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CLINICAL LETTER

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T cell involvement in the pathogenesis of drug-induced enterocolitis syndrome

Recently, Mori et al. published three new cases of drug-induced enterocolitis syndrome (DIES). A 6-year-old boy (*Case 1*) was investigated for a suspected IgE-mediated hypersensitivity reaction (angioedema of hands and feet) to amoxicillin/clavulanic acid (AMX/CL). He was orally provoked with AMX/CL with a graded challenge of 1/10 – 2/10 – 7/10 of the therapeutic dose every 30 minutes. After 2 hours of observation, the patient was discharged from the hospital in good conditions. After two hours and thirty minutes from the last drug intake, the child began vomiting, and his general condition deteriorated progressively. He was pale and lethargic, although vital signs were in range. For the severity of symptoms, he was admitted to our emergency care unit and needed intravenous saline solution infusion. During the acute phase, leukocytosis (20,480/μL; normal range 5000–15,000/μL) with neutrophilia 13,170/μL (not eosinophilia) and an increased methemoglobin level (1.1%; normal value 0.2–0.6%) were observed. In the following hours, he completely recovered and was discharged with the diagnosis of DIES to AMX/CL.[1]

It has been postulated that food protein–induced enterocolitis syndrome (FPIES) and DIES may share common pathogenetic aspects as they are both non-immediate hypersensitivity reactions involving adaptive immunity in addition to other still unknown mechanisms.[2] Here, we aimed to investigate the role of T cells in DIES by performing lymphocyte transformation test (LTT) toward AMX/CL and detecting cytokine production after hapten stimulation in hapten-specific T-cell lines.

Skin prick tests (SPTs) and intradermal tests (IDTs) with AMX/CL were completed. The mother solution and serial dilutions of the culprit drug were freshly prepared at the time of skin testing. IDTs were read after 20 minutes (immediate IDTs) and after 24–48 hours (delayed IDTs) according to the European Network of Drug Allergy Guidelines. Skin tests were considered positive when the diameter was greater than 3 mm from the initial wheal or increased from the initial wheal in association with a flare and negative saline control. The patient underwent the drug provocation test (DPT) with the culprit drug the day after the completion of the skin tests.

The DPT followed the following protocol: AMX/CL was orally administered at refracted doses (1/10-2/10-7/10 of the pediatric therapeutic dose per day [50/mg/kg/day]) every 30 minutes in an inpatient setting. The patient was observed for 2 hours after the last drug intake in case of a negative outcome. DPT was considered positive if, during the challenge or within 48 hours after the end of the challenge, any objective skin, respiratory and/or cardiovascular, neurologic, or gastrointestinal clinical manifestation was observed and documented with a picture by a physician or parents. In case of a reaction, the child was reevaluated in the allergy unit.

The culture medium was VLE-RPMI 1640 (Biochrom GmbH) supplemented with 2 mm endotoxin-free L-glutamine, 1% nonessential amino acids, 1% sodium pyruvate (Sigma-Aldrich), and $2x10^{-5}$ M 2-ME (Merck; complete medium). Penicillin (BP), ampicillin (AM), AMX (all from Sigma), and AMX/CL (GSK) were used; rIL-2 (Proleukin) was from Novartis; the polyclonal activator phytohemagglutinin (PHA) was from Biochrom AG; and the recall antigen streptokinase (SK) was from CSL Behring.

The LTT toward AMX/CL was performed 8 months afterward without the interference of corticosteroids. Peripheral blood mononuclear cells (PBMCs) were isolated by Lymphoprep (Sentinel Diagnostic). 2×10^5 cells were cultured in triplicate in round-bottomed 96-well plates in a complete medium with 5% autologous serum in a final volume of 0.2 mL for 5 days in the presence of 4 increasing doses of individual βLs (BP and AM 2.5–0.5–0.1–0.02 mg/mL, AMX 1–0.5–0.1–0.02 mg/mL, AMX/CL 0.5–0.1–0.02–0.004 mg/mL) or in a medium alone as a negative control at 37°C in 5% CO_2-humidified atmosphere. As positive controls, 2500 UI/mL SK and 1% vol/vol PHA were used. After 16-hour pulsing with 0.5 μCi/well ^{3}HTdR (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 positive.[3]

Additionally, 1×10^6 PBMCs were cultured in the presence of a single βL (BP, AM, or AMX 0.5 mg/mL, AMX/CL 0.1 mg/mL) for 6 days. Activated T cells were then expanded for a further 8 days by adding rIL-2 (25 U/mL). For the hapten specificity of T-cell lines, T-cell blasts were finally recovered, washed, counted, adjusted to 1×10^6/mL, and assessed for specificity by thymidine incorporation, cultured for 3 days in the presence of autologous irradiated PBMC (1:1 ratio) and the βL used for the induction (BP, AM, and AMX 0.5 mg/mL, AMX/CL 0.1 mg/mL) or cross-reactive. SI was considered positive when ≥3.

In assessing 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 μM) for 5 h, with the addition of brefeldin A (5 μg/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 min 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 min at room temperature with a fluorochrome-conjugated specific mAb: PB-anti-CD3, FITC-anti-IFN-γ, PE-anti-IL-4, (Becton Dickinson), APC-Cy7-anti-CD8 (Miltenyi Biotec), or FITC-anti-IL-17 (eBioscience). 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.[4]

LTT was positive to AMX (Figure 1A). AMX-specific short-term T-cell line strongly proliferated when assessed with AM (SI 8.4), AMX (SI 10.2), or AMX/CL (SI 4.4) in MHC-restricted conditions (data not shown). The flow cytometric analysis showed that high percentages of CD4+ T-cell blasts of the AMX-specific T-cell line produced IL-4 alone or co-expressed IL-4 and interferon (IFN)-gamma (TH2 and TH0 cells, respectively) (Figure 1B).

We report the first experience on DIES investigating the pathomechanisms involved and suggesting the possible role of T cells starting from the same hypothesis already postulated for FPIES.[5] It has been previously speculated that FPIES may be a T-cell-mediated reaction to food proteins, and we have already demonstrated the increase in IL-4 and the decrease in IFN-gamma expression in circulating T cells after a positive challenge with the causative food.[6] Indeed, TH2 cytokines other than IL-4, such as IL-3, IL-5, and IL-13, also have been found, and this may account for eosinophilia observed in early/late-onset FPIES patients.[2,7] Further, dysregulated production of other cytokines may favor large amounts of food allergens that are not captured by antigen-presenting cells to instead pass through the damaged epithelial barrier. In bioptic specimen, mononuclear cells expressing tumor necrosis factor-alfa (TNF-α) are present in the intestinal mucosa,[8] and TNF-α is a proinflammatory cytokine contributing synergically

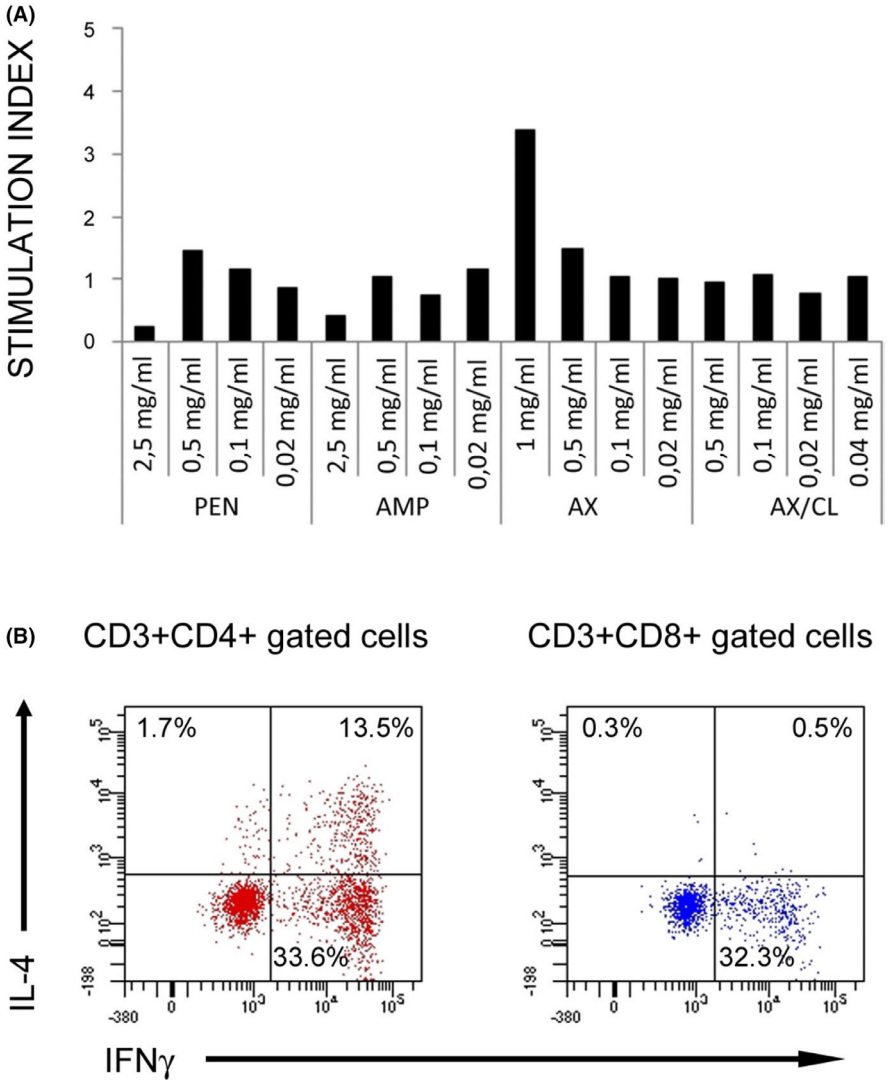

FIGURE 1 Results of the lymphocyte transformation test (LTT) toward amoxicillin/clavulanic acid (AMX/CL) in the patient with the diagnosis of DIES. (A) The LTT was strongly positive to both AMX and AMX/CL. (B) The flow cytometric analysis showed high percentages of AMX-specific T-cell blasts producing IL-4 alone or co-expressing IL-4 and interferon (IFN)-gamma (TH2 and TH0 cells)

with INF-γ to increase intestinal permeability.[5] Despite the possible involvement of T cells exhibiting a TH2 phenotype independently of food allergen specificity in FPIES, specific IgE is hardly found in the serum. However, local production of specific or unspecific IgE might be the case.[1]

This case demonstrates that a drug-specific T-cell-mediated response may be involved in DIES, which is a rare picture almost exclusively related to AMX or AMX/CL, thus confirming the classical features of a non-immediate hypersensitivity reaction. Sensitization of T cells after the binding to self-proteins and neoantigen formation[9] or direct stimulation of immune receptors (e.g., T-cell receptor or major histocompatibility complex antigens) may be equally involved, not differently from classical hypersensitivity reactions. The demonstration of drug-specific resident T cells into intestinal biopsies of these patients would be necessary to prove our speculation. More studies are needed to confirm our data and to better understand the immunopathogenesis of DIES, but this report gives a perspective for future research.

CONSENT FOR PUBLICATION

Written informed consent for publication of the clinical details was obtained from the patient's parents.

KEYWORDS

DIES, pathogenesis, pediatrics, rare allergic diseases, T cell

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests to disclose in relation to this paper.

AUTHOR CONTRIBUTION

Francesca Mori: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Supervision (supporting); Writing – original draft (lead); Writing – review & editing (lead). **Giulia Liccioli:** Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Writing – original draft (supporting); Writing – review & editing (supporting). **Manuela Capone:** Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Writing – original draft (supporting); Writing – review & editing (supporting). **Mattia Giovannini:** Formal analysis (supporting); Investigation (supporting); Writing – original draft (supporting); Writing – review & editing (supporting). **Simona Barni:** Formal analysis (supporting); Investigation (supporting); Writing – original draft (supporting); Writing – review & editing (supporting). **Lucrezia Sarti:** Formal analysis (supporting); Investigation (supporting); Writing – original draft (supporting); Writing – review & editing (supporting). **Elio Novembre:** Formal analysis (supporting); Investigation (supporting); Writing – original draft (supporting); Writing – review & editing (supporting). **Paola Parronchi:** Conceptualization (supporting); Data curation (lead); Formal analysis (lead); Investigation (lead); Supervision (lead); Writing – original draft (supporting); Writing – review & editing (supporting).

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