TITLE

Involvement of myeloid dendritic cells in the evaluation of immediate hypersensitivity reactions to betalactams

SHORT TITLE

Myeloid dendritic cells in immediate allergic reactions

AUTHORSHIP

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The authors approved the final version of the manuscript as submitted and agreed to be accountable for all aspects of the work.

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Dendritic cells, maturation, proliferation, lymphocyte transformation test, immediate drug hypersensitivity reactions

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ABSTRACT (250words)

Background: Amoxicillin (AX) and clavulanic acid (CLV) are the betalactam antibiotics (BLs) most used to treat bacterial infections, although they can trigger immediate hypersensitivity reactions (IDHRs). The maturation analysis of monocyte-derived dendritic cells (moDCs) and their capacity to induce proliferative response of lymphocytes are useful to test the sensitisation to a drug, although without optimal sensitivity. Nevertheless, this can be improved using directly isolated DCs such as myeloid DCs (mDCs).

Objective: To evaluate the specific recognition of drugs by mDCs, as well as their capacity to stimulate autologous lymphocytes in IDHRs to AX and CLV.

Methods: mDCs and moDCs were obtained from 28 allergic patients (AP), 14 to AX, 14 to CLV and from 10 healthy controls (HC). The expression of CCR7, CD40, CD80, CD83, and CD86 was analysed after stimulation with both BLs. We measured the capacity of these pre-primed DCs to induce drug-specific activation of different lymphocyte subpopulations, $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+$Th1, and $CD4^+$Th2, by flow cytometry.

Results: Higher expression of CCR7, CD40, CD80, CD83, and CD86 was observed on mDCs compared to moDCs from AP after stimulating with the culprit BL. Similarly, mDCs induced higher proliferative response, mainly of $CD4^+$Th2 cells, compared to moDCs, reaching up to 67% of positive results with AX, whereas of only 25% with CLV.

Conclusions: mDCs from selective AP efficiently recognise the culprit drug which trigger the IDHR. mDCs also trigger proliferation of lymphocytes, mainly those with a Th2 cytokine pattern, although these responses depend on the nature of the drug, mimicking the patient's reaction.

KEY MESSAGES (3 bullets)

- The analysis of activation, maturation, and migration markers on mDCs can be used to assess the sensitisation to amoxicillin and clavulanic acid, in a more efficiently way than using moDCs.

- Drug pre-primed mDCs have a greater capacity to activate autologous lymphocytes compared to moDCs.
- The inclusion of pre-primed mDCs in LTT induced proliferative response of $CD4^{+}$Th2 cells in IDHRs patients mimicking the acute reaction.

CAPSULE SUMMARY (35words)

This study provides a new *in vitro* approach consisting of using myeloid dendritic cells. These findings give us a more complete picture about betalactam recognition, with potential implications for the future diagnosis of hypersensitivity reaction.

ABBREVIATIONS AND ACRONYMS

Allergic patients, AP; amoxicillin, AX; anaphylaxis, ANA; antigen presenting cell, APC; betalactams, BLs; carboxyfluorescein succinimidyl ester, CFSE; clavulanic acid, CLV; dendritic cell, DC; drug hypersensitivity reaction, DHR; drug provocation test, DPT; European Academy of Allergy and Clinical Immunology, EAACI; Granulocyte-macrophage colony-stimulating factor, GM-CSF; healthy control, HC; Hospital Regional Universitario de Malaga, HRUM; immediate drug hypersensitivity reaction, IDHR; intradermal test, IDT; lipopolysaccharide, LPS; maturation index, MI; mean fluorescence intensity, LN, lymph node; MFI; lymphocyte transformation test, LTT; monocyte-derived dendritic cell, moDC; myeloid dendritic cell, mDC; nonimmediate drug hypersensitivity reaction, NIDHR; peripheral blood mononuclear cell, PBMC; penicillin V, PV; plasmacytoid dendritic cell, pDC; phytohemagglutinin, PHA; proliferation index, PI; skin prick test, SPT; skin test, ST; specific immunoglobulin E, sIgE; urticaria/angioedema, URT/ANG.

INTRODUCTION

Betalactam antibiotics (BLs) are the most frequent drugs used to treat bacterial infections, especially amoxicillin (AX), prescribed or not alongside clavulanic acid (CLV).[1, 2] Nevertheless, they are also the most frequent antibiotics responsible for drugs hypersensitivity reactions (DHRs) in both children,[3, 4] and adults.[5]

DHRs are commonly classified accordingly the time interval between drug administration and the onset of symptoms into immediate (IDHRs), in which symptoms appear from a few minutes to 6 hours, mainly mediated by specific immunoglobulin E (sIgE); and non-immediate (NIDHRs), in which symptoms appear from 6 hours to several days after drug administration, being mainly mediated by T cells.[6-8]

The diagnosis of DHRs to BLs is complex, based on clinical history, often unreliable, and by *in vivo* test.[9, 10] Skin test (ST) is the first-line diagnostic method in IDHRs, although its sensitivity is not optimal.[9, 11, 12] A confirmed diagnosis is vital for avoiding false allergic label,[13] because of this, drug provocation test (DPT) is required as gold standard to confirm it,[14] although, it is not risk-free and not recommended for the most severe reactions. Therefore, *in vitro* tests represent safe alternatives that can help to improve the diagnosis of IDHRs.[8, 15-18]

Although lymphocyte transformation test (LTT), which consists on evaluating the proliferation of lymphocytes after drug specific stimulation, has been mainly used in NIDHRs,[8, 15, 19] it can be also used for evaluating IDHRs.[20, 21] In spite of its good specificity, its sensitivity is not optimal, probably because of different factors, like the nature of the drug, their interaction with the immune system, the

heterogenicity of clinical manifestations, or the method used to analyse lymphocyte proliferation.[6, 19, 20, 22] In line with this, several studies have indicated that the inclusion of the specific antigen presenting cells (APCs) could be an important factor for improving this sensitivity.[20, 22, 23]

Dendritic cells (DCs) are professional APCs, and the most potent inducers and orchestrators of immune response,[24] due to their important role to bridge innate and adaptive immunity.[25] Immature DCs have the ability to recognise exo- and intracellular antigens.[26] Then, after processing, they undergo a complex maturation process that leads to migrate to the lymph nodes (LNs), where they present the antigens to naïve T and B cells through cytokine production,[27, 28] and expression of costimulatory molecules.[24, 27-29] Therefore, the analysis of specific maturation, activation and migration markers on DCs after their stimulation could represent a useful tool to assess a sensitisation. Two main circulating DCs can be found in blood, plasmacytoid DCs (pDCs) and myeloid DCs (mDCs),[26] with different recognition and migration behaviour.[30] The latter group can be further divided into CD1c (BDCA-1), $CD16^{+}$, and BDCA-3 DCs.[30] Nevertheless, because of the low frequency of circulating DCs,[25] most *in vitro* studies used monocyte-derived DCs (moDCs), which can be easily *in vitro* transformed from monocytes.[24, 26, 31] In line with this, some studies have analysed their specific response to drugs in both IDHRs,[20] and NIDHRs,[23, 32, 33] although their inclusion showed a suboptimal sensitivity.[23]

In the present study, we evaluated the recognition of two BLs (AX and CLV) by different DCs, in a well-defined group of patients with selective IDHRs to each drug. Herein, we used flow cytometry to analyse the expression of maturation, activation, and migration markers on moDCs, and mDCs in response to both

drugs. Moreover, we analysed their capacity to stimulate different lymphocyte populations, comparing the results with those obtained from traditional LTT.

METHODS

Patient and Healthy control selection

Patients who attended the Allergy Unit of Hospital Regional Universitario de Malaga (HRUM) between 2018 and 2019 with a suggestive history of IDHR after the administration of AX or AX-CLV were evaluated following the European Academy of Allergy and Clinical Immunology (EAACI) guidelines.[9, 11, 34, 35] After the allergological study, 28 confirmed allergic patients (AP), 14 to AX and 14 to CLV, were included. A group of 10 sex-age matched subjects with no reported allergy to any drug was included as healthy controls (HC). All participants were correctly informed about the study and signed an informed consent. The research was conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committee of Malaga.

Allergological study

A detailed clinical history was recorded, classifying the IDHR into urticaria/angioedema (URT/ANG) or anaphylaxis (ANA). The severity was classified according to Brown classification.[36] Skin prick test (SPT) and intradermal test (IDT) when the former was negative, were performed with benzilpenicilloyl octa-lysine (0.04 mg/mL), benzylpenicilloate (0.5 mg/mL), AX (20 mg/mL), and CLV (20 mg/mL) (all from Diater, Madrid, Spain), as previously described.[9, 11] Afterwards, DPT with penicillin V (PV) was performed in patients with SPT+ to AX, and with PV and/or AX in patients with SPT+ to CLV followed by home intake of daily therapeutic dose for 2 days.[11]

Dendritic cell isolation

Peripheral Blood Mononuclear Cells (PBMCs) were isolated by density gradient (Lymphoprep™, STEMCELL Technologies, Vancouver, Canada). CD1c (BDCA-1)$^{+}$ DCs (mDCs) were directly isolated from PBMCs by magnetic separation (CD1c (BDCA-1)+ Dendritic Cell Isolation Kit (Miltenyi Biotec, North Rhine-Westphalia, Germany). Viability dye, CD19-PECy7, CD4-, CD14-, CD16-, CD8-FITc, HLA-DR-VB and CD1c-AF647 were used to characterise mDCs (fig. S1A) before (fig. S1B) and after (fig. S1C) isolation.

Monocytes (CD14^{+} cells) were isolated from PBMCs by magnetic separation (Miltenyi Biotec). Then, monocytes were cultured with 100ng/ml of IL-4 and 200ng/ml of granulocyte-macrophage colony stimulating factor (GM-CSF) (both from R&D Systems Inc, Minnesota, USA) for 5 days to obtain moDCs. CD14-PerCP, HLA-DR-FITc, and CD1a-PECy7 were used to confirm differentiation from monocytes to moDCs (fig. S1D).

DC maturation assays

mDCs and moDCs were cultured with AX and CLV at 0.5 mM and 1mM for 72 hours. Lipopolysaccharide (LPS) (InvivoGen, California, USA) and culture media were used as positive or negative controls, respectively. Afterwards, DCs were labelled with CCR7-PerCP, CD40-PE, CD80-PECy7, CD83-APC, CD86-FITc, and LIVE/DEAD™ Fixable Near-IR Dead Cell Stain. DCs were acquired in a FACSCANTO II flow cytometer. Results were expressed as Mean Fluorescence Intensity (MFI) and as Maturation Index (MI), calculated as the ratio between % of stimulated and unstimulated DCs. MI ≥ 2 was considered positive.

Lymphocyte Transformation Test

After monocytes and mDCs isolation, the rest of PBMCs were labelled with carboxyfluorescein succinimidyl ester (CFSE) and cultured with autologous primed mDCs and moDCs, as showed above, for 7 days. Both mDC- and moDC-LTTs were compared with the traditional ones, consisting on the direct incubation of CFSE-labelled PBMCs with the drugs for 7 days. Phytohemagglutinin (PHA) (Sigma-Aldrich, Misuri, US) was used as positive control and cells without stimulus as negative control. Proliferation was assessed by measuring the % of $CFSE_{LOW}$ of $CD3^+$, $CD8^+$, and $CD4^+$ cells, with both, Th1 ($CD4^+CCR5^+$ cells) and Th2 ($CD4^+CRTH2^+$ cells) profile. Results were expressed as Proliferation Index (PI), as follows:[20, 22, 32]

$$PI = \frac{(\%\ CFSElow\ stimulated\ lymphocytes + DCs) - (\%\ CFSElow\ unstimulated\ lymphocytes + DCs)}{\%\ CFSElow\ unstimulated\ lymphocytes}$$

Statistical analysis

Mann-Whitney test was used to compare quantitative pairs of data without normal distribution (Kolmogorov–Smirnov test). For multiple quantitative comparisons, Kruskal-Wallis test with multiple comparisons was used. For qualitative comparisons, Chi-square (X^2) test was used. Differences statistically significant were considered when p-value ≤0.05 was obtained. Statistical analysis was performed by using GraphPad PRISM v8.

RESULTS

Twenty-eight patients with confirmed IDHRs to BLs, 18 females (64%) and 10 males (36%), were included in the study (Table 1). 14 were selective to AX (group A), and 14 to CLV (group B). The mean age was of 45.39±13.98 years. The reactions were ANA in 20/28 patients (71%), and URT/ANG in 8/28 (29%). Following Brown's severity classification,[36] 20/28 patients (64%) have a reaction of grade I or II (36% each one), and 8/28 (36%) have a reaction of grade III.

Time interval between the administration of the drug and onset of symptoms was 34.64±22.28 min. Time interval between reaction and the study was 4.68±4.04 years. Twenty out of 28 patients only reported one-episode (71%) whereas 8/28 (29%) reported two. 20/28 patients (71%) have a reaction after the administration of AX-CLV, whereas 8/28 (29%) have a reaction after AX administration. All patients from group A have positive ST with AX, except A9, who had positive result to AX in DPT. Similarly, patients from group B have positive results to ST to CLV and tested negative to DPT to AX to confirm the selective response to CLV. Tolerance to PV was confirmed in all patients by DPT as previously reported.

Migration and maturation marker expression on non-stimulated DCs.

Higher expression of CCR7 was observed on non-stimulated mDCs compared to non-stimulated moDCs. On the contrary, higher expression of CD40, CD80, and CD86 was observed on non-stimulated moDCs compared to non-stimulated mDCs. No differences were observed regarding the expression of CD83 (Fig. 1).

DC maturation assays

The stimulation with AX increased the expression of CCR7, CD40, CD80, CD83, and CD86 on mDCs in AX-AP compared to HC (fig. 2A-E). Moreover, the stimulation with AX only increased the expression of CCR7, CD83, and CD86, but not of CD40 or CD80 on moDCs from AX-AP compared to HC. In a similar way, the stimulation with CLV of mDCs from CLV-AP increased the expression of CCR7, CD80, CD83, and CD86, but no difference was observed in the expression of CD40 (fig. 2F-J). By contrast, the stimulation of moDCs with

CLV in CLV-AP increased significantly only the expression of CD83, but not any of the other markers analysed (fig. 2F-J).

The highest % of positivity in mDCs from AX-AP was found for CD80 (60%) followed by CCR7, CD40 and CD83 (47%), and CD86 (27%) (fig. 3A-E), whereas the positivity in moDCs was lower for all analysed markers (fig. 3A-D), except for CD86 (31%) (fig. 3E). In HC, the positivity induced by AX was lower than 10% for both mDCs and moDCs, independently the marker, except for CD86 on mDCs (17%) (fig 3A-J).

The positivity of mDCs from CLV-AP stimulated with CLV, for CCR7, CD40, CD80 and CD86 was higher than with moDCs (fig. 3F-H, J), except for CD83 (fig. 3I). In HC, the positivity was lower than 10% in both, mDCs and moDCs stimulated with CLV for all markers (fig 3F, H, I), except for CD40 which was of 25% with mDCs, and of 21% with moDCs (fig. 3G), and of 17% in mDCs for CD86 (fig. 3E).

Then, we analysed the specificity of maturation assays, by stimulating mDCs and moDCs with the non-culprit drugs, CLV in AX-AP or AX in CLV-AP. For AX-AP, similar MI was obtained for mDCs and moDCs, independently the marker analysed, except for CD40 and CD80 (fig. S2A-E). The positivity was lower than 25% with both, mDCs and moDCs, independently the marker analysed (fig. S3A, C-E), with exception of CD40 in mDCs (46%) (fig. S3B). For CLV AP, similar MI was obtained with mDCs and moDCs for CCR7, CD80 and CD86, although higher MI was obtained for CD40 and CD83 in moDCs compared to mDCs (fig. S2G, I). The positivity was lower than 25% for all the markers analysed, except for CD86 in moDCs (26%) (fig. S3F-J).

Lymphocyte proliferation assays

We analysed the capacity of these APCs, mDCs or moDCs, to present the culprit drug to autologous lymphocytes and to induce a specific proliferative response. Higher PI was obtained in $CD3^+$, $CD4^+$, and $CD4^+$Th2 cells (fig. 4A, B, E), after culturing mDCs with AX-pre-primed mDCs from AX-AP, compared to those from HC. The inclusion of AX-pre-primed moDCs only increased the PI of $CD4^+$Th2 cells from AX-AP compared to HC (fig. 4E). When all PBMCs were cultured with AX, higher PI was obtained in AX-AP than in HC in all cell subpopulations (fig.4A-C, E), except for $CD4^+$Th1 cells (fig. 4D). Similarly, CLV-pre-primed mDCs increased the PI in $CD3^+$, $CD4^+$, and $CD4^+$Th2 cells from CLV-AP compared to HC (fig. 4F, G, J), but not in $CD8^+$ and $CD4^+$Th1 cells (fig. 4H, I). By contrast, no differential proliferative response was observed with direct CLV stimulation of PBMCs or after including CLV-pre-primed moDCs, independently the lymphocyte population analysed (fig. 4F-J).

Analysing positivity of the results in AX-AP, the highest LTT positivity was found with AX-pre-primed mDCs in $CD3^+$, $CD4^+$, and the two subpopulations, $CD4^+$Th1 and $CD4^+$Th2 cells (67%) (fig. 5). The use of AX-pre-primed moDCs reported a positive proliferation in $CD4^+$cells (33%) (fig. 5B), followed by $CD4^+$Th1 and $CD4^+$Th2 cells (22% for both) (fig. 5D, E). When PBMCs from AX-AP were cultured with the culprit drug, the highest positivity was obtained for $CD4^+$Th2 cells (50%) (fig. 5E), followed by $CD4^+$Th1 and $CD8^+$cells (25% for both) (fig. 5C, D) and $CD3^+$ and $CD4^+$cells (13% for both) (fig. 5A, B).

In CLV-AP, when CLV-pre-primed mDCs were included, positive proliferation was observed in $CD3^+$, $CD4^+$ and $CD4^+$Th2 cells in 25% of cases (fig. 5F, G, J). Nevertheless, when CLV-pre-primed moDCs or CLV-stimulated PBMCs were

used, the positivity was lower than 10% for all cell subpopulation analysed (fig.5F-I), except for $CD4^+$Th2 cells with pre-primed-moDCs (20%) (fig. 5J).

Then we analysed the specificity of the proliferative response of $CD4^+$Th2 cells by using different APCs: mDCs or moDCs; or monocytes/B cells included in PBMCs, by stimulating with the non-culprit drugs, CLV in AX-AP or AX in CLV-AP. Data showed that in AX-AP, higher PI was observed with the direct drug stimulation of PBMCs, compared to using pre-primed-mDCs or -moDCs (fig. S4A). Similarly, for CLV-AP, higher PI was obtained for PBMCs, compared to the use of pre-primed -mDCs or -moDCs (fig. S4B).

The % of positive cases after CLV stimulation of PBMCs from AX AP was much higher (38%) compared to using moDCs (11%), and mDCs (0%) (fig. S4C). Similarly, positivity of PBMCs from CLV AP stimulated with AX was high (21%), whereas no positive cases were detected using moDCS or mDCs (fig. S4D).

DISCUSSION

IDHRs to BLs, and concretely to AX and CLV have increased in the last decades,[5 37-40] probably because of their higher prescription.[18] There is an unmet need regarding the diagnosis, since it is a complex process based on clinical history, not always reliable, and *in vivo* tests (ST and DPT), with not optimal sensitivity and not recommended for most severe reactions respectively, driving to a high rate of overdiagnoses.[11, 13] Given the importance of AX and CLV use, and that inappropriate allergy diagnose leads to using second-line treatments with high consequences to the patient and health services.[18] Therefore, the use of safe *in vitro* tests could be reliable alternatives to improve the diagnosis of IDHRs to AX and CLV.[8, 15, 17] However, the current

329 available tests, both immunoassays and cellular tests, although specific, they do
330 not have optimal sensitivity.[4, 17, 22, 33, 41-45]
331 In relation to cellular tests, one factor that could increase the *in vitro* response is
332 the inclusion of APCs that process and present the drugs in an efficient way to
333 be recognised by specific lymphocytes. In this regard, the analysis of migration
334 and maturation markers on DCs after the stimulation with the culprit drug may
335 represent a useful *in vitro* alternative to assess the sensitisation to a concrete
336 drug. mDCs have been reported as the true “sentinels” against internal and
337 external antigens in blood and tissues,[25] however, most research have used
338 moDCs, which can be easily transformed *in vitro* in high number from circulating
339 monocytes.[26] Although moDCs are present in nature, they only developed
340 during inflammation, and after resolution, they disappear.[26, 28, 46] Because of
341 their different nature, their response to drugs can also be different, having
342 important implications in DCs maturation *in vitro* assays sensitivity.
343 In steady state, higher expression of CCR7 was observed on mDCs compared
344 to moDCs, whereas higher expression of CD40, CD80, and CD86 were
345 observed in moDCs. These data suggest that, although both immature DCs
346 have important phagocytic, and antigen processing capacity,[47, 48] moDCs are
347 much activated at baseline. This may indicate that moDCs are ready to present
348 antigens in the inflammation tissue, with lower capacity to migrate from blood or
349 tissues to LNs,[49, 50] whereas mDCs need to migrate prior the presentation to
350 naïve T cells. Therefore, after drug stimulation, mDCs expressed higher levels
351 of CCR7 compared to moDCs in AP. This observation is in line with a recent
352 study in patients with IDHRs to CLV, in which although higher expression of
353 CCR7 on moDCs from AP was observed after the inclusion of the drug and/or

its metabolites, its expression was much lower than other maturation markers analysed.[20] Nevertheless, this lower expression seems not to affect the capacity of moDCs to induce activation of lymphocytes, indicating that this marker may be not so essential for moDCs in *in vitro* assays.

Different studies have shown that DCs maturation markers like CD40, CD80, CD83, and/or CD86 were highly expressed on stimulated moDCs from AP with NIDHRs to AX,[23] and other drugs.[32] Related to this, Sanchez-Quintero and colleagues reported a positivity of 45.8% with CD86 expression in AX-AP with NIDHRs.[51] In our study, we found a positivity of 31% for CD86. Nevertheless, the expression of these markers was much higher after AX stimulation of mDCs (47%-60%). The lower expression of activation and maturation markers on moDCs, compared to previous studies, could be explained because of the different involvement of moDCs depending on the type of the reaction, IDHRs or NIDHRs.

Similarly, mDCs from CLV-AP after stimulation with the drug expressed higher maturation markers compared to HC. Although the positivity between CLV- and AX-AP in mDCs is comparable, their expression on moDCs was much lower in CLV-AP compared to AX ones. This lower reaction could be explained by the higher degradative rate of CLV compared to other BLs, as it has been demonstrated before.[52-55] Because of its degradation, different CLV metabolites can be formed, with different abilities to form protein-carrier conjugates and therefore with different efficiency to be recognised by the immune system,[20, 37, 56] as we have previously demonstrated.[20, 37] Herein, we hypothesised that mDCs are more sensitive and ready-to-act than moDCs, and can need lower concentration of these reactive CLV metabolites to be activated.

LTT has been mainly used to evaluate NIDHRs, with a high variability of sensitivity,[22, 23, 57, 58] mainly because of multiple factors. Mori and colleagues reported a LTT sensitivity of 52% with 92% of specificity in a group of children with NIDHRs to AX or AX/CLV.[3] Similar sensitivity was found by Prieto et al. (52.9%), nevertheless, important differences were observed between AX (63.6%) compared to CLV (33.3%) suggesting the importance of the nature of the drug included in the LTT,[4] similar to our observations.

To our knowledge, only two previous studies used LTT to evaluate IDHRs to BLs.[20, 21] The sensitivity found by Luque and colleagues was of 64.5%, higher than in our study.[21] Differences between studies may be explained by the methodology used and by the clinical characteristics of patients due to Luque et al. included more than 30% of AP to penicillin and none to CLV, with most of them being cross-reactors. This sensitivity was like ours by using mDCs (67%) for AX. In a recent study with CLV in IDHRs, the used of moDCs reported a low sensitivity (19%) when proliferation of $CD4^{+}$Th2 cells was analysed,[20] like our observations in this study (20% with moDCs and 25% with mDCs). In the present study, the highest sensitivity was achieved when the proliferative response of $CD4^{+}$Th2 cells was analysed, without the need to combine results from different cell populations, in agreement with a previous study in our group with IDHRs to CLV,[20] and contrary to NIDHRs, where the inclusion of the proliferative response of different cell populations increased the overall LTT sensitivity.[22] This highlights the high heterogenicity of immune mechanisms of NIDHRs compared to IDHRs, and the importance of analysing the proliferation of specific subpopulations involved in each concrete immune mechanism.[20, 22]

It has been demonstrated that the use of pre-primed moDCs trigger higher proliferation of autologous T cells in response to a drug in patients with NIDHRs to AX, than other APCs,[23] similarly to another study carried out with heparins.[33] Nevertheless, LTT sensitivity is still not optimal. It might be possible that AX and CLV are not strong stimulators. Because of this, DCs only achieve a semimature status,[30] being possible the rapidly expression of maturation markers, but weak cytokine response.[30] In our study, only 20% of sensitivity was observed after including moDCs, probably due to the different mechanism involved in IDHRs and NIDHRs. Nevertheless, the inclusion of pre-primed mDCs increased LTT sensitivity up to 67% for AX, in line with other studies which showed that mDCs have a strong ability to trigger T cell proliferation and activation.[30]

In conclusion, we have demonstrated the importance of the inclusion of APCs to increase the *in vitro* lymphocyte response to drugs. Specifically, we found that AX and CLV interact with mDCs, in a specific way, and the analysis of activation markers on their surface could be used to assess the sensitisation in patients with IDHRs to AX or CLV, in a more efficiently way than using moDCs. Moreover, the inclusion of pre-primed mDCs increased LTT sensitivity to evaluate IDHRs compared to previous approaches with both drugs, mainly analysing the proliferative response of $CD4^{+}$Th2 cells by flow cytometry, mimicking the allergic response of the patients. Although more studies are required, this research gives us a more complete picture about the specific recognition of drugs by the immune system, which might have implications for future diagnosis of IDHRs to BLs.

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