

Drug fever after a single dose of amoxicillin-clavulanic acid



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Clinical Implications

- Drug fever may occur after a single dose of amoxicillin-clavulanic acid. The pathogenic mechanism could be mediated by hapten-specific T cells that highly produce tumor necrosis factor- α , which is known to induce fever.

TO THE EDITOR:

Although amoxicillin-clavulanic acid (AMX/CLV) can be associated with hypersensitivity reactions, ranging from minor and self-limiting to anaphylaxis,¹ this is the first reported case of a reproducible maculopapular exanthema (MPE) with drug fever occurring after a single therapeutic dose, not associated with drug reaction with eosinophilia and systemic symptoms (DRESS). This case presented with delayed onset skin hypersensitivity reactions and fever that were finally associated with increased neutrophil counts plus high C-reactive protein (CRP) levels.

The patient was a 14-year-old Caucasian girl with a history of AMX/CLV-associated reactions. In 2012, during an Epstein Barr virus infection, she had an MPE during AMX/CLV therapy.

In February 2014, she was given AMX/CLV for a febrile bacterial infection and noted another itchy MPE 12 hours after the first dose. She stopped the drug, and in May 2014, she was referred to the Allergy Unit of Anna Meyer Children's Hospital for evaluation.

Skin tests were performed according to the European Network for Drug Allergy² recommendations in the following sequence: (a) skin prick testing (SPT) with 20 mg/mL AMX and AMX/CLV; and (b) an intradermal (ID) testing with 1/100 and 1/10 dilutions of the full-strength (200 mg/mL) AMX and AMX/CLV concentration at 20- to 30-minute intervals. Positive and negative controls for SPT and ID were obtained using histamine (ALK-Abellò, Milan, Italy: 10 and 1 mg/mL) and normal saline. A positive skin test result was when the SPT wheal was greater than 3 mm and was accompanied by erythema with a negative saline control result or 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 and a negative saline control result within 15 minutes. ID tests were also read after 24-72 hours for delayed T-cell-mediated reactions.

Specific IgE to amoxicillin, ampicillin, and penicillin G and V were measured (Immunocap RAST, Uppsala, Sweden) and were all negative.

Because all of the skin and *in vitro* tests were negative, an open challenge drug provocation test (DPT) to AMX/CLV using 1/10, 2/10, and then 7/10 of the therapeutic dose every 30 minutes (50/mg/kg/day in 2 doses) was administered following the

current guidelines.³ She had no acute reactions, and after 2 hours of observation, she was discharged.

After 3 hours from the last dose, she was vomiting and had a fever (39.4°C), headache, and generalized erythema. After 8 hours, she had diarrhea and difficulty sleeping. She was transported to the emergency department of Anna Meyer Children's Hospital where her vital signs, complete blood count, and CRP levels were found to be within the normal range. She was discharged with the suspicion of a concurrent viral infection.

In October 2014, the ID tests with AMX/CLV were again negative. The DPT with AMX/CLV was repeated with the same graded challenge protocol, and after 3 hours, she again noted vomiting and had a fever, headache, and an itchy MPE. Later she had difficulty sleeping and diarrhea. The next day, she was admitted to the Anna Meyer Children's Hospital Allergy Unit, where her blood was drawn and the following results were observed: 12.3 mg/mL CRP (normal value < 0.50 mg/dL); white blood cells count (WBC) of 14,950 mmc; neutrophils 90%; and liver function and a erythrocyte sedimentation rate within the normal ranges. After 2 days, the MPE was reduced and the girl no longer had a fever (Figure 1).

After a month, a lymphocyte transformation test (LTT), induction of hapten-specific short-term T-cell lines, and cytokine production measurements were performed⁴ (see this article's Online Repository at www.jaci-inpractice.org).

The LTT was strongly positive to both AMX and AMX/CLV (Figure 2, A), and the T-cell lines that were specific for AMX and AMX/CLV could be induced. The LTT positivity and induction of the hapten-specific T-cell lines were also confirmed 6 months apart from the reaction (data not shown). A flow cytometric analysis showed high percentages of TNF- α producing T-cell blasts (Figure 2, B). No significant production of CXCL8, IL-6, or IL-17 could be found (data not shown).

Drug fever is a rare event occurring in only approximately 3%-5% of adverse drug reactions.⁵ In 18%-29% of patients who

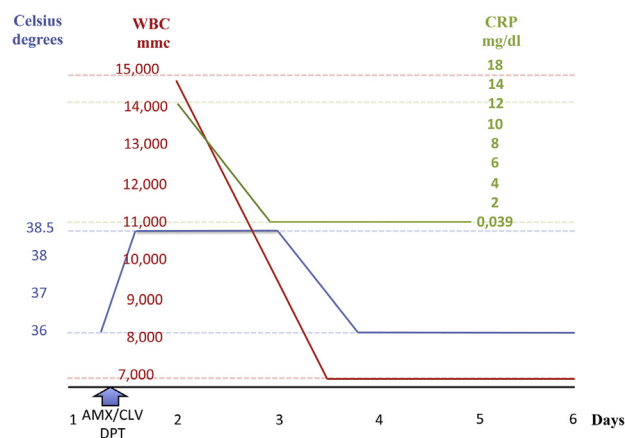


FIGURE 1. Fever, WBC, and CRP course. WBC, White blood cell; CRP, C-reactive protein.

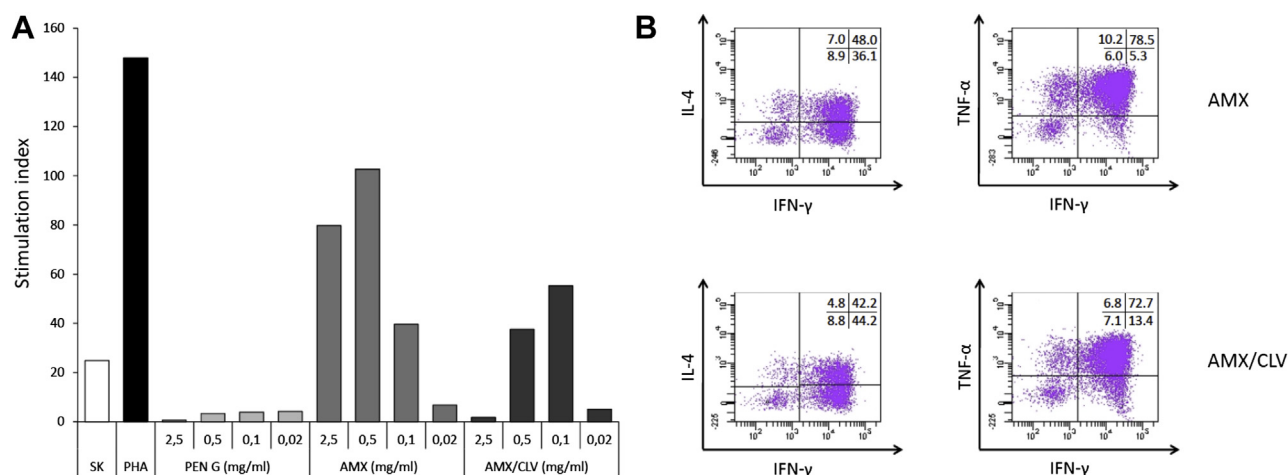


FIGURE 2. Laboratory findings. **A**, Lymphocyte transformation test (LTT). **B**, Intracellular cytokine expression by polyclonally stimulated hapten-specific T-cell lines.

develop drug fever, they can have skin involvement that resembles a hypersensitivity reaction.⁶ Fever is an idiosyncratic reaction to antimicrobials that typically occurs as a delayed event, at least 6 days from the beginning of a treatment course. However, despite the worldwide usage of AMX/CLV, no reports have been published in adults or in children regarding β -lactam-induced drug fever in the absence of DRESS.¹ In our patient, pyrexia was accompanied by leukocytosis and increased CRP, which pointed toward an acute inflammatory reaction, with prostaglandin and/or cytokine release in the peripheral tissues. In this case, however, a T-cell-mediated reaction to AMX/CLV has been proven, initially, by LTT positivity. The specificity and sensitivity of LTT are still controversial, although in nonimmediate reactions to β -lactams, they range between 58% and 88.8% and 85% and 100%, respectively.^{7,8} The induction of AMX- and AMX/CLV-specific T-cell lines not only confirmed the role of T cells but also implicated the involved mechanism as a significant number of the hapten-specific T-cell blasts produced TNF- α . TNF- α is a cell signaling protein (cytokine) that is involved in systemic inflammation and acute phase reactions, and it is chiefly produced by activated macrophages, in addition to many other cell types, including CD4⁺ lymphocytes. In addition, TNF- α is able to induce fever as it is an endogenous pyrogen.⁹ A possible role for TNF- α in sleep disturbances and migraines has also been recently suggested.

In conclusion, the present case can be mostly categorized as a hypersensitivity reaction to AMX/CLV with a well-defined pathogenic mechanism involving T lymphocytes and their related cytokines, in particular TNF- α . Awareness of this rare adverse event to AMX/CLV, a commonly used drug, could help in the prompt recognition of the reaction. Moreover, *in vitro* tests, specifically the LTT and induction of specific T-cell lines, provided interesting and useful results in the diagnosis of this reaction, and if these tests were performed before the second drug provocation test, then the patient would have avoided being at risk again.

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Materials and Methods

Reagents. The medium used was VLE RPMI 1640 (Biochrom GmbH, Berlin, Germany) supplemented with 2 mM endotoxin-free L-glutamine, 1% nonessential amino acids, and 1% sodium pyruvate (Sigma-Aldrich, St. Louis, Mo) and 2×10^{-5} M 2-ME (Merck, Darmstadt, Germany) (complete medium). Amoxicillin (AMX) and penicillin (PEN) were provided by Sigma. Amoxicillin/clavulanic acid (AMX/CLV; Clamoxyl) was from VIDAL (France). Phorbol 12-myristate 13-acetate (PMA) ionomycin, brefeldin A, and saponin were all purchased from Sigma. rIL-2 was purchased from Novartis (Proleukin). Streptokinase (SK) was from CSL Behring (Marburg, Germany), and phytohemagglutinin (PHA) was from Biochrom AG (Berlin, Germany).

Lymphocyte transformation test. LTT was performed as already described.⁴ In brief, PBMCs were isolated using a Lymphoprep (Sentinel Diagnostics, Milan, Italy) density gradient, followed by 2 washings with PBS at pH 7.2. A total of 2×10^5 cells/well were cultured in triplicate in round (U)-bottomed well plates in complete medium plus 5% heat-inactivated autologous serum at a 0.2 mL final volume for 5 days at 37°C in 5% CO₂ atmosphere in the presence of 1 of 4 increasing doses of haptens (PEN, AMX and AMX/CLV at 2.5-0.5 to 0.1-0.02 mg/mL, corresponding to 0.5-0.1 to 0.02-0.04 mg/well, respectively) or medium alone as a negative control. As positive controls, the recall antigen SK (2500 UI/mL) and the polyclonal activator PHA (1% vol/vol) were used. After 16 hours of pulsing with 0.5 μ Ci³ HTdR per well (PerkinElmer, Waltham, Mass), the cultures were harvested, and radionuclide uptake was measured by scintillation counting. The stimulation index was calculated as the ratio of the radioactivity in the stimulated cultures to the radioactivity in the unstimulated cultures.

Generation of hapten-specific short-term T-cell lines. Hapten-specific short-term T-cell lines were generated as described by Brugnolo et al.⁴ In brief, 1×10^6 PBMCs were cultured in 1 mL complete medium containing 5% autologous

serum in the presence of the drug (AMX or AMX/CLV at 0.5 mg/mL/well) for 6 days after 1 mL of fresh complete medium containing 5% autologous serum and rIL-2 (25 U/mL) was added. Activated T-cell blasts were thus expanded for further 8 days by replacing 5% autologous serum and rIL-2 (25 U/mL) medium every 3 days. At the end of the culture period, T-cell blasts were recovered, washed, and counted. Hapten specificity was performed by incubating 1×10^5 /well T-cell blasts in the presence of 1×10^5 /well irradiated autologous peripheral blood mononuclear cells as antigen-presenting cells and the specific hapten (0.1 mg/well) in round-bottomed plates in 200 μ L final volume in 5% CO₂ at 37°C for 3 days. Tritiated thymidine (0.5 μ Ci/well) was finally added and the culture stopped after 16 hours.

Intracytofluorimetric analysis of cytokine production by hapten-specific T-cell lines.

To assess cytokine synthesis by the hapten-specific T-cell lines at the single-cell level, 1×10^6 T-cell blasts were polyclonally stimulated with PMA (10 ng/mL) plus ionomycin (1 μ M) for 5 hours, with the addition of brefeldin A (5 μ g/mL) during the last 2 hours, and were analyzed for the expression of cytokines and surface molecules.

After polyclonal stimulation, the T-cell blasts were washed twice with PBS at pH 7.2, fixed for 15 minutes with formaldehyde (2% in PBS), washed twice with 0.5% BSA in PBS, permeabilized with PBS containing 0.5% BSA and 0.5% saponin, and finally incubated for 15 minutes at room temperature with a fluorochrome-conjugated specific mAb: PB-anti-CD3, FITC-anti-IFN- γ , FITC-anti-TNF- α , PE-anti-IL-4, PE-anti-CXCL8, or PE-anti-IL-6 (Becton Dickinson, San Jose, Calif); APC-Cy7-anti-CD8 (Miltenyi Biotec, Bergisch Gladbach, Germany); or FITC-anti-IL-17 (eBioscience, San Diego, Calif). After washing, the cells were analyzed, and the area of positivity was determined using an isotype-matched mAb, a FACSCalibur cytofluorimeter, and CellQuest software (Becton Dickinson). In all cytofluorimetric analyses, a total of 10^4 events, gated as CD3⁺CD8⁻ and CD3⁺CD8⁺ cells, were acquired for each sample.

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