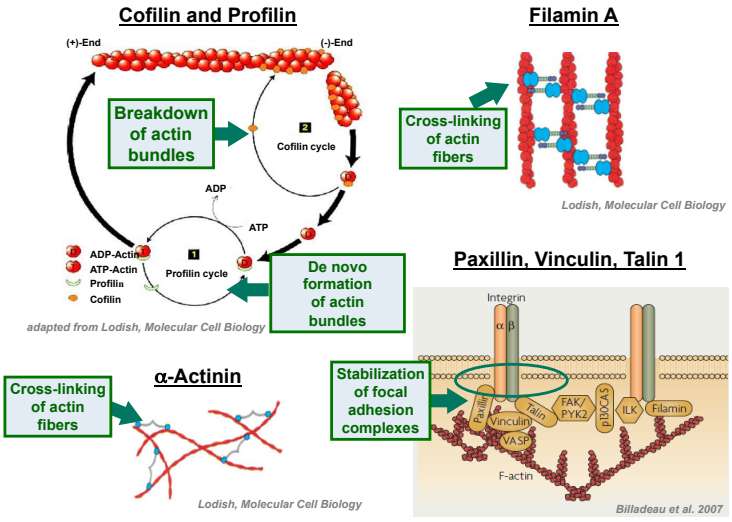
Background

- Ex vivo expansion of T lymphocytes is a component of cell production for adoptive therapy, but may induce functional defects
- Deficits in the migration potential and function of *ex vivo* expanded cells including actin cytoskeletal components have been reported e.g. in keratinocytes *(EMBO Mol Med. 2013 5:640-53)* or induced pluripotent stem cells *(J Biosci Bioeng. 2014 118:716-22)*

Questions

- What is the effect of *ex vivo* expansion on cell volume of T lymphocytes?
- How are transcription and protein expression of actin-binding proteins (ABPs) affected during ex vivo expansion in T lymphocytes?
  - i.e. actin-polymeririzing factors cofilin and profilin,
  - i.e. the small intercalating molecules filamin A and alpha actinin
  - i.e. components of adhesion complexes paxillin, talin and vinculin

Functions of the Investigated Actin Binding Proteins



Methods

- T Lymphocytes were isolated from peripheral blood using anti-CD3 microbeads
- T-Lymphocytes were expanded under standard tissue culture conditions. Cells were stimulated to grow by anti-CD3/anti-CD28 and Interleukin-2
- Cell size was determined using calibrated microbeads by flow cytometry
- mRNA was quantified using qPCR
- Proteins levels were assessed using flow cytometry after titration of fluorescence-coupled antibodies to antigen saturation

Results

Expansion of T Lymphocytes Results in Increased Cell Volume

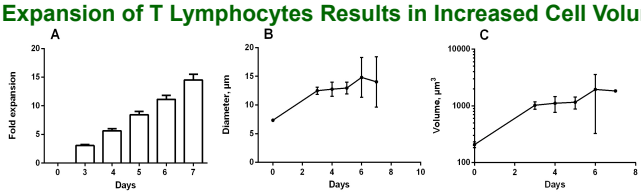


Fig. 1: T lymphocytes in culture during 7 days. **A**. Cell numbers of CD3+ T lymphocytes increased 15±1 fold within 7 days; **B** The mean diameter of T lymphocytes increased from 7±0.3 to 15±4.4 µm; **C**. 9-fold increase in cell volume (from a mean V=208µm³ to 1828µm³). T lymphocytes were expanded using anti-CD3 and anti CD28 microbeads. N=5, Values ±SD.

On a per Cell Basis, ABP Transcripts are Decreased and ABP Proteins Remain Stable During Expansion of T Lymphocytes

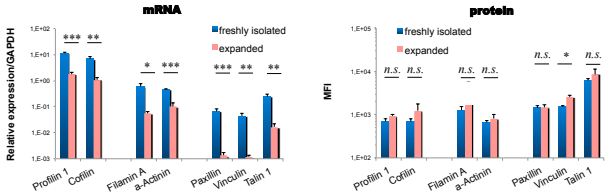


Fig. 2: Expression of ABPs in freshly isolated and ex vivo expanded T lymphocytes. Left panel: mRNA levels of ABPs decreased between 5 and 50 fold. Detection was performed with Taqman-Primers. Right hand panel: absolute protein levels of ABPs remained constant, except for Vinculin, which showed 1.6-fold increase. Detection with indirect immunochistochemical staining and flow cytometry. N=5; Values: means ±SEM;*p≤0.05; **p≤0.005; ***p≤0.0005.

Relative to Cell Volume, ABP Transcripts and Proteins Decrease During Expansion of T Lymphocytes

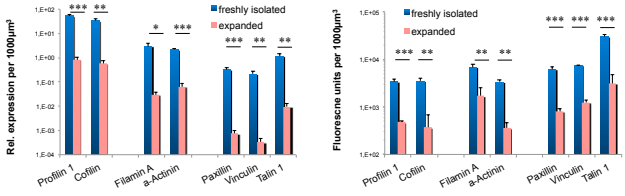


Fig. 3: Changes in the expression of ABPs in T lymphocytes during expansion culture per cell volume. Expression levels of mRNA (left) and protein (right) shown in Fig. 3 were related to the cell volumes of fresh and expanded T lymphocytes that had been determined in Fig. 1 and 2. N=5; Values ± SEM ;*p≤0.05; **p≤0.005, ***p≤0.0005.

Conclusions & Future Perspectives

- T lymphocytes expanded ex vivo within 7 days showed a 2.2-fold increase in diameter, reflecting a 9-fold increase in cell volume
- Culture expansion resulted in maintenance of absolute levels of ABPs in T lymphocytes, but drastic loss in transcripts of the investigated ABPs
- Our data indicates major alterations in the turnover of ABPs protein in T lymphocytes during the expansion phase.
- Further research may delineate critical contributions of alterations in ABPs to somefunctional deficits observed in cultured cells used for immunotherapies.