

# P-384. Lymphocyte Transformation Test Effectivity based on the Antigen presenting Cells Employed in Immediate Allergic Reactions to Betalactams.

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## RATIONALE

Lymphocyte transformation test (LTT) is a reliable *in vitro* assay, useful for the diagnosis of nonimmediate allergic reactions to drugs (NIRD). Nevertheless, its use in immediate allergic reactions to drugs (IRD) seems not to have enough sensitivity, which impairs its use in clinical routine. LTT is usually carried-out stimulating peripheral blood mononuclear cells (PBMCs) with the culprit drug. This approach does not take into account the interaction between different cell populations and the drug, which can influence its processing by antigen presenting cells (APCs) and its presentation to lymphocytes. Moreover, LTT was usually measured by the inclusion of radioactive thymidine (3H) whereas the use of other flow cytometry-based techniques could improve its implementation as well as decrease the risk.

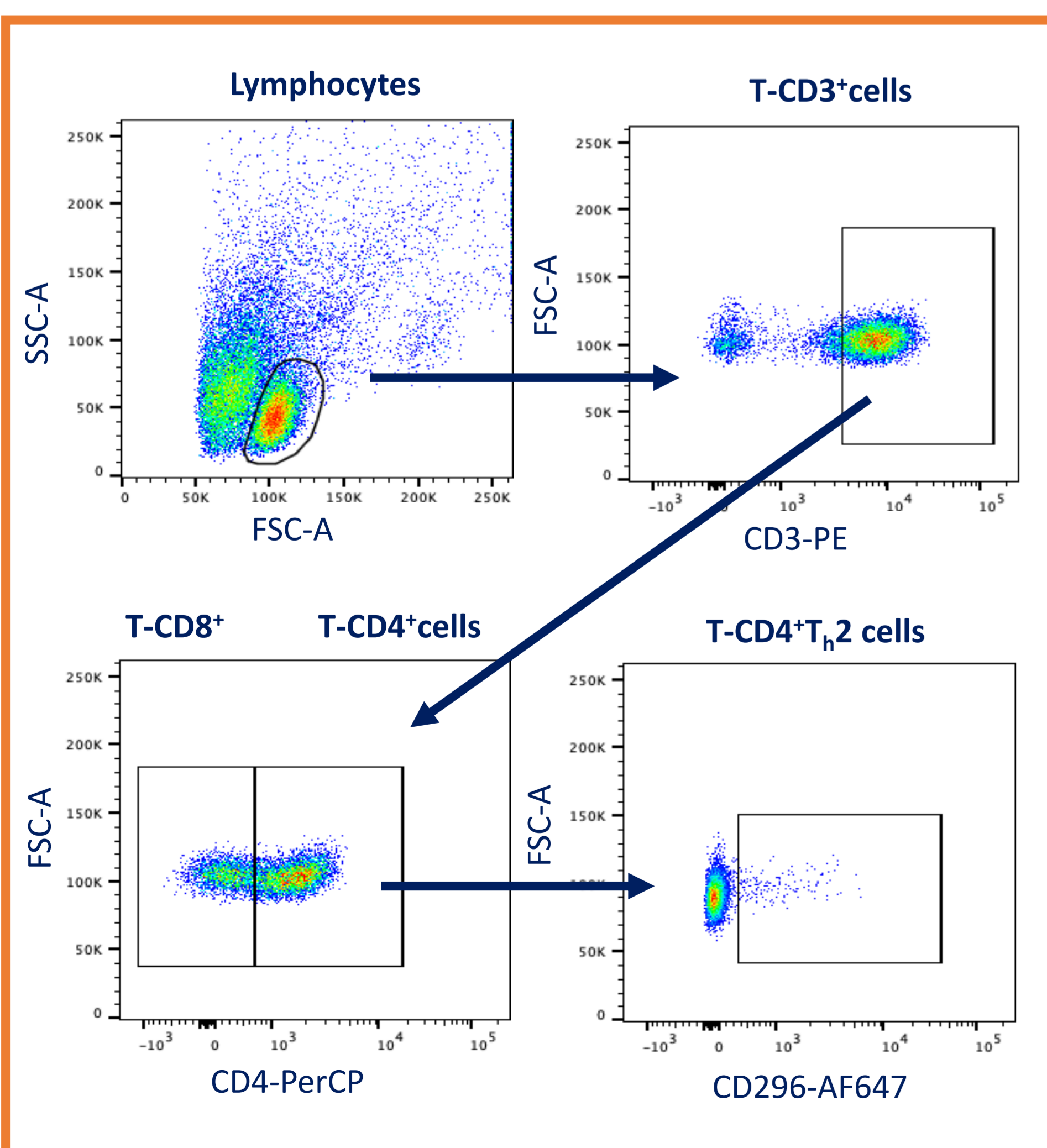
## METHODS

Peripheral mDCs (CD1c<sup>+</sup>) and monocytes (CD14<sup>+</sup>) were isolated from 10 allergic patients with IRD to amoxicillin (AX), 10 to clavulanic acid (CLV), and from 10 Healthy Controls (HC). Monocytes were cultured with GM-CSF and IL-4 to differentiate into moDCs. Immature mDCs and moDCs were pre-primed with each betalactam for 72h. Afterwards, they were cultured with autologous lymphocytes. The proliferative response of CD3<sup>+</sup>, CD4<sup>+</sup>, CD4<sup>+</sup>T<sub>H</sub>2 and CD8<sup>+</sup> lymphocytes was assessed by flow cytometry, measuring the dilution of Carboxyfluorescein succinimidyl ester (CFSE<sub>Dim</sub>). Both assays were compared with the traditional LTT in which PBMCs were directly cultured with each drug. Results were expressed as proliferation index (PI), calculated as the ratio between %CFSE<sub>Dim</sub> stimulated population and %CFSE<sub>Dim</sub> unstimulated population. PI>2 was considered positive.

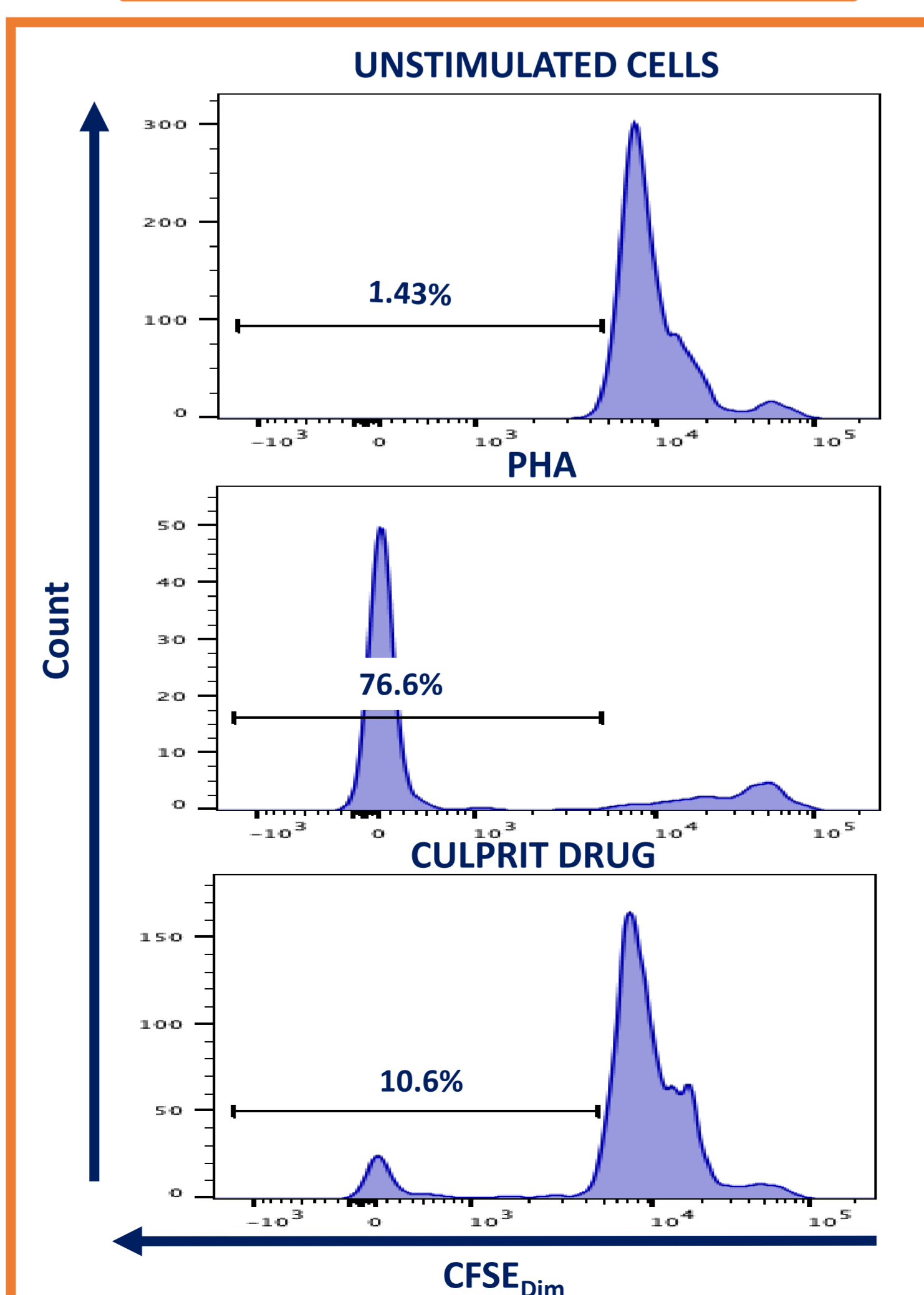
## GOAL

To assess the sensitivity of specific lymphocyte proliferation tests by flow cytometry using as APCs: monocytes and B-cells or different pre-primed dendritic cells, myeloid and monocyte-derived dendritic cells (mDCs and moDCs).

### Cell population strategy

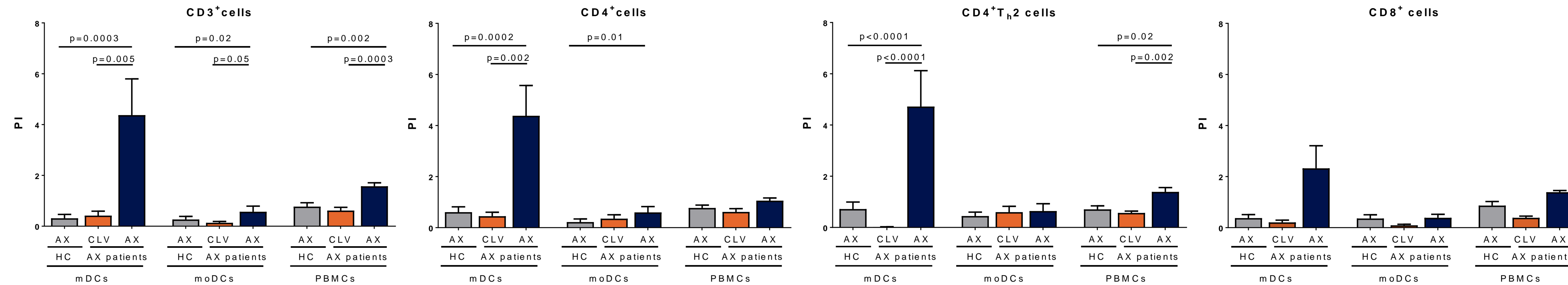


### Proliferation analysis



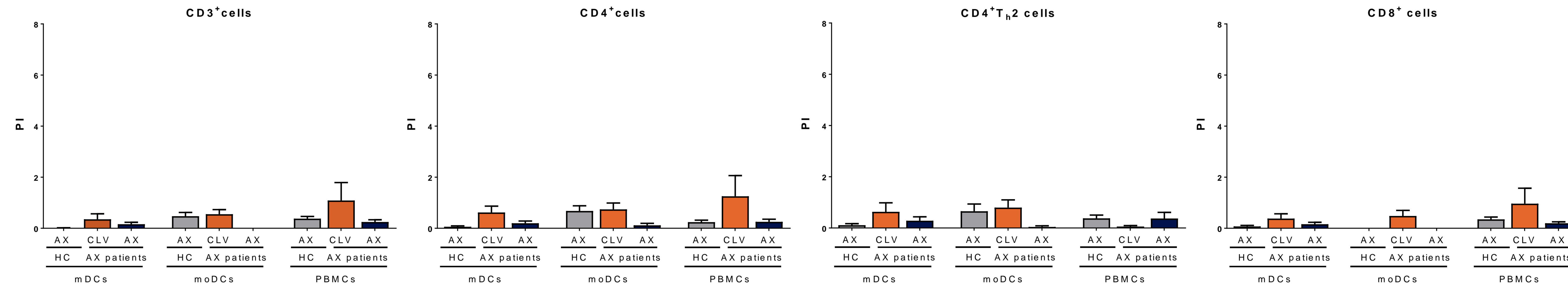
## RESULTS

### AX-allergic patients



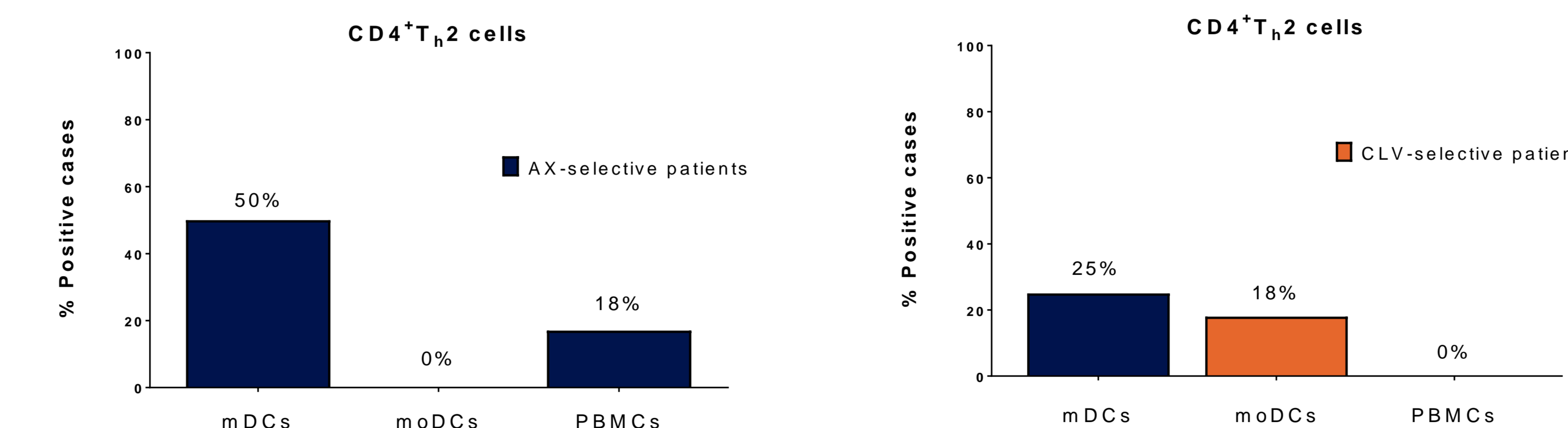
Higher PI was observed in lymphocytes from AX allergic patients compared with HC independently of the APCs and only with the culprit (AX) (Figure A). Higher response was observed when pre-primed mDCs with AX were used, specially in T-CD4<sup>+</sup> cells with a Th2 pattern (Figure B, D). Interestingly, lower proliferation was observed after presenting AX with moDCs or PBMCs. Negative proliferation was observed after stimulation with CLV.

### CLV-allergic patients



No statistic differences were found for all T cell subpopulations, independently of the APCs used. Moreover, lower proliferation was observed in these cells after the stimulation with AX.

### POSITIVITY



Proliferation with mDCs showed positive results (PI>2) in 50% and 25% of cases in AX and CLV allergic patients respectively, whereas using others APCs, moDCs or monocytes and B-cells, was lower than 20%. We did not find positive results in controls for any drug and APCs.

## CONCLUSION

The use of mDCs improves the sensitivity of LTT for the diagnosis of immediate reactions to AX. Nevertheless, no difference was observed in CLV allergic patients. This lower sensitivity could be caused because of the non inclusion of the specific CLV determinants that cause the reactions.

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