Hematopoietic Innovative Therapies

ADVANCED Therapies Unit

Lentiviral vector-mediated gene complementation for the diagnosis of LAD-I.

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Introduction

- Leukocyte Adhesion Deficiency Type I (**LAD-I**) is an autosomal recessive primary immunodeficiency caused by mutations in the ***ITGB2*** gene that encodes for **CD18**, the common subunit of β_2 integrins. Reduced or absent expression of CD18 prevents normal leukocyte trafficking to the infection sites, thus these patients suffer from recurrent and severe bacterial and fungal infections, which cannot be properly resolved.
- A 5 year old Pakistani boy born to consanguineous parents, has been diagnosed as potential LAD-I since flow cytometry analyses showed less than 1% of $CD18^+$ peripheral blood (PB) leukocytes. His clinical picture is defined by leukocytosis (17-22x10^6/L) and neutrophilia (10-13x10^6/L) as well as by a history of severe chronic gingivitis and periodontitis.

Objective

Confirm the LAD-I diagnosis by studying the implication of CD18 in the phenotype of the patient´s cells.

Results

Characterization of β_2 integrins expression in peripheral blood

Evaluation of CD18 membrane expression in PB revealed differences among antibody clones (**Figure 1**), indicating a possible non-functional protein as the cause of the deficiency.

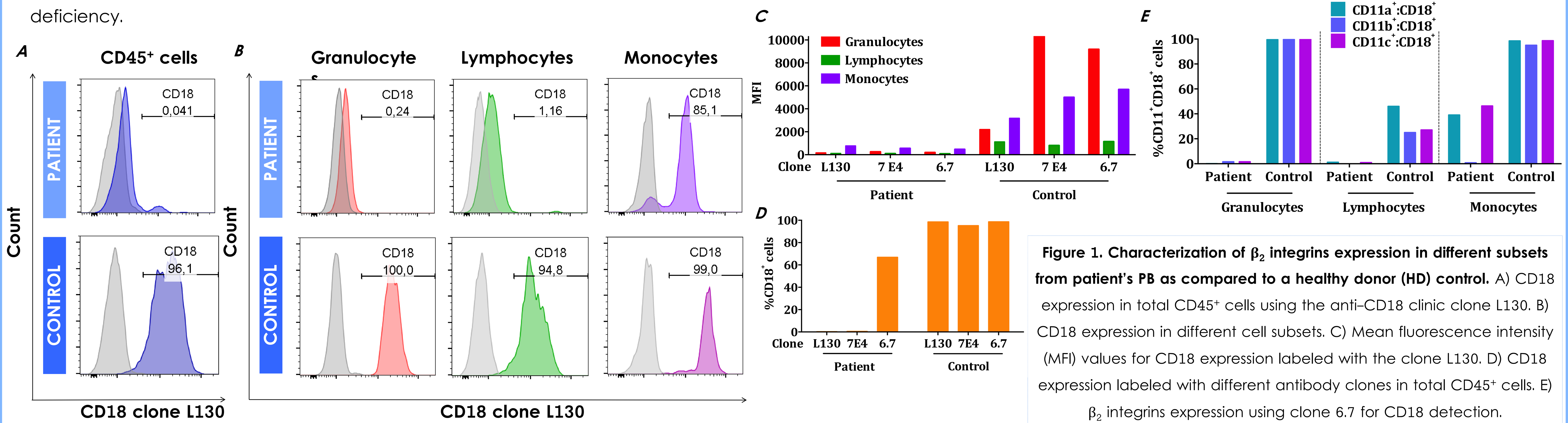

Figure 1. Characterization of β_2 integrins expression in different subsets from patient's PB as compared to a healthy donor (HD) control. A) CD18 expression in total $CD45^+$ cells using the anti-CD18 clinic clone L130. B) CD18 expression in different cell subsets. C) Mean fluorescence intensity (MFI) values for CD18 expression labeled with the clone L130. D) CD18 expression labeled with different antibody clones in total $CD45^+$ cells. E) β_2 integrins expression using clone 6.7 for CD18 detection.

Gene complementation

To confirm the implication of CD18 in the cell phenotype of this patient, T-cells were transduced (**Figure 2**) with the therapeutic Chim.hCD18 lentiviral vector (LV) (**Figure 3**) aimed for the *ex vivo* gene therapy (GT) of LAD-I patients. Transduction of PB T-cells with the Chim.hCD18-LV restored mRNA values (**Figure 4A**) and membrane expression of CD18 and CD11a (**Figure 4B**). Recovery of β_2 integrin expression was accompanied by the restoration of its functionality, measured as specific CD18:CD11a (LFA-1) binding capacity to soluble ICAM (sICAM) *in vitro* (**Figure 4C**) .

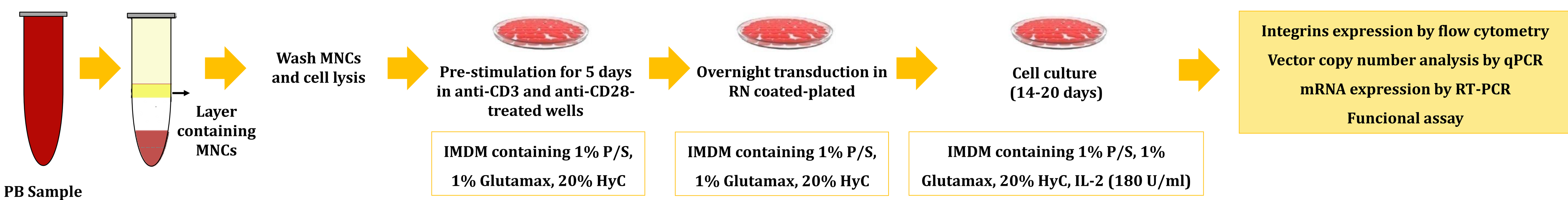

Figure 2. Protocol for LV transduction of mononuclear cells from peripheral blood samples. Mononuclear cells (MNCs) from PB samples are isolated in a ficoll density gradient and pre-stimulated for 5 days at 37°C and 5% O_2 in supplemented IMDM medium in plates previously treated with anti-CD3 and anti-CD28 antibodies. Then, cells are washed and suspended in semi-conditioning medium (prepared by diluting half conditioning medium in which cells were pre-stimulated and half new IMDM supplemented with IL-2). Cells are then transduced in retronectin (RN)-coated plates with the Chim.hCD18-LV. After transduction, cells were washed and maintained in the semi-conditioning medium for up to 14-20 days for the different analyses.

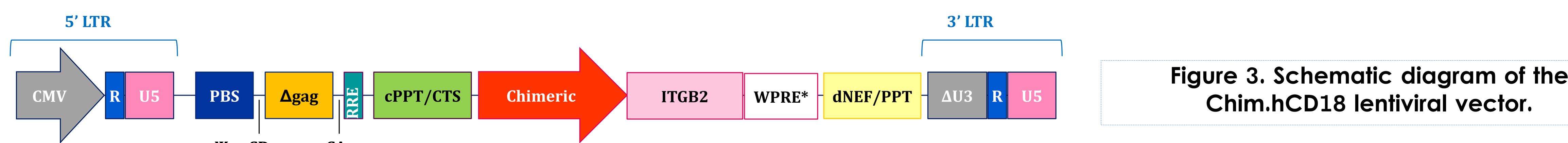

Figure 3. Schematic diagram of the Chim.hCD18 lentiviral vector.

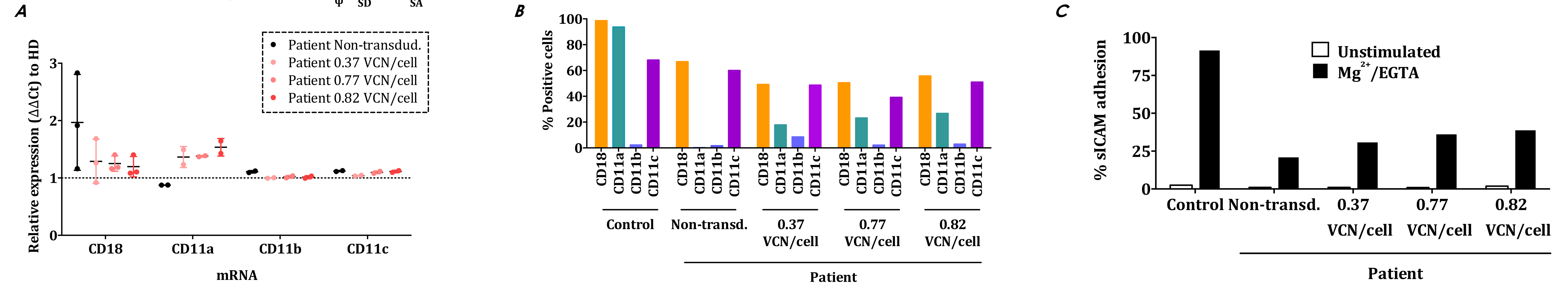

Figure 4. *In vitro* results from Chim.hCD18-LV transduction of PB sample from a patient. A) Relative mRNA expression. Levels of CD18, CD11a, CD11b and CD11c in non-transduced (Non-transd.) and transduced cells from the patient were analyzed by RT-PCR and relativized to a HD control. B) Flow cytometry analysis of β_2 integrins expression. Figure shows the percentage of cells positive for CD18 (clone 6.7), CD11a, CD11b and CD11c markers in control cells from a HD as well as in non-transduced or transduced cells at different vector copy number (VCN/ cell. C) sICAM binding assay. Adhesion functional assay was performed in controls and transduced cells to measure the ability of LFA-1 expressing cells to bind to their ligand ICAM-1.

Conclusions

Although flow cytometry studies with specific monoclonal antibodies did not consistently confirm a defective CD18 expression in PB cells from this patient, endogenous CD18 was incapable of facilitating the expression of CD11a. Remarkably, the lentiviral complementation with a therapeutic LV facilitated the endogenous expression of CD11a, demonstrating the restoration of functional β_2 integrins. Our results therefore indicate that detection of CD18 does not discard a diagnosis of LAD-I, and reveals the convenience of performing functional complementation in those cases in which clinical signs and phenotypic or mutational screening are not concordant.

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