

Sensitization to amoxicillin/clavulanic acid may underlie severe rashes in children treated for infectious mononucleosis



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Clinical Implications

- Patients with skin eruptions after drug intake during Epstein-Barr virus infection may exhibit true sensitization to penicillins. *In vitro* screening may help the diagnosis of drug allergy in skin-negative patients with contraindications to oral challenge.

TO THE EDITOR:

Infectious mononucleosis (IM) is acute febrile pharyngitis with lymphadenopathy caused by the Epstein-Barr virus (EBV). Maculopapular rash of the trunk usually occurs during IM with an incidence of 4.2% to 13%.¹ However, when aminopenicillins were used concomitantly, the incidence of skin rashes was initially reported as ranging between 80% and 100%,² although recently much lower rates have been reported^{3,4}; in a recent extensive review taking into account 1980-2016 case reports, epidemiological data and underlying mechanism of antibiotic-induced skin rash in IM, only 17 cases were identified.⁵ However, selection criteria might represent a relevant bias in this study. Very recently, in a large pediatric population of 221 children, the rash incidence during IM was 18.6% and skin

involvement developed after antibiotic treatment in 20 patients.⁶ More importantly, *in vivo* allergy workup showed a proven sensitization against amoxicillin/clavulanic acid (AX/CL) in 3 of 10 children.⁶ In the past, skin rashes after aminopenicillins intake in florid mononucleosis were considered as benign, nonallergic phenomena due to a transient loss of tolerance toward antibiotics.⁵ Thus, skin tests were neither required nor recommended. However, the possibility of drug hypersensitivity emerging concomitantly to IM cannot be dismissed, given recent reports.⁶⁻⁹

In this study, we investigated the possibility of real sensitization to AX/CL in 10 patients (4 males, 6 females; mean age 8.1 years) with moderate-to-severe skin eruptions during IM via *in vivo* (skin prick tests [SPTs], intradermal tests [IDs], and patch tests [PTs]) and *in vitro* tests (lymphocyte transformation test [LTT]) and induction of hapten-specific short-term T-cell lines (see this article's Online Repository at www.jaci-inpractice.org). All the children had no previous hypersensitivity reaction to antibiotics, including AX/CL. Ten drug-exposed nonallergic individuals without a history of EBV infection (2 females, 8 males; mean age 10.3 years) were included as a control group.

Four of 10 patients had severe reactions: 2 had Stevens-Johnson syndrome (SJS) and 2 drug reaction with eosinophilia and systemic symptoms (DRESS). Four of 10 patients had long-lasting (7- to 10-day) maculopapular exanthema (MPE) involving more than 50% of the body surface (upper and lower limbs, trunk in all the patients, face included in 2), and 2 developed urticarial vasculitis (Table I). Three of 10 patients had a subsequent reaction to AX/CL at a distance from the first episode before being evaluated.

In 2, the second reaction was comparable with that reported during IM (patients 2 and 3), but in 1 case (patient 7), the second reaction was a DRESS that was much more severe than the first one (MPE). The mean time interval between the adverse

TABLE I. Clinical features of the patients and results of the *in vivo* and *in vitro* tests

Patient no.	Sex/age (y)	Reaction	Timing of the reaction	Time interval	PTs	IDs	LTT	TCLs
1	M/3	SJS	10 d	2 y	neg	neg	neg	pos
2	F/12; 15	MPE*	1 d	2 y	n.p.	neg	pos	pos
		MPE*	1 d	6 mo				
3	F/12; 15	MPE*	3 d	4 y	pos (48 h)	pos (24 h)	pos	pos
		MPE*	1 d	6 mo				
4	M/4	DRESS	15d	6 mo	n.p.	neg	neg	pos
5	M/3	U-V	8 d	2 y	n.p.	neg	pos	pos
6	F/4	SJS	12 d	6 mo	n.p.	neg	neg	neg
7	M/11; 13	MPE*	2 d	3 y	pos (48-72 h)	pos (72 h)	pos	pos
		DRESS	2 d	6 mo				
8	F/8	MPE†	4 d	6 mo	n.p.	pos (24 h)	neg	neg
9	F/11	MPE†	1 d	2 y	n.p.	neg	neg	neg
10	F/17	U-V	7 d	6 mo	n.p.	neg	pos	pos

Ten children with skin rash after AX/CL treatment during EBV infection were screened *in vivo* by means of patch (PTs) and intradermal (IDs) tests with AX/CL and *in vitro* by means of lymphocyte transformation test (LTT) or induction of drug-specific short-term T-cell lines (TCLs) to assess drug sensitization. The timing of the reaction indicates the time interval between administration of the culprit drug and the adverse reaction. The time interval indicates the time between the adverse reaction and the allergic workup. AX, Amoxicillin; CL, clavulanic acid; DRESS, drug reaction with eosinophilia and systemic symptom; EBV, Epstein-Barr Virus; MPE, maculopapular exanthema; neg, negative; n.p., not performed; pos, positive; SJS, Stevens-Johnson Syndrome.

*Trunk, upper and lower limbs involving but face-sparing MPE.

†Generalized face-including MPE.

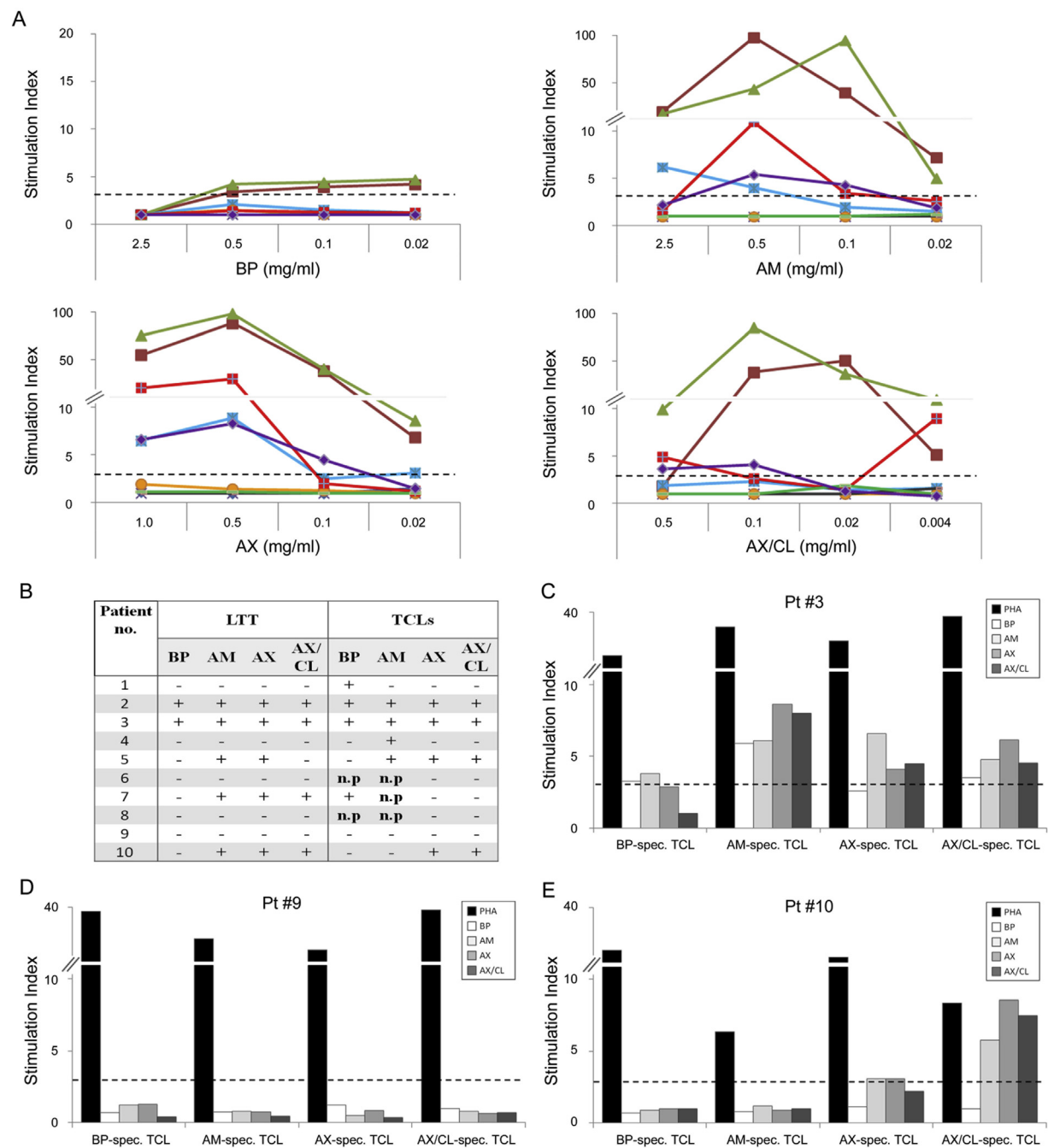


FIGURE 1. **A**, Dose-curve-dependent proliferative response of PBMCs to the single β -lactams in the 10 patients (individually represented). **B**, Results from LTT and induction of TCLs; n.p.= not performed. Specificity of drug-induced TCLs was assessed in MHC-restricted conditions in response to single β -lactams. Three representative cases of TCL proliferation are shown (patients 3; **C**, patient 9; **D**, patient 10; **E**). Dotted lines represent the cutoff of the positivity of SI (≥ 3). *AM*, Ampicillin; *AX*, amoxicillin; *BP*, penicillin; *CL*, clavulanic acid; *LTT*, lymphocyte transformation test; *n.p.*, not performed; *PHA*, phytohemagglutinin; *SI*, stimulation index; *TCL*, T-cell line.

reactions and allergy workup was 22.2 months (range 6-54, median 24 months).

All the children underwent SPT with AX/CL with negative results. Three of 10 exhibited positive delayed ID tests. PTs were performed in 3 of 10 patients obtaining positive results in 2 (Table I).

Five of 10 children had positive LTT to at least one of the β -lactams tested (Table I, Figure 1, A). Seven of 10 patients had

positive hapten-specific T-cell lines (TCLs) to at least one of the β -lactams tested (penicillin, ampicillin, AX, or AX/CL) (Table I, Figure 1, B). Drug-specific TCLs from 3 representative cases (positive patients 3 and 10, negative patient 9) are illustrated (Figure 1, C-E).

LTT and induction of drug-specific TCLs were both positive in 5 children. Two patients had concordant positive *in vivo* (IDs)

and *in vitro* tests (LTT and TCLs). Three children were negative for both LTT and TCLs (Table 1, Figure 1, B).

PBMCs from the control group (10 children) never showed any drug-specific lymphocyte reactivity (data not shown).

This is the first study in a pediatric population to show that real sensitization to AX/CL can occur during EBV infection, by using LTT. Drug-specific T memory lymphocytes were demonstrated *in vitro* in 7 of 10 patients although *in vivo* tests (IDs and/or PTs) were positive in only 3 children.

Drug-related skin rash is a well-known complication in IM commonly attributed to β -lactams.^{3,5} However, the pathogenic mechanisms are still unclear. A delayed-type time-limited hypersensitivity reaction and transient loss of tolerance to the antibiotic due to the immunostimulatory properties of EBV have been hypothesized.^{7,9} Therefore, allergic diagnostic tests are not routinely performed and their accuracy is still debated. Limited studies support the persistence of drug allergy.⁶⁻⁹ DRESS and Severe Cutaneous Adverse Reactions (SCARs) after antibiotic intake concomitant to EBV infection have been seldom described and are presumably rare, but differentiating drug-induced from virus-induced manifestations is important.¹⁰ In our study, 3 of 10 patients experienced a second reaction to AX/CL at a distance from the EBV infection and, in 1, clinical manifestations were even more severe than previously observed. This suggests that a persistent sensitization to the culprit drug may occur and may be unpredictably followed by more severe reactions in case of readministration, as it happens in nonviral-associated drug sensitization. Thus, the virus may simply play the role of “bystander” in these cases.

As regards the reliability of *in vivo* diagnostic tests, positive IDs or PTs were reported in a high percentage of adults⁸; however, T-cell proliferation was not performed⁹ or was assessed using a low-sensitive colorimetric technique.⁸ Vice versa, positive skin test-concordant LTT was reported in 3 of 4 MPE.⁷ In the largest pediatric cohort,³ children were not screened for drug sensitization. In the paper by Dibek Misirlioglu et al,⁶ *in vivo* screening was indeed able to assess true sensitization to antibiotics after IM and maculopapular rash, but in only 3 of 10 patients with further 5 reacting after re-exposure, suggesting a low rate of sensitivity of skin tests in delayed reactions. Our study is the first to combine *in vivo* and *in vitro* assays to test drug sensitization in a homogeneous pediatric population (AX/CL-restricted intake, nonremote proven IM, serious skin involvement), although restricted in number. Our results seem in keeping with previous reports.⁷⁻⁹ Positive delayed IDs were found in only 3 of 10 patients. Actually, the low number of children studied did not allow reaching an absolute conclusion as a result of the limited accuracy of *in vivo* tests in this setting. Despite high specificity, sensitivity of *in vivo* tests is indeed crucial in drug allergies, with positive PTs and delayed IDs not exceeding 30% in MPE.¹¹ Because of the severe reactions, oral challenge with the culprit drug was not recommended in skin-negative children,¹¹ resulting in unproven sensitization in 7 of 10 patients. Conversely, *in vitro* tests helped to demonstrate β -lactam-specific memory T cells in 5 of 10 patients and 7 of 10 via LTTs and drug-specific TCLs, respectively. Delayed-type drug allergy is suitable for investigation via LTTs as it shows 40% to 70% sensitivity and is more sensitive than skin testing in mild/moderate reactions.^{10,12} In our study, LTT was positive in 3 patients not identified by skin tests. Interestingly, although AX/CL was the culprit drug during

IM, the proliferation of PBMCs could also be stimulated by other cross-reactive aminopenicillins suggesting a side-chain nonspecific (patients 2 and 3) or specific (patients 5, 7, and 10) sensitization. As the frequency of drug-specific T lymphocytes is low, their *in vitro* amplification may be more effective in detecting adaptive responses. Actually, we were able to identify 2 additional drug-sensitized patients (patients 1 and 4) by inducing specific TCLs. However, in 2 patients (patients 6 and 9), neither *in vivo* nor *in vitro* tests could demonstrate any drug sensitization, despite a severe skin reaction (SJS) in one (patient 6) usually exceptional in EBV infection. Furthermore, the possible role of isolate CL recognition, better known to associate with immediate reactions in the adult population,¹³ could not be assessed.

In conclusion, our data suggest that hypersensitivity to penicillins may develop after EBV infection. In particular, drug allergy should be suspected when severe skin eruptions, usually rare in primary EBV infection, occur in the context of IM. Limitations of this work include the relatively small number of children studied and the possible selection bias (severe cases). Confirmation of these results in large populations is thus needed. The current data do suggest that extension of the allergic workup to *in vitro* screening, although expertise-requiring, may be useful in ascertaining drug allergy in those skin-negative patients experiencing severe manifestations that contraindicate oral challenge.

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ONLINE REPOSITORY MATERIALS AND METHODS

Patients

Children were consecutively recruited from January 2014 to December 2017 from patients referred to the Allergy Unit of A. Meyer Children's University Hospital in Florence for the allergic workup. The pediatric Allergy Unit usually screens approximately 200 children per year for suspected amoxicillin/clavulanic acid (AX/CL) adverse reactions. Maculopapular exanthema (MPE) was defined as the occurrence of macules and/or papules based on photographic documentation and/or description by colleagues. The duration of the cutaneous involvement was always longer than 7 days after the last drug intake. In all the patients with MPE, both upper and lower limbs were bilaterally involved with extension to the face in 2 (patients 8 and 9) (Table I). Drug reaction with eosinophilia and systemic symptoms was diagnosed when presenting widespread purpuric infiltrative papules and plaques in the context of skin erythroderma, facial edema, lips erosions, biochemical abnormalities, and final desquamation at resolution following the European Registry of Severe Cutaneous Adverse Reactions to Drugs and Collection of Biological Samples (RegiSCAR) criteria.^{E1}

Mononucleosis diagnosis

Diagnosis of IM was confirmed by heterophilic IgM antibodies using the monospot test.

In vivo tests

Skin prick tests and intradermal tests (IDs) with AX/CL were carried out on all the patients. Mother solution and serial dilutions of the drug were freshly prepared at the time of skin testing. IDs were read after 20 minutes and at 24-48-72 hours to detect immediate and delayed type reactions according to European Network on Drug Allergy (ENDA).^{E2} Patch tests (PTs) with AX/CL at 5% and 20% in petrolatum with readings at 24-48-96 hours were performed in 3 of 10 patients. A positive PT was defined whenever an infiltrate was detected. Skin testing was considered positive when the ID was greater than 3 mm from the initial wheal or an increase in the diameter of the initial wheal was associated with a flare, together with a negative saline control result.

Reagents

The culture medium was VLE-RPMI 1640 (Biochrom GmbH, Berlin, Germany) supplemented with 2 mM endotoxin-free L-glutamine, 1% nonessential amino acids, 1% sodium pyruvate (Sigma-Aldrich) and 2×10^{-5} M 2-ME (Merck, Darmstadt, Germany) (complete medium). Penicillin (BP), ampicillin (AM), and AX were purchased from Sigma, AX/CL from GSK, rIL-2

(Proleukin, GlaxoSmithKline, London, UK) from Novartis (Basel, Switzerland), the polyclonal activator phytohemagglutinin (PHA) from Biochrom AG, and the recall antigen streptokinase (SK) from CSL Behring.

Lymphocyte transformation test

PBMCs were isolated by Lymphoprep (Sentinel Diagnostic, Milano, Italy): 2×10^5 cells were cultured in triplicate in round-bottomed 96-well plates in complete medium plus 5% heat-inactivated autologous serum in a final volume of 0.2 mL for 5 days in the presence of 4 increasing doses of individual β -lactam (BP and AM 2.5-0.5-0.1-0.02 mg/mL, AX 1-0.5-0.1-0.02 mg/mL, AX/CL 0.5-0.1-0.02-0.004 mg/mL) or medium alone as negative control at 37°C in 5% CO₂ humidified atmosphere. As positive controls, 2500 UI/mL SK and 1% vol/vol PHA were used. After 16-hour pulsing with 0.5 μ Ci/well ³HTdR (PerkinElmer), the cultures were harvested, and radionuclide uptake was measured by scintillation counting. A stimulation index (SI) (ratio between the radioactivity from stimulated and unstimulated cultures) ≥ 3 was considered as positive.^{E3}

Generation of hapten-specific short-term T-cell lines (TCLs)

TCLs were generated by culturing 1×10^6 PBMCs in the presence of single drugs (BP, AM, and AX 0.5 mg/mL, AX/CL 0.1 mg/mL) for 6 days.^{E3} Activated T cells were expanded for further 8 days by the addition of rIL-2 (25 U/mL). T-cell blasts were finally recovered, washed, counted, adjusted to 1×10^6 /mL, and assessed for specificity via thymidine incorporation in the presence of the same drug used for the TCL induction (BP, AM, and AX 0.5 mg/mL, AX/CL 0.1 mg/mL) or the other cross-reactive β -lactams plus autologous irradiated PBMC (1:1 ratio) for 3 days. SI was considered as positive when ≥ 3 .

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