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RATIONALE

Lymphocyte Transformation Test (LTT) is a reliable *in vitro* technique that allows determining the specific proliferative response of different lymphocyte populations after stimulation with the culprit drug. Despite that it has been mainly used for evaluating delayed hypersensitivity reactions, its use in immediate reactions to betalactams also shows similar sensitivity, although not optimal. Over the last years, allergic reactions to other betalactams as clavulanic acid (CLV) have been specially analyzed using basophil activation test (BAT). The results indicate that, besides the native drug, the inclusion of some antigenic determinants (ADs) could be useful for improving the sensitivity of the test from 41% to 69% (Barbero *et al. Allergy*. 2019). Moreover, in other studies we had previously demonstrated that the use of dendritic cells (DCs) as antigen presenting cells improves the specific positive results in LTT (Rodriguez-Pena *et al. J Allergy Clin Immunol*. 2006). We hypothesize that some of the CLV ADs generated and presented by DCs are effectively recognized by the immune system of allergic patients and their presence in the LTT could increase its sensitivity, and therefore, be useful for clinical routine.

AIM OF THE STUDY

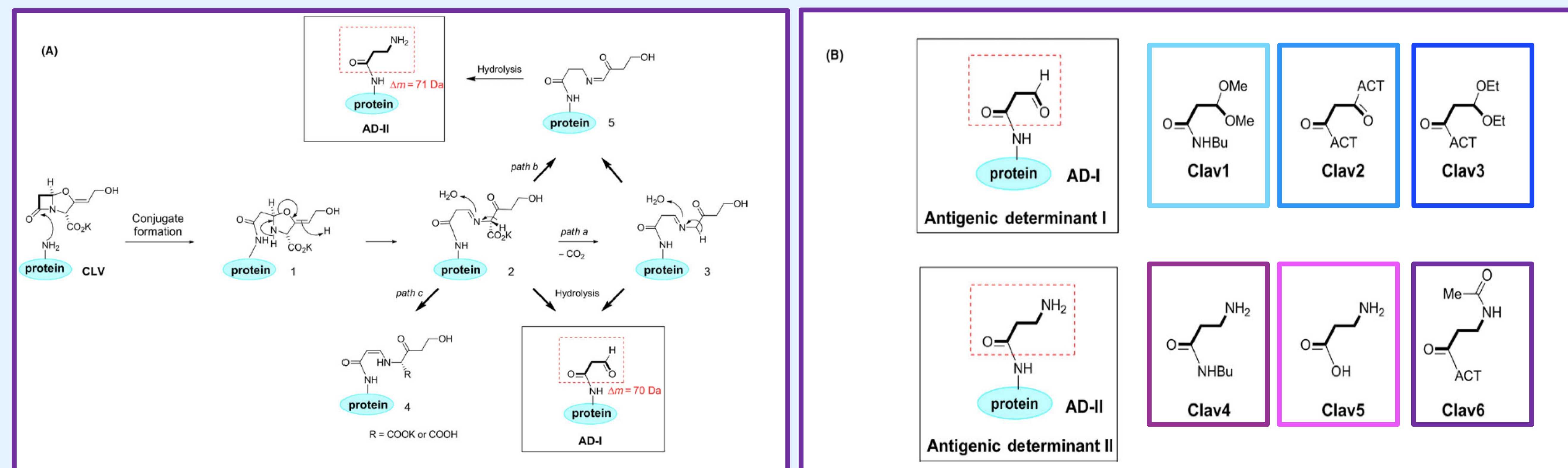
To evaluate the sensitivity and specificity of LTT using pre-primed DCs in the study of IR to CLV as well as to different ADs of CLV.

METHODS

Synthesis of ADs of CLV. Two ADs of CLV were synthesized based on their degradation pathways: AD-I (N-protein, 3-oxopropanamide), and AD-II (N-protein, 3-aminopropanamide) (Figure A). Three different synthetic analogues were designed for each AD, with different reactivity and ability to couple to proteins (Figure B).

CLV and AD immune recognition by monocyte-derived dendritic cells (mo-DCs). Monocytes (CD14⁺) from 11 allergic patients with IR to CLV, and from 10 healthy controls (HC) were isolated and cultured with GM-CSF and IL-4 for 5 days at 37°C and <5%CO₂ to differentiate into immature mo-DCs. Then, mo-DCs were pre-primed with each AD and CLV itself for 72 hours. Afterwards, mo-DCs were cultured for 7 days with autologous lymphocytes, which were previously labelled with carboxyfluorescein succinimidyl ester (CFSE).

LTT. The specific proliferation of T-lymphocytes (CD3⁺), as well as of CD8⁺, CD4⁺, and T_H2-CD4⁺ subpopulations, was assessed by flow cytometry analyzing the dilution of CFSE (%CFSE_{Dim}). Results were given as Proliferation Index (PI), calculated as the ration between stimulated and unstimulated cells. Positive results were considered when PI>2.

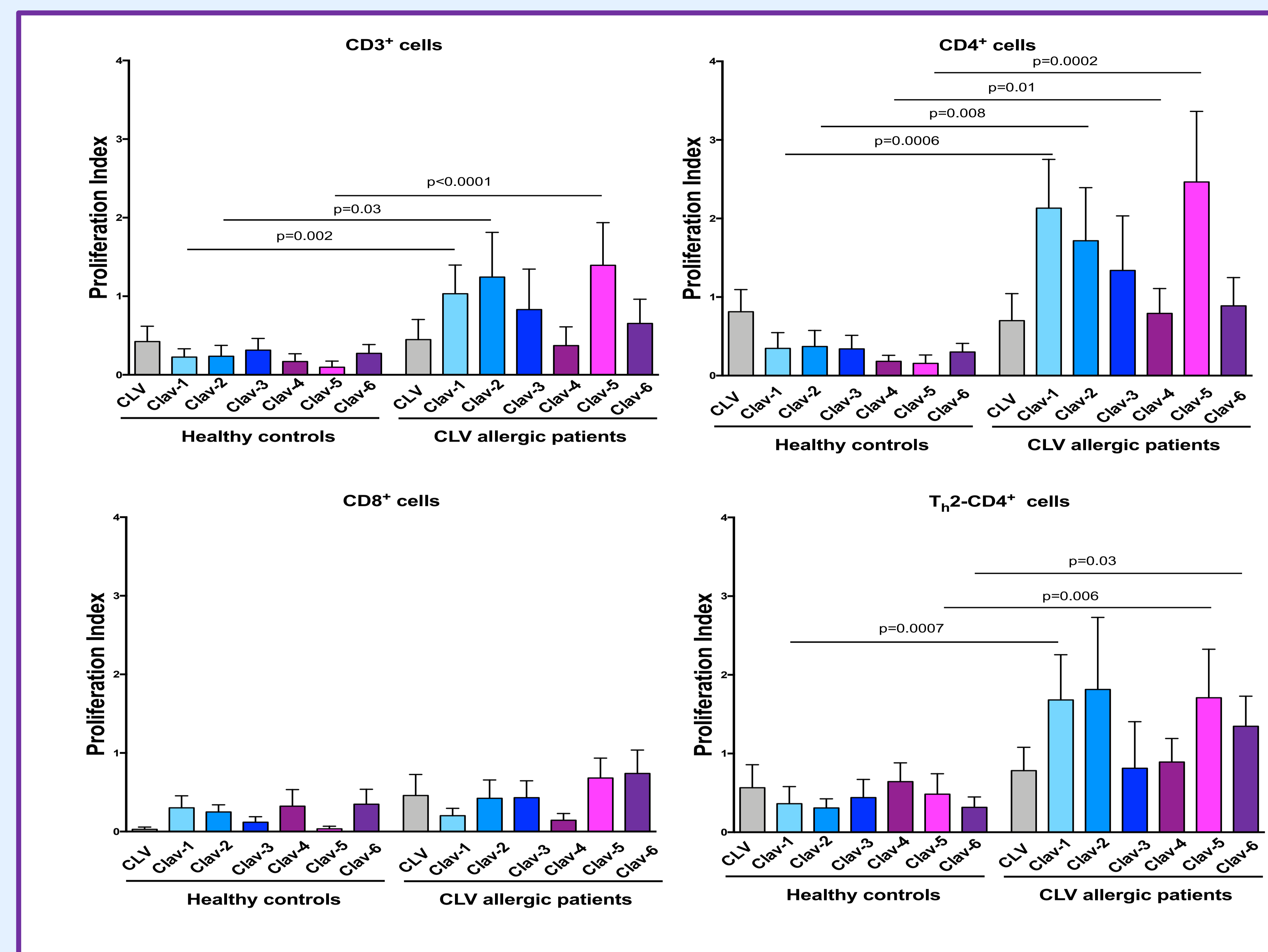


CONCLUSIONS

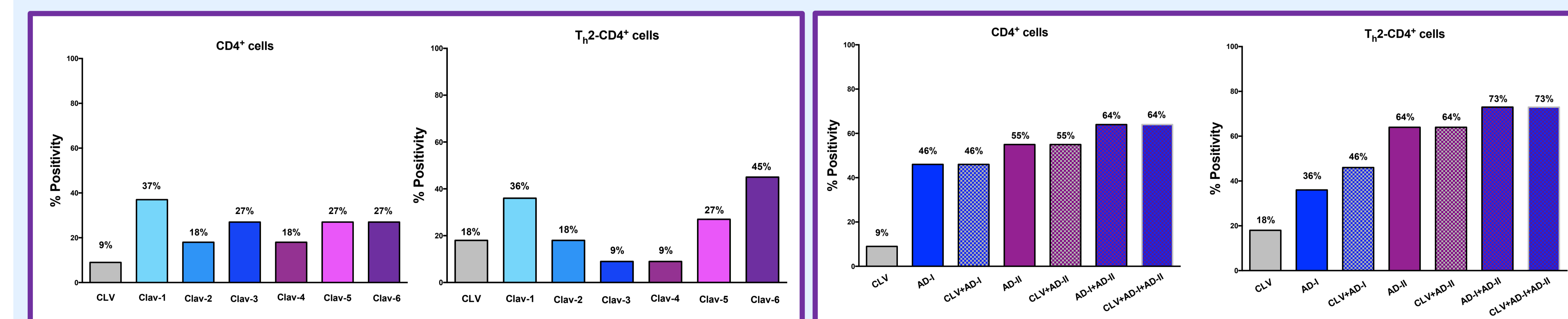
The inclusion of synthetic CLV ADs improves LTT sensitivity with a high specificity, compared with the inclusion of CLV itself in immediate allergic reactions to this drug. These results suggest a different pattern of recognition to the CLV ADs in CLV allergic patients; therefore, the inclusion of both AD reports better results.

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RESULTS



Higher proliferation of CD3⁺ cells was observed in CLV allergic patients compared with HC after the inclusion of the analogues Clav-1, Clav-2, and Clav-5. These differences were also higher in CD4⁺ and T_H2-CD4⁺ allergic patients' cells after the inclusion of Clav-1, Clav-2, Clav-4, Clav-5, and Clav-6. No proliferation was observed in CD8⁺ cells, neither with CLV, nor with any analogue, suggesting their lower implication in IR reactions. Interestingly, no different proliferative response was observed after the inclusion of CLV itself, independently of the cell population analyzed, between patients and HC.



LTT with CLV showed positive results only in 9% and 18% of allergic patients in CD4⁺ and T_H2-CD4⁺ cells, respectively. Regarding the ADs, there was a range of positivity between 9-45%. By evaluating the data after combining the results with the analogues from the different ADs, we observed that the inclusion of AD-I (Clav 1-3) increased LTT sensitivity to 46% and 36%, and the inclusion of AD-II (Clav 4-6) to 55% and 64% in CD4⁺ and T_H2-CD4⁺ cells, respectively. Interestingly, the inclusion of both ADs increased LTT sensitivity to 64% in CD4⁺ cells and to 73% in T_H2-CD4⁺ cells. The inclusion of CLV does not produce an added value in terms of sensitivity. Moreover, no positive results were obtained with HC independently of the analogue included or CLV itself.

The present study has been supported by Institute of Health "Carlos III" of the Ministry of Economy and Competitiveness (grants cofunded by European Regional Development Fund) PI15/01206, RETICS ARADyAL (RD16/0006/0001), Andalusian Regional Ministry of Health (PI-0241-2016). Authors declare that they do not have any conflict of interest.