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Successful Application of the Mast Cell Activation Test in Immediate Hypersensitivity to Amoxicillin

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ABSTRACT

Background: Immediate drug allergic reactions (IDAR) to betalactams are frequent, yet mislabelling remains common and negatively impacts clinical decisions. Conventional diagnostics such as STs and drug provocation are effective but limited by time, risk, and contraindications in severe cases. In vitro alternatives—sIgEquantification and basophil activation tests (BAT)—offer safer options, although performance may be affected by biological variability and suboptimal sensitivity with an important drawback for the latter in patients with non-releaser basophils. This study aimed to evaluate a mast cell activation test (MAT) based on human $CD34^+$-derived mast cells (dMCs) for IDAR diagnosis to amoxicillin (AX) using both free AX and dendrimeric amoxicilloyl conjugates (G4/G5-AXO).

Methods: $CD34^+$ cells were cultured for 10–12 weeks to generate dMCs. After passive sensitization with sera from AX-allergic patients ($N = 28$) or tolerant controls ($N = 11$), dMCs were stimulated with free AX, G4-AXO, or G5-AXO. Activation was quantified by CD63 expression via flow cytometry. Diagnostic performance was compared with BAT and sIgE determination by ImmunoCAP.

Results: MAT with free AX achieved 53.57% sensitivity, G4/G5-AXO reached 46.43%, and all methods maintained 100% specificity. Combining results with AX or dendrimeric stimuli increased sensitivity to 75% while preserving specificity. Importantly, MAT identified positive cases among BAT non-releasers and patients with undetectable sIgE.

Conclusions: Under optimized conditions, MAT using dMCs and different AX determinants reached 75% sensitivity and 100% specificity. The complementary use of free AX and dendrimeric conjugates expands detection across heterogeneous IgE reactivity profiles from AX-allergic patients, reinforcing the diagnostic value of advanced cellular models and engineered allergens.

Abbreviations: AX, amoxicillin; AXO, amoxicilloyl; G4/G5-AXO, dendrimeric amoxicilloyl conjugate; hSCF, human stem cell factor; IL, interleukin; LDL, ow density lipoprotein.

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1 | Introduction

Global antibiotic consumption increased 16.3% and is projected to rise [1]. In Europe, β-lactam (BLs), specifically penicillins, are the most consumed group of antibiotics (47% of total), followed by cephalosporins and other BLs (12%) [2]. This consumption correlates with a higher incidence of hypersensitivity reactions, resulting in 10%–15% of the total population labeled as allergic to BLs [3–6]. Amoxicillin (AX) causes most immunoglobulin E (IgE)-mediated immediate drug allergic reactions (IDAR) [3, 4]. However, 90%–95% of penicillin labels are false, and over 90% tolerate BLs [5, 7]. Such mislabeling leads to suboptimal prescribing, broader-spectrum use, greater toxicity, and antimicrobial resistance [8, 9]. Given these challenges, accurate diagnosis represents the cornerstone for effective treatment and prevention strategies.

Standard diagnosis includes clinical history, skin tests (STs)—skin prick (SPT) and intradermal (IDT)—and, if necessary, drug provocation test (DPT) [3, 10]. Though widely endorsed by guidelines (European Academy of Allergy and Clinical Immunology (EAACI), European Network in Drug Allergy (ENDA)), these methods have drawbacks: time-consuming, risky, and sometimes contraindicated. STs, particularly for BLs, may lack optimal sensitivity, requiring multiple determinants to improve accuracy [3]. As a safer alternative, in vitro tests such as sIgE determination by immunoassays, basophil activation test (BAT), and more recently mast cell activation test (MAT) have emerged to complement the in vivo diagnosis of IgE-mediated IDAR [11, 12]. sIgE determination by commercial immunoassays was evaluated in penicillin-allergic patients from Spanish and Italian populations, showing 39%–52% sensitivity and up to 16% false positives for penicillin G [13]. Additional limitations include time-dependent decreases in sIgE levels (within 6 months or more [14]) and limited availability of specific drug–carrier conjugates [15]. To overcome these limitations, BAT is recommended as an alternative for diagnosis [11]. Meta-analysis showed BAT for penicillin allergy has 51% sensitivity and 89% specificity [16], and maximizing specificity to 100% may drop sensitivity to 13% [17]. While useful, BAT discloses other limitations: dependency on basophil presence and responsiveness, with 10%–15% being non-releasers, as for immunoassays, potential sensitivity loss due to time-dependent decreases in sIgE levels and the lack of standardized drug protocols [11]. These constraints prevent BAT from serving as a standalone test.

MAT is a strong alternative, leveraging mast cells' effector role in IgE-mediated responses. Mast cells express high levels of FcεRI and are central to immediate allergic responses. Therefore, these cells can be used after sensitization with sIgE from allergic patients to determine the drug recognition. Various cell sources have been explored, including cell lines [18] or humanized MC progenitor lines [19], which offer short culture time or less variability [20], as well as primary mast cells derived in vitro from $CD34^+$ progenitors (dMCs) [21] used in this study, which display a phenotype closer to primary MCs than cell lines [20]. dMCs exhibit phenotypic differences depending on their origin [22], since dMCs from peripheral blood are more similar to skin MCs, contain and release more histamine, and express more FcεRI than dMCs from cord blood, being more mature cells [23].

However, in vitro dMCs may not fully recapitulate the complexity of the in vivo microenvironment. The possibility of experimentally modulating the cytokine context can be an advantage or a challenge. Alarmins like Interleukin-33 (IL-33) and Thymic Stromal Lymphopoietin (TSLP) significantly influence mast cell biology and activation. For instance, IL-33 can acutely prime mast cells for increasing degranulation, enhancing their activities and cytokine release [24–27]. TSLP modulates mast cell function and supports their survival, and it can also potentiate responses to other stimuli [26–29]. Given the importance of the cytokine milieu in shaping the outcome of the reaction, several protocols have been tested to activate dMCs in vitro by several authors [24–26, 28, 30, 31].

On the other hand, another major limitation of cell-based in vitro diagnostics in IDAR lies in the nature of the antigen itself. Under the hapten hypothesis, stable drug–carrier conjugates are often required to enable effective IgE recognition and cross-linking as well as the subsequent cell activation [32]. Synthetic nanostructures like dendrimers can offer advantages over traditional carriers like human serum albumin (HSA) or poly-L-lysine (PLL) due to their defined dimensions, multivalent and symmetrical architecture, allowing precise peripheral multi-hapten display that improves antigen accessibility, recognition, and IgE cross-linking on the cell surface [33–35]. This improves immune recognition and reproducibility by yielding a more reliable cellular in vitro test.

Considering these factors, in this study, we sought to develop and evaluate MAT using human dMCs for the in vitro diagnosis of AX allergy, whose performance is further enhanced by incorporating amoxicilloyl (AXO)-conjugated dendrimers (higher-generation dendrimers were selected based on our prior optimal in vitro results [35, 36]). The system demonstrated high specificity and enhanced sensitivity, offering broader diagnostic coverage than current methods. This work supports the integration of advanced cellular models and engineered allergens into the diagnostic algorithm for BL allergy, particularly for complex or ambiguous cases.

2 | Methods

2.1 | Allergological Workup

We designed a retrospective observational study including a total of 28 patients with confirmed IDAR [37] to AX and 11 controls who tolerated this drug, who were recruited from the Allergy Unit of the *Hospital Regional Universitario de Málaga*. Sampling among patients with IDAR to AX was stratified by the AX-sIgE immunoassay profile (high/intermediate/low/negative), owing to its strong correlation with MAT results, and by the BAT phenotype (positive, negative, non-releasers). Diagnostic assessment followed the recommendations of the EAACI [3]. The study was conducted following the principles of the Declaration of Helsinki and was approved by the institutional review board (PI1800095) and by the Provincial Ethics Committee of Malaga. Further details on the diagnostic evaluation of patients, clinical history, and severity can be found in the Methods section of the article's Online Repository.

2.2 | Dendrimers-AXO Synthesis

The poly(amidoamine) (PAMAM) dendrimer (ethylenediamine core) with amino surface groups (PAMAM 4th (G4) and 5th generation (G5)) (Sigma-Aldrich) were incubated with sodium AX (0.02 M carbonate buffer, pH 10.8, at 4°C), stirred for six days, and then purified by gel filtration using Sephadex G-10 and distilled water as the eluent. The solvent was then freeze-dried to obtain the corresponding pure PAMAM-based AXO conjugates: G4-AXO and G5-AXO containing 64 and 128 AXO units/dendrimer, respectively. The purity and structural characterization were confirmed by ^{1}H- and ^{13}C-Nuclear Magnetic Resonance (NMR) techniques. Further details on the conjugate's synthesis including those with HSA can be found in the Methods section of the article's Online Repository.

2.3 | BAT

BAT was performed as previously described [38]. Cells were stimulated with AX at 1.25 mg/mL and stained with CD63-FITC, CD203c-PE, and CCR3-APC (Biolegend). At least 500–1000 basophils per sample were analyzed in a FACSCalibur flow cytometer (BD Bioscience) following the gating strategy for basophil selection, SSC-A_{low}/CCR3^{+}/CD203c^{+}, and for activated basophils, CD63^{+}. Results were considered positive when the percentage of CD63^{+} was higher than the cut-off selected from ROC analysis.

2.4 | AX-sIgE Determinations

To determine serum AX-sIgE (c6) and BP-sIgE, FEIA ImmunoCAP (Thermo-Fisher) was used following the manufacturer's instructions in allergic patients and controls. Results of AX-sIgE were considered positive when results were ≥ 0.35 kUA/L.

2.5 | Mast Cell Derivation From CD34^{+} Peripheral Blood Cells

CD34^{+} peripheral blood cells were obtained from STEMCELL Technologies (Canada). Cells were cultured for 10–12 weeks in two phases: expansion and differentiation [21, 39]. Step I (Weeks 0–4): Cells were cultured at 1×10^{6}cells/mL in StemSpan SFEM II (STEMCELL Technologies), supplemented with 100 ng/mL human stem cell factor (hSCF), 50 ng/mL IL-6, 10 ng/mL IL-3, and 10 μg/mL LDL. Hemi-depletions were performed to maintain concentrations between $0.5–1 \times 10^{6}$ cells/mL. Step II (Weeks 5–12): Cells were cultured in IMDM with 100 ng/mL hSCF, 50 ng/mL IL-6, 55 μM 2-mercaptoethanol, 0.5% BSA, and 1% insulin-transferrin-selenium (Thermo Fisher) until derived mast cells (dMCs) reached ≥ 90% purity (CD117^{+}/FcεRI^{+}), typically after 4–6 additional weeks (Figure S1).

2.6 | Mast Cell Activation Test

dMCs were seeded at 50,000 cells per condition and sensitized overnight at 37°C with sera from AX-allergic patients or controls in medium II plus 100 ng/mL TSLP (PeproTech) at a 1:1 cell-to-serum ratio. After washing, cells were incubated for 1 h with 100 ng/mL IL-33 (Peprotech) in fresh medium II, followed by stimulation for 30 min and subsequent cooling on ice for at least 5 min at 4°C. In the MAT experiments, 10,000–20,000 mast cells were acquired and analyzed per sample to ensure robustness of the results.

Specific dMC activation was assessed using AX or G4/5-AXO nanostructures. AX at 1,25 and 2,5 mg/mL (3420 and 6840 μM, respectively) based on prior experience and BAT data shows optimal responses [38, 40]. G4/5-AXO nanostructures (64 and 128 AX units/dendrimer, respectively) were tested at 3.045, 6.09, and 30.450 μg/mL for G4-AXO and 3.063, 6.126, and 30.640 μg/mL for G5-AXO (5, 10, or 50 μM AX-equivalent). Lower concentrations of dendrimers showed suboptimal activation and therefore were not included in further analysis. Additionally, for comparison with nanostructures AX-equivalent, we tested AX at 3,65 μg/mL (10 μM) and HSA-AXO at 119,4 μg/mL (10 μM AX-equivalent).

PBS and anti-IgE (1 μg/mL, Invitrogen) served as negative and positive controls, respectively. Cells were washed, stained with Zombie NIR, CD63-FITC clone H5C6, CD117-PerCP clone 104D2, CD203c-PE clone NP4D6, and FcεRI-APC clone AER-37 (BioLegend), fixed with 2% paraformaldehyde (15 min, 4°C, dark), and resuspended in flow cytometry buffer (PBS 1X, 2% FBS, 4 mM EDTA, 0.02% sodium azide). Activation was defined as %CD63^{+} (Figure S1) above the ROC-derived cut-off, based on raw values without subtraction of the negative control.

2.7 | Statistical Analysis

Normality was assessed using the Kolmogorov–Smirnov test. Non-normally distributed paired and unpaired quantitative variables were compared using Wilcoxon and Mann–Whitney tests, respectively. Qualitative variables were analyzed with Fisher's exact test (independent samples) or McNemar test (paired samples). ROC curves defined cut-offs optimizing sensitivity and specificity. Patient age and the time interval between reaction and blood sampling are presented as median and interquartile range (IQR). $p < 0.05$ was considered significant. Analyses were performed with Prism 10 (GraphPad Software, San Diego, CA, USA).

3 | Results

3.1 | Study Population

Twenty-eight subjects (15 females, 13 males) with confirmed IDAR to AX were included, as well as 11 subjects (4 males, 7 females) with confirmed tolerance to AX. Patients were classified as AX-selective if they had positive in vivo tests to AX and tolerated BP or as cross-reactive if they had positive in vivo tests to BP. Based on this, 12 were AX-selective, 7 cross-reactive, and 9 remained unclassified because DPT could not be performed, either due to contraindications or patient refusal. Clinical manifestations were distributed as follows: 50% grade III, 25% grade II, and 21.43% grade I-URT/ANG. One patient could not be

classified because the patient did not remember details about the reported reaction. The mean time interval between the reaction and blood sampling for in vitro testing was 1574.8 days (IQR: 98–400.5). The median age for AX-allergic patients was 57 years (IQR: 53.25–68.25). Symptom onset latency after drug intake was classified into <15 min ($N=14$); 15-30 min ($N=4$); 30-60 min ($N=4$); 1-6 h ($N=2$); UNK ($N=4$) (Table S1).

3.2 | dMCs Are Activated With AX and/or G4/5-AXO Dendrimers in AX-Allergic Patients

To evaluate the usefulness as a tool for in vitro diagnosis for IDAR to AX, we conducted MAT with AX and AXO-conjugates (nanostructures or HSA) using dMCs. dMCs were sensitized with patients' sera with AX-sIgE ranging from 0 to 10.3 kUA/L (median: 0; IQR: 0.0–3.1) and control sera. Results indicated that all three concentrations of AX elicited statistically significant activation differences between AX-allergic patients and controls ($p=0.0018$; $p<0.0001$ and $p=0.0003$, respectively) (Figure 1A). Cut-offs from ROC curves were applied (Figure S2). At 10 μM AX, a cut-off of 3.2 yielded a sensitivity of 32.14% ($p=0.0025$); at 3420 μM, a cut-off of 3.8 provided a sensitivity of 53.57% ($p=0.0002$); and at 6840 μM, a cut-off of 4.6 resulted in a sensitivity of 53.57% ($p=0.0007$). All AX concentrations demonstrated 100% specificity (Figure 1C).

dMCs challenged with G4/G5-AXO also showed significant differences between AX-allergic patients and controls ($p=0.007$, $p=0.0052$, and $p=0.0064$ for G4-AXO; $p=0.037$, $p=0.0064$, and $p=0.0025$ for G5-AXO) (Figure 1B). ROC curve analysis of G4-AXO showed sensitivities of 42.86%, 46.43%, and 39.29% at 5, 10, and 50 μM AX-equivalent concentrations, respectively ($p=0.008$, 0.0063, and 0.0076), with 100% specificity at all concentrations. For G5-AXO, sensitivities were 32.14%, 46.43%, and 31.14% at 5, 10, and 50 μM, respectively ($p=0.0047$, 0.0076, and 0.0033), also with 100% specificity across all concentrations. G4/5-AXO nanostructures as carriers were compared to an alternative carrier for AX, has, at the same AX equivalent concentration (10 μM) as G4 and G5-AXO, as well as free AX. Results indicated that G4-AXO elicited stronger responses than HSA-AXO ($p=0.03$). No significant differences were observed for the rest of the comparisons (Figure S3). No activation was observed in unsensitized dMCs (Table 1).

3.3 | The Combination of AX and G4/5-AXO Enhances MAT Sensitivity

AX at 3420 μM achieved the highest individual sensitivity (53.57%). Both G4-AXO and G5-AXO at 10 μM equivalent of AX yielded comparable sensitivities (43.46%) (Figure 2A), and subsequent results are therefore reported using these concentrations. Interestingly, some patients responded exclusively to either AX or dendrimeric conjugates, but not to G4-AXO and G5-AXO, suggesting complementary diagnostic profiles (Figure 2B). Accordingly, we evaluated whether combining the results from both approaches improved sensitivity while preserving specificity. Consequently, the results showed that overall sensitivity increased up to 75% when AX and G4/5-AXO results were combined, while maintaining 100% specificity (Figure 2A). Moreover, when the false negative cohort for AX was re-classified considering G4/5-AXO results, 42.86% of them were identified as positive only when dendrimeric stimuli were used ($p=0.03$) (Figure 2C), underscoring the complementary effect of G4/5-AXO nanostructures to AX in MAT.

3.4 | MAT Results With Different Stimuli Diverge Depending on the Clinical Response

When MAT results were analyzed depending on the clinical response of the patients, selective or cross-reacting, it was observed that free AX elicited positive responses more frequently in AX-selective patients (66.67%) than in cross-reactive ones (42.87%). In contrast, nanostructures showed the opposite trend, with statistically significant differences for G4-AXO responses observed in 85.71% of cross-reactive patients versus 25% of AX-selective ones ($p=0.02$), and G5-AXO in 83.3% versus 27.3%, respectively (Figure 3A).

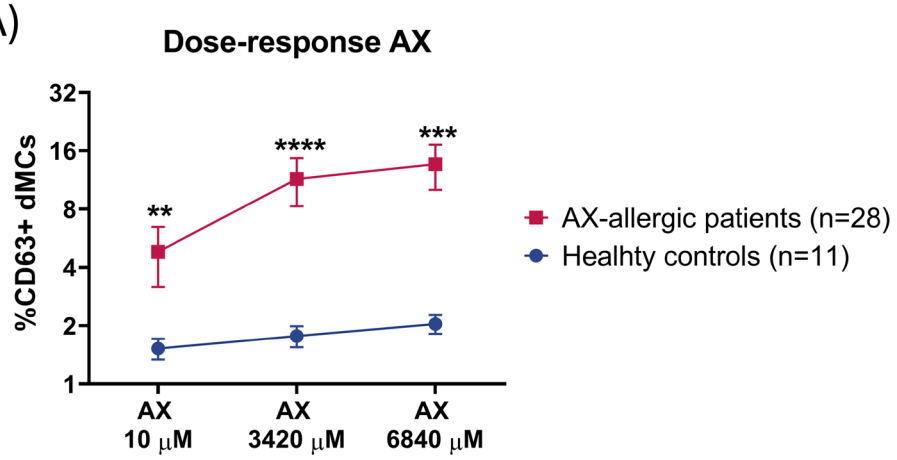
No significant differences were observed in positivity rates or activation levels according to severity grade (Figure 3B), nor did reaction latency affect assay results (Figure 3C). There was also no correlation between the time elapsed since the clinical reaction and the in vitro results (Figure 3D). Only a trend was seen of decreasing %$CD63^+$ with longer intervals for G4/5 nanostructures, while AX levels remained stable.

3.5 | MAT With AX and G4/5-AXO Nanostructures Combination Outperforms ImmunoCAP

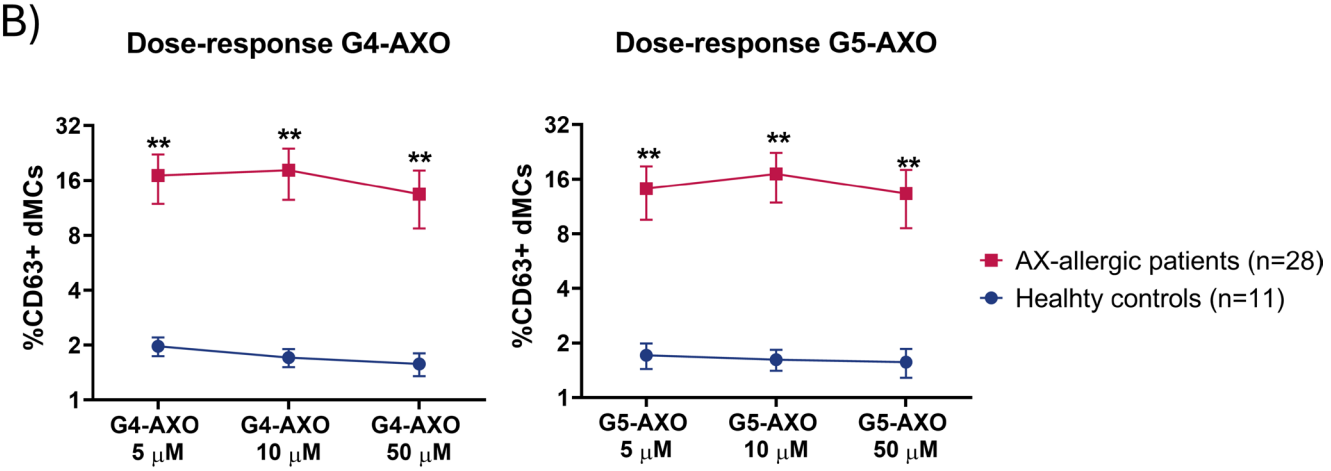
MAT results using AX and/or G4/5-AXO stimuli were compared to AX-sIgE determinations. The sensitivity of AX-sIgE determination was 46.43% (Figure 4A), identical to that of MAT with G4/5-AXO (Figure 4A), and slightly lower than that of MAT with free AX (Figure 4A). As mentioned before, when the result of either free AX or G4/5-AXO was considered positive in MAT, sensitivity reached 75% (Figure 4A), which was significantly higher ($p=0.039$). It is noteworthy that while MAT demonstrated a specificity of 100%, ImmunoCAP showed a specificity of 90.9%.

Given the apparent differences in the pattern of positive responders to AX and G4/5-AXO in MAT, suggesting a complementary role between both stimuli, we assessed the correlation between the MAT results and ImmunoCAP. MAT responses to AX did not correlate with AX-sIgE (Spearman $r=-0.23$, $p=0.23$). In contrast, MAT responses to G4-AXO and G5-AXO showed moderate/strong correlations with AX-sIgE, with Spearman r values of 0.65 ($p=0.0002$) and 0.53 ($p=0.0041$), respectively; this correlation was also observed with BP-sIgE for AX (Spearman $r=-0.24$, $p=0.19$) and for G4/5-AXO (Spearman r0.64, $p=0.0002$ and Spearman $r=0.67$, $p=0.0001$ respectively). Interestingly, among the 13 patients who tested positive by ImmunoCAP, 11 also showed a positive response to G4-AXO, 10 to G5-AXO, and only 5 to AX, resulting in 2 patients who tested positive by ImmunoCAP but negative in MAT. Conversely, among the 15 patients who were false negatives by ImmunoCAP, 2 were positive to G4-AXO, 3 to G5-AXO, and 10 to AX (Table 1), indicating the

(A)



(B)



(C)

	AUROC	Cut-off	Sensitivity (%)	95 %CI	Specificity (%)	95 %CI	p-value
AX 10 μM	0.8149	3.2	32.14	17.93% to 50.66%	100	74.12% to 100.0%	0.0025
AX 3420 μM	**0.8929**	**3.8**	**53.57**	**35.81% to 70.47%**	**100**	**74.12% to 100.0%**	**0.0002**
AX 6840 μM	0.8539	4.6	53.57	35.81% to 70.47%	100	74.12% to 100.0%	0.0007
G4-AXO 5 μM	0.776	4	42.86	26.51% to 60.93%	100	74.12% to 100.0%	0.008
G4-AXO 10 μM	**0.7841**	**3.4**	**46.43**	**29.53% to 64.19%**	**100**	**74.12% to 100.0%**	**0.0063**
G4-AXO 50 μM	0.7776	3.5	39.29	23.57% to 57.59%	100	74.12% to 100.0%	0.0076
G5-AXO 5 μM	0.7938	4.5	32.14	17.93% to 50.66%	100	74.12% to 100.0%	0.0047
G5-AXO 10 μM	**0.7776**	**3.5**	**46.43**	**29.53% to 64.19%**	**100**	**74.12% to 100.0%**	**0.0076**
G5-AXO 50 μM	0.8052	4.5	32.14	17.93% to 50.66%	100	74.12% to 100.0%	0.0033

AUROC: area under the receiver-operating curve; CI: confidence interval; AX: amoxicillin; G4: 4th generation poly(amidoamine) dendrimer; G5: 5th generation poly(amidoamine) dendrimer; AXO: amoxicilloyl.

FIGURE 1 | (A) Dose–response curve in MAT with AX (B) and with G4-AXO (left) and G5-AXO (right) at AX-equivalent concentrations. Results are expressed as the %CD63 and mean ± SEM (red squares: Healthy controls, $n = 11$; blue circles: AX-allergic patients, $n = 28$). $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. (C) Cut-off values to discriminate AX-allergic and healthy controls selected by ROC analysis.

similarity in detection patterns between ImmunoCAP and the nanostructure-based MAT, and the distinct profile observed with AX alone, probably because the AX adducts in ImmunoCAP and the nanostructures in MAT are more similar than those produced with free AX during the MAT performance [15].

Taken together, these findings emphasize the diagnostic value of MAT. Using nanostructures (G4/5-AXO), MAT shows the same sensitivity as ImmunoCAP and identifies patients' profiles similarly, likely reflecting shared IgE reactivity. Crucially, MAT also incorporates AX as a stimulus, capturing additional responders and markedly increasing sensitivity.

TABLE 1 | Values of MAT and BAT for the in vitro assessment.

	MAT (%CD63+)												BAT (%CD63+)
			HSA-AXO	AX			G4-AXO			G5-AXO			AX
ID	Ctrl - (PBS 1×)	αIgE 1μg/mL	10μM	10μM	3420μM	6840μM	5μM	10μM	50μM	5μM	10μM	50μM	3420μM
Pt. 1	2.6	**98.8**	7.4	3	**6.8**	**9.5**	**75.7**	**81.4**	**73.4**	**58.2**	**79.8**	**77.2**	**40.1**
Pt. 2	1.9	**98.6**	12.1	2.6	2.6	2.3	**97.5**	**97.5**	**96.4**	**97.8**	**95.4**	**92.5**	1.49
Pt. 3	1.3	**98.9**	1.8	1.4	**72.5**	**85.8**	**48.8**	**70.3**	**47.3**	**56.2**	**56**	**61.9**	Not valuable
Pt. 4	1.5	**99.4**	0.8	1.2	1.4	1.3	**54.8**	**67.3**	**34.3**	**16.5**	**62.1**	**20.2**	**10.6**
Pt. 5	1.3	**99.6**	1.2	1.8	**4.8**	**6.9**	**17.2**	**19.9**	**6.9**	4.1	**22.9**	**11.1**	**5**
Pt. 6	1.6	**98.6**	2.2	2.2	1.9	2.06	**14**	**4.9**	1.9	**14.8**	**19.7**	3.5	2.5
Pt. 7	3.6	**98.8**	5.2	2.9	3	2.67	**30.4**	**35.2**	**9.9**	**41.2**	**20.8**	**8.2**	2.2
Pt. 8	1.6	**86.8**	5.7	**5.3**	**8.9**	**10.5**	**5.7**	**5.5**	**5.9**	**4.7**	**5.3**	**5.6**	**66.7**
Pt. 9	2.0	**94.6**	3.9	**46.2**	**59**	**58.7**	**7.3**	**6.3**	**5**	**7.7**	**7**	**4.2**	**7**
Pt. 10	2.1	**96.6**	3.3	2.2	**17.8**	**19.7**	3.3	**3.5**	2.9	3.1	3.1	2.7	Non-releaser
Pt. 11	3.0	**95.6**	1.4	0.8	1.8	2.94	1.5	1.2	1	1.6	1	1.6	**11.5**
Pt. 12	1.0	**95.8**	1.2	**3.3**	**9.2**	**9.4**	1.4	1	1.2	1.2	1.1	1.2	**79.2**
Pt. 13	1.0	**93.5**	0.9	**17.8**	**21.1**	**25.7**	0.9	0.9	1	1	1.2	0.9	**55.2**
Pt. 14	1.7	**92.2**	1.6	1.5	1.5	1.48	1.7	1.7	1.3	1.5	1.5	1.3	**5.9**
Pt. 15	2.5	**94.6**	3.3	1.7	1.9	2.12	**76.8**	**78.7**	**57.9**	**58**	**69.5**	**51.8**	Non-releaser
Pt. 16	1.0	**93.3**	0.7	0.9	**14.1**	**20.3**	1	1.3	0.9	1	0.8	0.8	**40.6**
Pt. 17	3.0	**98.6**	2.7	2.5	3	2.3	2.1	2.3	2.2	2	1.6	2	2.5
Pt. 18	2.2	**98.4**	2.4	3	3.2	3.2	10	6.4	2.9	2	6.5	1.8	**11**
Pt. 19	2.9	**98.4**	2.3	2.1	2.2	2.3	2	1.9	1.7	2	1.3	1.5	**5**
Pt. 20	3.2	**98.6**	2	1.9	2.2	2.6	2.6	2.2	2.7	2.6	2.4	2.5	5.9
Pt. 21	2.3	**88.1**	3.3	**4.3**	**13.6**	**14.8**	3.6	**3.8**	**4.7**	4.2	**4**	**4.8**	2.5
Pt. 22	1.8	**95.4**	3	**3.6**	**4.3**	**5.6**	**4.1**	3.3	**3.6**	4.1	**3.5**	3.5	Non-releaser
Pt. 23	1.5	**98.3**	1.9	2.6	2.1	2.1	2.4	2.1	2.2	1.6	2	2.1	2.2
Pt. 24	1.9	**41.9**	2.8	**3.9**	3.1	3	3.3	2.2	2.8	2.6	2.4	2.6	Non-releaser

(Continues)

TABLE 1 | (Continued)

	MAT (%CD63+)												BAT (%CD63+)
			HSA-AXO	AX			G4-AXO			G5-AXO			AX
ID	**Ctrl - (PBS 1×)**	**αIgE 1 μg/mL**	**10 μM**	**10 μM**	**3420 μM**	**6840 μM**	**5 μM**	**10 μM**	**50 μM**	**5 μM**	**10 μM**	**50 μM**	**3420 μM**
Pt. 25	2.4	**90.4**	2	2	**13.4**	**17.8**	2.8	2.1	1.9	1.8	2.2	2.2	2.2
Pt. 26	2.3	**98**	3.1	**3.3**	**11.1**	**23.2**	2.8	2.8	2	2.6	2.5	2.2	1.2
Pt. 27	1.9	**92.5**	2.4	2.1	**6.2**	**14.3**	1.9	2.2	1.9	1.8	1.6	1.8	**32.2**
Pt. 28	1.9	**95.7**	2.5	**8.9**	**28.2**	**29.4**	2.2	2.1	0.8	1.6	1.3	1.3	**17.6**
Ctrl. 1	1.8	**92.2**	2.6	1.3	1.7	2	1.4	1.5	1.9	1.6	1.3	1.3	Negative
Ctrl. 2	1.1	**94.2**	1.5	1.3	2.9	3.2	1.2	1.3	1.2	1.4	1.1	1	Negative
Ctrl. 3	1.4	**85.8**	1	1	1.1	1.1	1.4	1.3	0.6	0.9	0.8	0.9	Negative
Ctrl. 4	1.5	**79.9**	1.3	1.2	1.4	1.4	1.5	1.4	0.9	1.2	2	1.2	Negative
Ctrl. 5	2.3	**92.1**	4.6	3.1	3.4	3.6	3.9	3.3	3.4	4.3	3.4	4.3	Negative
Ctrl. 6	2.2	**94.9**	1.9	1.2	1.6	2	1.7	1.8	1.5	1.9	1.7	1.9	Negative
Ctrl. 7	1.9	**86.3**	3.4	1.4	1.4	1.3	2	1.1	1.4	1.6	1.3	1.6	Negative
Ctrl. 8	1.6	**93.8**	1.6	1.2	1.2	1.6	2.7	1.5	1.7	2	1.3	1.6	Negative
Ctrl. 9	2.5	**94.9**	2.1	2.1	1.9	2.1	2.2	2.2	1.9	1.6	1.6	1.1	Negative
Ctrl. 10	1.4	**97**	2.1	2	1.8	2.3	1.9	2.3	1.8	1.4	2	1.5	Negative
Ctrl. 11	1.4	**88.2**	1.4	1.1	1.2	1.4	1.7	1.4	1.1	1.1	1.4	1.1	Negative
dMCs uns.	0.9	0.9	0.9	0.9	0.9	0.9	0.6	1.0	0.8	0.9	0.7	0.8	

Note: Data in bold indicate positive results for each test according to their respective cut-off points.
Abbreviations: αIgE, anti-immunoglobulin E; AX, amoxicillin; BAT, basophil activation test; Ctrl, control; dMCs, derived mast cells; G4, PAMAM nanostructure generation 4th; G5, PAMAM nanostructure generation 5th; HSA, human serum albumin; Pt, patient; uns, unsensitized.

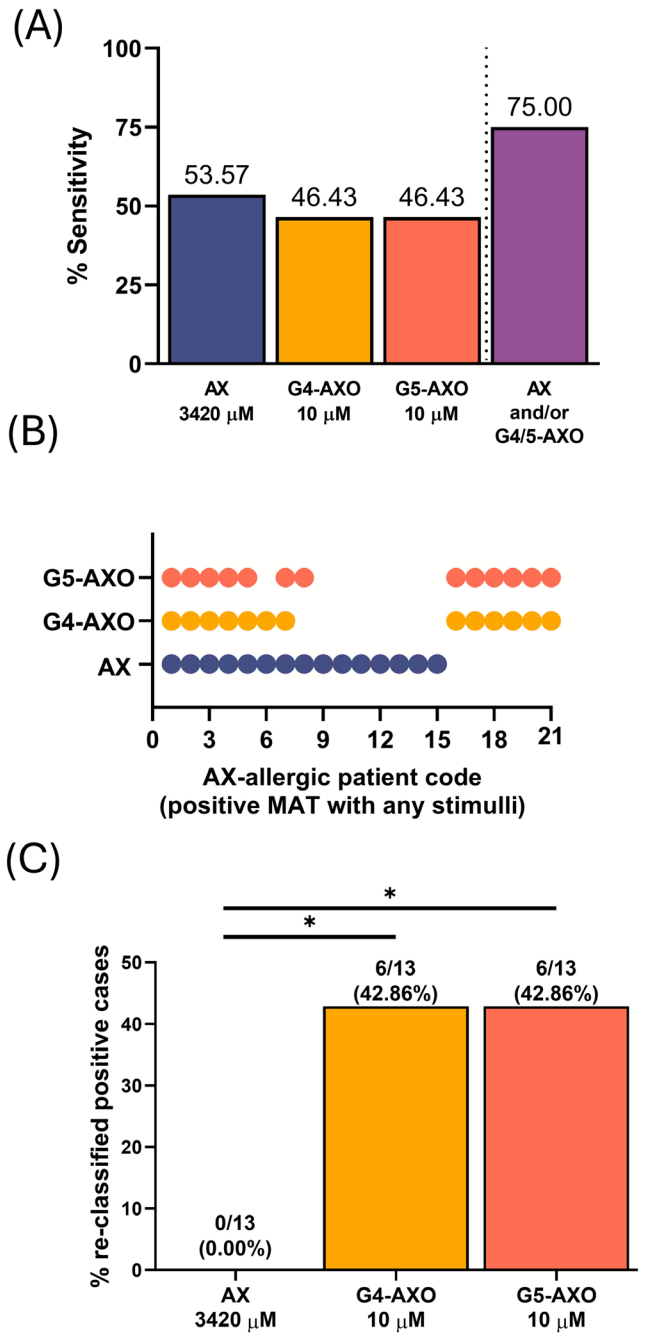


FIGURE 2 | (A) Sensitivity of the MAT with each stimulus at its most sensitive concentration, and the combination of all three. Results are expressed as the percentage of positive cases based on the ROC cut-off. *$p < 0.05$, ***$p < 0.001$. (B) Scheme of all patients with positive MAT for any of the stimuli used. Dots indicate the strategy with which the positive result was obtained. (C) Re-classified positive cases among AX-allergic patients: Percentage of individuals with negative MAT results to free AX but positive responses to G4/5-AXO.

3.6 | MAT Complements BAT in AX In Vitro Diagnostic and Show Positive Results for BAT Non-Releasers

Afterward, we compared the diagnostic performance of MAT concerning other established cellular assays, BAT, using free AX. In the 23 patients with valuable results in BAT, MAT showed a sensitivity of 52.17% with AX and 43.48% with G4/5-AXO, which was slightly lower than that of the BAT (60.87%, Figure 5A). In both BAT and MAT, specificity was 100%. However, when both stimuli were combined, MAT sensitivity increased to 73.91%, confirming its ability to detect IDAR to AX with good sensitivity. No correlation was observed between BAT with AX and MAT responses to either AX, G4-AXO, or G5-AXO (Spearman r, $p > 0.05$) (Figure 5B).

Given the known limitations of the BAT in individuals whose basophils do not respond to IgE-mediated stimulation, we tested sera from AX-allergic patients previously classified as non-releasers ($n = 4$) or with non-evaluable BAT results due to poor sample quality ($n = 1$) in the MAT using AX and/or G4/G5-AXO nanostructures. Positive activation to AX and/or G4/G5-AXO was observed in 1 non-valuable and 3/4 non-releasers' sera tested using the MAT (Table 1). These results support the applicability of the MAT in cases where the BAT cannot be reliably performed.

4 | Discussion

This study proposes MAT as a reliable in vitro support for AX-IDAR, reaching 75% sensitivity and 100% specificity while avoiding some limitations of ImmunoCAP and BAT, which would play a complementary role.

Although MAT using LAD2 cells worked well in food allergy [41, 42], they underperformed when applied to IDAR (Table S2), showing lower effectiveness particularly in the case of BL hypersensitivity. These results are somewhat unexpected, since this technique is highly dependent on IgE, and the relatively low levels of drug-sIgE usually observed in AX allergy could partly explain the limited responses. However, previous studies reported that LAD2 cells express a comparable, or even slightly higher, density of FcεRI receptors per cell than dMCs [19], suggesting that receptor availability alone does not account for the poor performance observed. Therefore, the underlying reasons for these unsatisfactory outcomes remain unclear, highlighting the need for further research to elucidate the intrinsic limitations of LAD2 cells in this context.

Other emerging models, such as Hoxb8 MAT, have shown promising outcomes [19, 43] but remain unexplored for IDAR. dMCs have proven effective in IgE-mediated IDAR, including chlorhexidine allergy [44]. Notably, its application to AX yielded moderate results [30], illustrating the challenges of this setting and the value of our optimized approach overpassing some of the claimed limitations of dMCs MAT in IDAR to AX.

Compared to other studies using MAT [30], our approach yielded satisfactory results with sensitivities comparable to those obtained with BAT. In our hands, the use of dMCs was essential to achieve these outcomes. Several aspects may underlie the different results. First, we used a cytokine-enriched medium (Medium II) containing hSCF, which enhances IgE-mediated mast cell activation [25, 45]. Protein content in the medium may also allow free AX to form covalent conjugates with carrier molecules. Furthermore, cytokine priming during sensitization and prior to activation likely increased cell responsiveness. Second and most notably, we

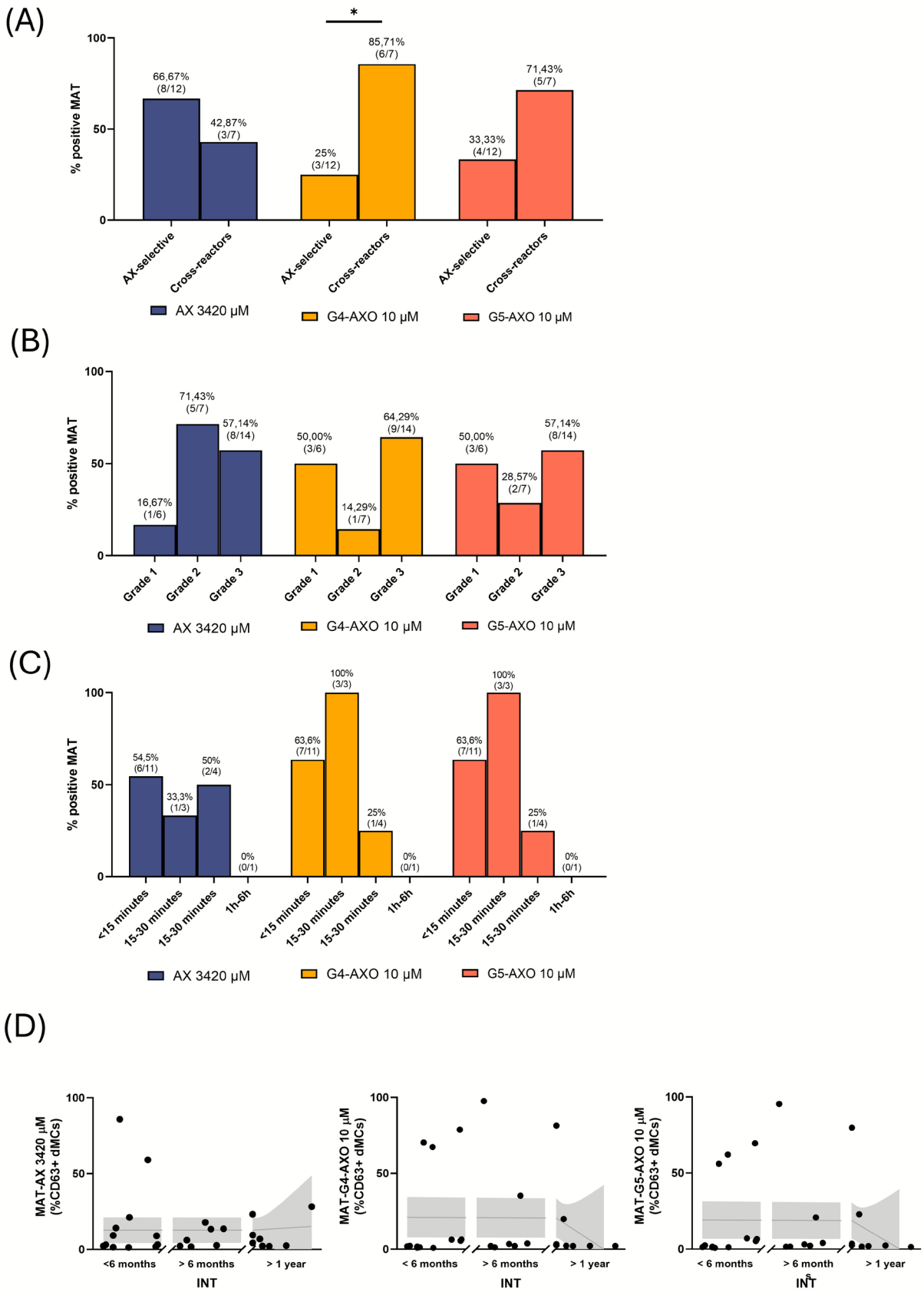


FIGURE 3 | (A) Proportion of positive cases according to AX-selective or cross-reactor response. (B) Correlation between interval time (INT) between reported reaction and study expressed in days, and MAT results for each stimulus, expressed as percentage of CD63+ cells. (C) Proportion of positive cases according to the severity grade of the allergic reaction. Grade 1: Urticaria/angioedema-anaphylaxis grade 2 (URT/ANG – I); Grade 2: Anaphylaxis grade 2 (II); Grade 3: Anaphylaxis grade 3 (III). (D) Proportion of positive cases according to allergic reaction latency time.

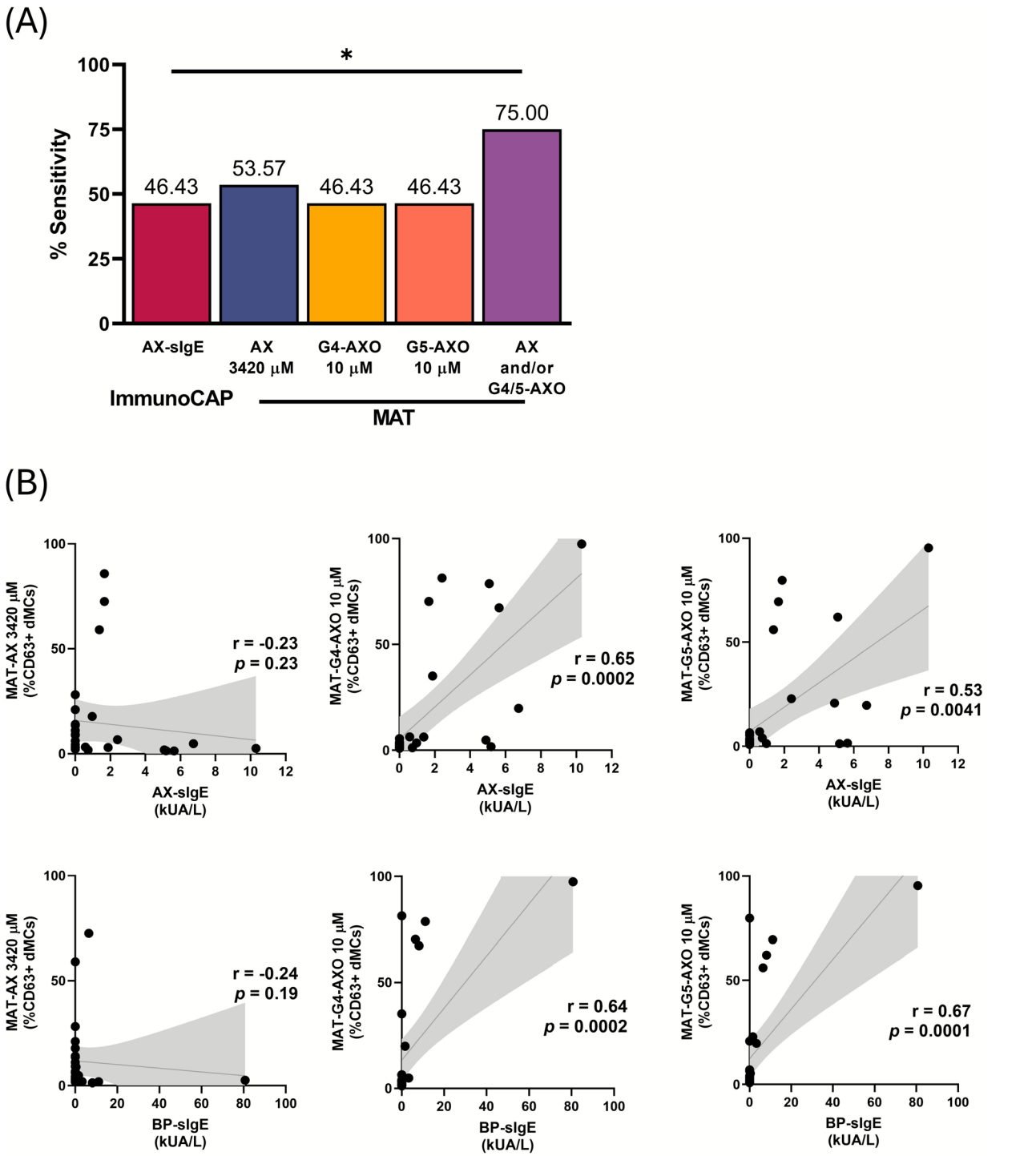


FIGURE 4 | (A) Comparison of ImmunoCAP sensitivity and MAT sensitivity with each stimulus at its optimal concentration, as well as the combination of all three. *$p < 0.05$. (B) Correlation between ImmunoCAP results, expressed as kilo allergen-specific units per liter (kUA/L) for c6 (AXO), and MAT results for each stimulus, expressed as percentage of CD63+ cells.

incorporated AXO-dendrimer conjugates, which overcome limitations of conventional hapten presentation by improving IgE cross-linking. Although we did not assess the individual contribution of each factor, their combination likely contributed to the robust responses observed in our MAT approach.

The highest sensitivities obtained with each type of stimulus were comparable: 53.57% for AX and 46.43% for the nanostructures. Interestingly, although responses partially overlapped, some patients reacted only to AX or AX-decorated nanostructures. Then, combining both types of stimuli, the sensitivity of MAT improved to 75% with high specificity. These findings support the notion that, although some patients respond irrespective of the stimulus, others may only be identified by a specific approach depending on their recognition pattern.

Compared with sIgE in ImmunoCAP, MAT with free AX showed slightly higher sensitivity, and the combined MAT

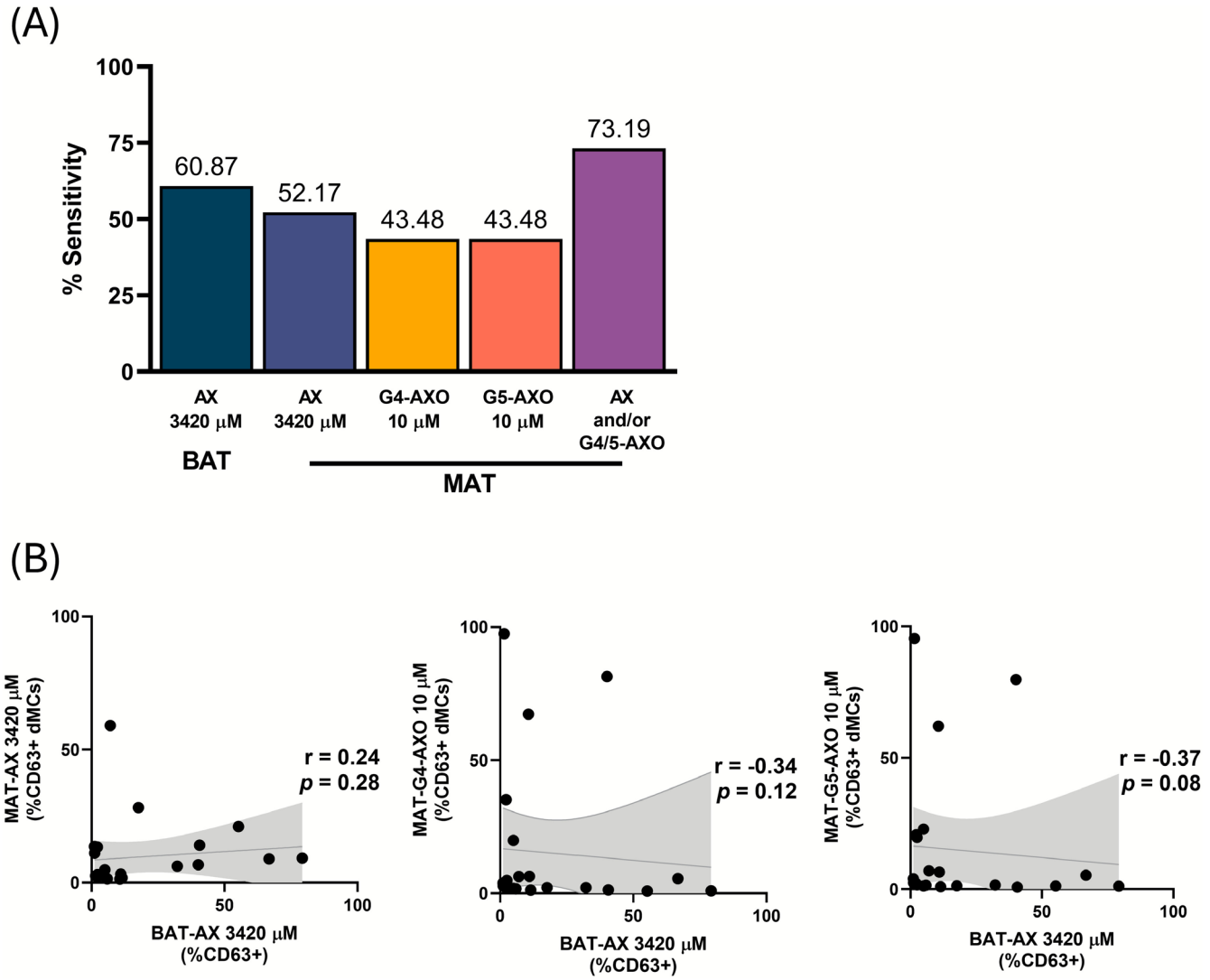


FIGURE 5 | (A) Comparison of BAT sensitivity and MAT sensitivity with each stimulus at its optimal concentration, as well as the combination of all three. (B) Correlation between BAT and MAT results for each stimulus, expressed as percentage of CD63+ cells.

approach (free AX plus G4/G5-AXO) was significantly superior. MAT specificity remained 100%, whereas sIgE yielded one false positive (90.9% specificity). Notably, MAT results for nanostructures strongly correlated with ImmunoCAP for AX-sIgE, potentially tackling its limitations, such as a lack of drug conjugates or false positives. Interestingly, ImmunoCAP results did not correlate with MAT using free AX but did with G4/G5-AXO. This suggests that G4/G5-AXO and the PLL-AXO conjugates used in ImmunoCAP are structurally more similar—both featuring a polyamide backbone and high AXO density—than the heterogeneous, low-density AX-protein adducts formed with free AX during MAT, affecting the recognition as previously described [15]. This variability may impact recognition, particularly in patients with broader sensitization profiles. In our cohort, the use of free AX in MAT resulted in a higher proportion of positive responses among AX-selective patients, whereas nanostructures detected all cross-reactive cases, with statistically significant differences between groups. This pattern is consistent with previous observations [15], where IgE from selective responders preferentially recognized AX itself, while cross-reactive patients responded more broadly to pre-conjugated AX structures, independent of the carrier. These findings suggest that selective responders may depend on limited or transient haptenation events, whereas cross-reactive profiles require stable, multivalent presentation of the antigenic determinant.

MAT was compared with the other in vitro cellular test, BAT, which, despite suboptimal sensitivity, is highly recommended by EAACI for IDAR [11]. MAT did not outperform BAT with individual stimuli, but their combination improved sensitivity. No correlation was found between the two tests. Moreover, MAT was effective in patients who were non-evaluable (1/1) or non-releasers (3/4) in BAT, reinforcing its clinical utility in difficult cases. One limitation of the study is that, as BAT requires fresh whole blood, nanostructures could not be assessed in this cohort, restricting direct comparison. Moreover, it is important to consider that the diagnostic metrics reported here stem from a retrospective observational design, which is optimal for evaluating the sensitivity of new in vitro tools in confirmed IDAR to AX cases. While these results demonstrate the high potential of the optimized MAT, a prospective analysis for getting data about

sensitivity, specificity, and predictive values is needed in order to make MAT applicable in their clinical practice.

Trends in MAT showed nanostructures best for grade 1, AX for grade 2, and similar responses in grade 3, though differences were not significant. Larger cohorts are needed to confirm. No clear effect of time since reaction on AX responses, which stayed stable, while nanostructure responses declined, reflecting decreased IgE-test sensitivity beyond 6–12 months [14]. In patients over one year post-reaction, 63% were AX-positive versus 25%–38% for nanostructures, indicating two response profiles.

In conclusion, MAT using dMCs under optimized conditions reached 75% sensitivity and 100% specificity, supporting its use as an early diagnostic step to reduce full work-ups. Moreover, our results in this context suggest that two profiles or combinations of them can be found in an AX-allergic patient cohort, shedding further light on why these types of reactions are so difficult to reproduce using an IgE-mediated approach, as illustrated by the low detection rates of AX-sIgE and the limited activation observed in cellular assays. Nevertheless, further analyses are needed to clarify these mechanisms and improve in vitro diagnostic strategies for IDAR to AX.

Author Contributions

C.M., C.J.A., and J.A.C. designed the study and coordinated the work of the rest of the authors. G.B. and M.S. recruited the study individuals, managed the clinical procedures, and obtained clinical data. P.T., A.T.A., and M.I.M. designed, synthesized, and characterized the AX-conjugated compounds used in the study. J.A.C., C.L.-M., and L.V.-A. performed the in vitro experiments and analyzed the experimental results. C.M., C.J.A., M.J.T., and J.A.C. wrote the manuscript and figures, which were reviewed by the rest of the authors.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. E. Y. Klein, I. Impalli, S. Poleon, et al., "Global Trends in Antibiotic Consumption During 2016-2023 and Future Projections Through 2030," *Proceedings of the National Academy of Sciences of The United States of America* 121, no. 49 (2024): e2411919121.

2. Control ECfDPa, *Antimicrobial Consumption in the EU/EEA—Annual Epidemiological Report 2023* (European Centre for Disease Prevention and Control, 2024).

3. A. Romano, M. Atanaskovic-Markovic, A. Barbaud, et al., "Towards a More Precise Diagnosis of Hypersensitivity to Beta-Lactams—An EAACI Position Paper," *Allergy* 75, no. 6 (2020): 1300–1315.

4. I. Doña, M. J. Torres, G. Celik, E. Phillips, L. K. Tanno, and M. Castells, "Changing Patterns in the Epidemiology of Drug Allergy," *Allergy* 79, no. 3 (2024): 613–628.

5. M. Fu, L. Hu, K. Han, et al., "The Burden of β-Lactam Allergy Labels in Health Care: A Systematic Review and Meta-Analysis," *Lancet Infectious Diseases* 25 (2025): 896–908.

6. L. Zhou, N. Dhopeshwarkar, K. G. Blumenthal, et al., "Drug Allergies Documented in Electronic Health Records of a Large Healthcare System," *Allergy* 71, no. 9 (2016): 1305–1313.

7. E. Minaldi, E. J. Phillips, and A. Norton, "Immediate and Delayed Hypersensitivity Reactions to Beta-Lactam Antibiotics," *Clinical Reviews in Allergy & Immunology* 62, no. 3 (2022): 449–462.

8. C. A. Stone, Jr., J. Trubiano, D. T. Coleman, C. R. F. Rukasin, and E. J. Phillips, "The Challenge of de-Labeling Penicillin Allergy," *Allergy* 75, no. 2 (2020): 273–288.

9. A. Arnold, L. L. Coventry, M. J. Foster, J. J. Koplin, and M. Lucas, "The Burden of Self-Reported Antibiotic Allergies in Health Care and How to Address It: A Systematic Review of the Evidence," *Journal of Allergy and Clinical Immunology. In Practice* 11, no. 10 (2023): 3133–3145.e3133.

10. A. Barbaud, L. H. Garvey, M. Torres, et al., "EAACI/ENDA Position Paper on Drug Provocation Testing," *Allergy* 79, no. 3 (2024): 565–579.

11. C. Mayorga, G. E. Çelik, M. Pascal, et al., "Flow-Based Basophil Activation Test in Immediate Drug Hypersensitivity. An EAACI Task Force Position Paper," *Allergy* 79, no. 3 (2024): 580–600.

12. J. Elst, C. Mertens, M. Van Houdt, et al., "Flow Cytometry-Assisted Analyses of Individual Human Basophils, Mast Cells and T Cells in the Diagnosis of Immediate Drug Hypersensitivity: A Review," *Allergy* 80, no. 5 (2025): 1242–1255.

13. A. Ariza, C. Mayorga, G. Bogas, et al., "Detection of Serum-Specific IgE by Fluoro-Enzyme Immunoassay for Diagnosing Type I Hypersensitivity Reactions to Penicillins," *International Journal of Molecular Sciences* 23, no. 13 (2022): 6992.

14. T. D. Fernández, M. J. Torres, N. Blanca-López, et al., "Negativization Rates of IgE Radioimmunoassay and Basophil Activation Test in Immediate Reactions to Penicillins," *Allergy* 64, no. 2 (2009): 242–248.

15. A. Ariza, C. Mayorga, M. Salas, et al., "The Influence of the Carrier Molecule on Amoxicillin Recognition by Specific IgE in Patients With Immediate Hypersensitivity Reactions to Betalactams," *Scientific Reports* 6 (2016): 35113.

16. M. R. Bennett, A. G. Mathioudakis, J. Wu, et al., "Performance Characteristics of Basophil Activation Tests for Diagnosing Penicillin Allergy: A Meta-Analysis," *Journal of Allergy and Clinical Immunology. In Practice* 12, no. 3 (2024): 714–723.e715.

17. K. Heremans, A. Toscano, J. Elst, et al., "Basophil Activation Test Shows Poor Sensitivity in Immediate Amoxicillin Allergy," *Journal of Allergy and Clinical Immunology. In Practice* 11, no. 2 (2023): 500–505.

18. E. Passante, "Mast Cell and Basophil Cell Lines: A Compendium," *Methods in Molecular Biology* 2163 (2020): 127–144.

19. N. Zbären, D. Brigger, D. Bachmann, et al., "A Novel Functional Mast Cell Assay for the Detection of Allergies," *Journal of Allergy and Clinical Immunology* 149, no. 3 (2022): 1018–1030.e1011.

20. D. G. Ebo, R. Bahri, A. Eggel, V. Sabato, C. Tontini, and J. Elst, "Flow Cytometry-Based Basophil and Mast Cell Activation Tests in Allergology: State of the Art," *Journal of Allergy and Clinical Immunology* 155, no. 2 (2025): 286–297.

21. R. Bahri, A. Custovic, P. Korosec, et al., "Mast Cell Activation Test in the Diagnosis of Allergic Disease and Anaphylaxis," *Journal of Allergy and Clinical Immunology* 142, no. 2 (2018): 485–496.e416.

22. T. Derakhshan, J. A. Boyce, and D. F. Dwyer, "Defining Mast Cell Differentiation and Heterogeneity Through Single-Cell Transcriptomics Analysis," *Journal of Allergy and Clinical Immunology* 150, no. 4 (2022): 739–747.

23. H. B. Andersen, M. Holm, T. E. Hetland, et al., "Comparison of Short Term in Vitro Cultured Human Mast Cells From Different Progenitors - Peripheral Blood-Derived Progenitors Generate Highly Mature and Functional Mast Cells," *Journal of Immunological Methods* 336, no. 2 (2008): 166–174.

24. R. Joulia, F. E. L'Faqihi, S. Valitutti, and E. Espinosa, "IL-33 Fine Tunes Mast Cell Degranulation and Chemokine Production at the Single-Cell Level," *Journal of Allergy and Clinical Immunology* 140, no. 2 (2017): 497–509.e410.

25. Z. Wang, S. Guhl, K. Franke, M. Artuc, T. Zuberbier, and M. Babina, "IL-33 and MRGPRX2-Triggered Activation of Human Skin Mast Cells-Elimination of Receptor Expression on Chronic Exposure, but Reinforced Degranulation on Acute Priming," *Cells* 8, no. 4 (2019): 341.

26. P. W. West, R. Bahri, K. M. Garcia-Rodriguez, et al., "Interleukin-33 Amplifies Human Mast Cell Activities Induced by Complement Anaphylatoxins," *Frontiers in Immunology* 11 (2020): 615236.

27. B. Salcman, R. Bahri, P. W. West, C. Tontini, K. Affleck, and S. Bulfone-Paus, "P2X7 Receptor-Induced Human Mast Cell Degranulation Is Enhanced by Interleukin 33," *International Journal of Molecular Sciences* 25, no. 3 (2024): 1730.

28. M. Babina, Z. Wang, K. Franke, and T. Zuberbier, "Thymic Stromal Lymphopoietin Promotes MRGPRX2-Triggered Degranulation of Skin Mast Cells in a STAT5-Dependent Manner With Further Support From JNK," *Cells* 10, no. 1 (2021): 102.

29. N. R. Han, H. A. Oh, S. Y. Nam, et al., "TSLP Induces Mast Cell Development and Aggravates Allergic Reactions Through the Activation of MDM2 and STAT6," *Journal of Investigative Dermatology* 134, no. 10 (2014): 2521–2530.

30. D. G. Ebo, R. Bahri, C. Tontini, et al., "Potential and Limitations of the Human Mast Cell Activation Test in Amoxicillin Hypersensitivity," *Allergy* 80, no. 2 (2025): 594–597.

31. E. Rönnberg, A. Ghaib, C. Ceriol, et al., "Divergent Effects of Acute and Prolonged Interleukin 33 Exposure on Mast Cell IgE-Mediated Functions," *Frontiers in Immunology* 10 (2019): 1361.

32. A. Ariza, C. Mayorga, T. D. Fernandez, et al., "Hypersensitivity Reactions to β-Lactams: Relevance of Hapten-Protein Conjugates," *Journal of Investigational Allergology and Clinical Immunology* 25, no. 1 (2015): 12–25.

33. C. Mayorga, E. Perez-Inestrosa, J. Rojo, M. Ferrer, and M. I. Montañez, "Role of Nanostructures in Allergy: Diagnostics, Treatments and Safety," *Allergy* 76, no. 11 (2021): 3292–3306.

34. V. Gil-Ocaña, I. M. Jimenez, C. Mayorga, et al., "Multiepitope Dendrimeric Antigen-Silica Particle Composites as Nano-Based Platforms for Specific Recognition of IgEs," *Frontiers in Immunology* 12 (2021): 750109.

35. N. Molina, A. Martin-Serrano, T. D. Fernandez, et al., "Dendrimeric Antigens for Drug Allergy Diagnosis: A New Approach for Basophil Activation Tests," *Molecules* 23, no. 5 (2018): 997.

36. A. Tesfaye, A. Rodríguez-Nogales, S. Benedé, et al., "Nanoarchitectures for Efficient IgE Cross-Linking on Effector Cells to Study Amoxicillin Allergy," *Allergy* 76, no. 10 (2021): 3183–3193.

37. P. Demoly, N. F. Adkinson, K. Brockow, et al., "International Consensus on Drug Allergy," *Allergy* 69, no. 4 (2014): 420–437.

38. J. A. Céspedes, R. Fernández-Santamaría, A. Ariza, et al., "Diagnosis of Immediate Reactions to Amoxicillin: Comparison of Basophil Activation Markers CD63 and CD203c in a Prospective Study," *Allergy* 78, no. 10 (2023): 2745–2755.

39. J. Folkerts, N. Gaudenzio, M. Maurer, et al., "Rapid Identification of Human Mast Cell Degranulation Regulators Using Functional Genomics Coupled to High-Resolution Confocal Microscopy," *Nature Protocols* 15, no. 3 (2020): 1285–1310.

40. J. A. Céspedes, R. Fernández-Santamaría, G. Bogas, et al., "Lipopolysaccharides in Combination With Amoxicillin Increases Basophil Activation Test Sensitivity to Amoxicillin IgE-Mediated Hypersensitivity," *Allergy* 79, no. 9 (2024): 2537–2542.

41. J. A. Céspedes, C. Lebrón-Martín, R. García-Otón, et al., "In Vitro Supporting Diagnostic Tools in Plant-Food Allergy," *Allergy* 78, no. 9 (2023): 2540–2543.

42. A. F. Santos, N. Couto-Francisco, N. Bécares, M. Kwok, H. T. Bahnson, and G. Lack, "A Novel Human Mast Cell Activation Test for Peanut Allergy," *Journal of Allergy and Clinical Immunology* 142, no. 2 (2018): 689–691.e689.

43. N. Bachmeier-Zbären, A. Celik, R. van Brummelen, et al., "Clinical Utility Analysis of the Hoxb8 Mast Cell Activation Test for the Diagnosis of Peanut Allergy," *Allergy* 80, no. 1 (2025): 215–226.

44. J. Elst, N. Moonen, M. M. van der Poorten, et al., "The Passively Sensitized Mast Cell Activation Test Is a Reliable Diagnostic for Chlorhexidine Allergy," *Journal of Allergy and Clinical Immunology: In Practice* 9, no. 10 (2021): 3826–3828.e3822.

45. A. M. Gilfillan and C. Tkaczyk, "Integrated Signalling Pathways for Mast-Cell Activation," *Nature Reviews Immunology* 6, no. 3 (2006): 218–230.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1**. **Data S2**.