

Cytokine gene expression profiling to help identify a safe antibiotic in a patient with drug rash with eosinophilia and systemic symptoms

Neeti Bhardwaj, MD, MS^a, and

Faoud T. Ishmael, MD, PhD^b

Clinical Implication

- There are few assays available to assess drug hypersensitivity. This case presents an assay that may be used in severe drug hypersensitivity reactions to identify the culprit agent, assess cross-reactivity with other drugs, and assist in finding a safe agent.

TO THE EDITOR:

The diagnosis of T-cell-mediated drug hypersensitivity reactions remains a challenge due to the lack of sensitive and specific assays.¹ These reactions are so rare that it is difficult to determine the positive and negative predictive values of any specific test. In particular, the syndrome of drug rash with eosinophilia and systemic symptoms (DRESS) can be difficult to sort out. Patients with DRESS are often on multiple medications.² There is a poor understanding of the utility of available diagnostic tests for DRESS. It occurs so rarely that individual groups find it difficult to develop the needed expertise and

confidence in the workup of DRESS. DRESS can be life threatening, so rechallenge is generally contraindicated. Skin testing is of no utility because DRESS is not mediated through IgE.³ *In vitro* testing remains the primary modality, although patch testing has also been attempted.³ We present a case report using profiling of T-cell cytokine gene expression changes by real-time quantitative PCR (qPCR) to determine the culprit drugs and a safe alternative in a patient with suspected DRESS syndrome.

This case involves a 53-year-old man with methicillin-sensitive *Staphylococcus aureus* infection of knee prosthesis. He had no history of drug intolerances and had used penicillins and cephalosporins multiple times in the past. The prosthesis was removed, and he was started on intravenous cefepime and vancomycin. During week 3 of treatment, he developed fevers and an erythematous, macular rash that involved the back, trunk, and extremities. Serologic testing showed an eosinophil count of 7130 cells/mL (36% of differential), elevated creatinine of 1.38 mg/dL (glomerular filtration rate of 54 L), and elevated C-reactive protein level (1.21 mg/dL). Liver function was not assessed at that time. This is a systemic reaction with eosinophilia, which, based on the regiSCAR scoring system,⁴ is consistent with DRESS (score of 4, which makes the diagnosis of DRESS “probable”). Both antibiotics were discontinued, and the rash improved over the course of 3 weeks. The eosinophil count (200 cells/mL), and renal function (creatinine level of 1.0 mg/dL) were normal 8 weeks later.

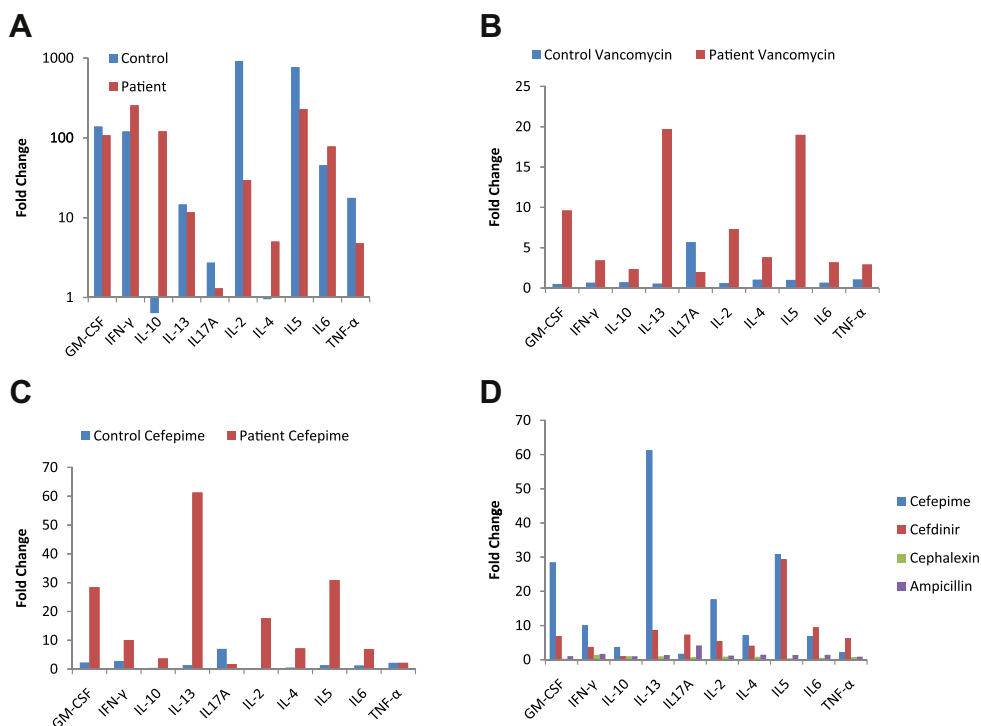


FIGURE 1. Changes in PBMC cytokine gene expression. **A**, Fold change in expression of cytokines in the patient and the control after phytohemagglutinin stimulation. Changes in cytokine mRNA expression were measured after incubation of cells with vancomycin (**B**) or cefepime (**C**). **D**, Cross-reactivity between different generations of cephalosporins and ampicillin was assessed in patient PBMCs.

The knee infection persisted, and the patient reported receiving a single dose of vancomycin months later at an outside hospital. A pruritic, macular rash developed hours later, per his account, but this information could not be verified from outside records. On another occasion, he developed a macular rash 6 to 8 hours after receiving cefepime infusion.

The patient was first evaluated in the outpatient allergy clinic approximately 6 months after the index reaction. *In vitro* testing, described below, was subsequently undertaken. He had no atopic diseases. His physical examination was significant for swelling and warmth of the right knee but was otherwise unremarkable. Laboratory work was normal, without eosinophilia or renal dysfunction.

The patient's initial reaction was suspicious for DRESS syndrome, a very severe and rare partially T-cell-mediated reaction. His history was unusual because readministration of both antibiotics led to a rash. We sought to further characterize the reaction to identify the inciting agent(s). We hypothesized that incubation of peripheral blood mononuclear cells (PBMC) with the culprit drug would lead to activation of T cells and that measurement of induction of cytokine gene expression by qPCR would be a sensitive method to assess T-cell stimulation. The experiments were conducted in parallel by using PBMCs obtained from a healthy control without a history of drug intolerance or atopy. Cells were incubated for 18 hours in the presence of phytohemagglutinin as a positive stimulation control, vancomycin (0.14 mg/mL), or cefepime (0.14 mg/mL). Total RNA was isolated, and gene expression changes in cytokines were measured by using qPCR array as previously described.⁵

As expected, phytohemagglutinin induced robust changes in cytokine expression in both the patient and control PBMCs (Figure 1, A, B, C). An increase in expression of GM-CSF, IL-5, IL-13, and IL-2 was observed in patient PBMCs stimulated with cefepime (mean fold increase, 34.5 ± 9.3) and vancomycin (13.9 ± 3.2) but not in cells of the control subject (cefepime, 1.35 ± 0.4 ; vancomycin, 0.7 ± 0.1). Increases in IFN γ were observed for cefepime (10-fold), vancomycin (3.4-fold), and cefdinir (3.7-fold) but not to the extent of the Th2 cytokines, which suggests that the latter was the more dominant mechanism in the patient's reaction.

These results indicated that both drugs were capable of inducing T-cell activation, consistent with the patient's history. This was an unexpected finding because the chance of both drugs inducing significant T-cell activation is predicted to be extremely low. Although vancomycin has been reported to be the cause of DRESS in several patients,⁶ individual cephalosporins have rarely been implicated. We speculated that the additive effects of both drug reactions may explain the severity of the patient's findings. Reaction to both drugs is, in fact, a novelty of this case. Many patients develop systemic drug reactions while on multiple medications, and it is possible that hypersensitivity to more than one agent is more common than perceived. However, this has not been investigated, in part due to a lack of good diagnostic tools.

We next sought to determine whether this assay could be used to detect cross-reactivity to penicillins and other cephalosporins. PBMCs from the patient and control were incubated with cefdinir, cephalexin, or ampicillin. There was a 29.4-fold increase in IL-5 in the cefdinir-treated sample compared with a 0.4-fold change in the cephalexin-treated sample and a 1.4-fold change in the ampicillin-treated cells (Figure 1, D). Because cefdinir and

cefepime contain similar R2 side chains, these side chains may constitute an important epitope in this type of drug reaction. Based on this, we recommended avoidance of cephalosporins with similar side chains, including third- and fourth-generation cephalosporins. If the patient needed a first- or second-generation cephalosporin with a different R2 side chain, then we recommended drug provocation testing to ensure safety. He was challenged with amoxicillin, which was well tolerated and was treated with amoxicillin-clavulanate for the methicillin-sensitive *Staphylococcus aureus* infection.

Conclusive diagnosis of drug intolerance continues to be a problem in clinical practice, but the method documented here appears effective. Cytokines play a central role in the effector phase of immune response, and their measurement may have diagnostic implications.^{1,7} Measurement of gene expression changes by qPCR is an inexpensive and extremely sensitive methodology, and this technique has the capability of detecting changes in a single cell.⁸

In this case, we used the qPCR measurement of cytokine mRNA changes to identify the culprit drugs, assess cross-reactivity between cephalosporins, and select a safe agent with which to challenge. The main limitation of this study is that the data presented are from a single patient with reported delayed drug hypersensitivity. Larger studies that involve more subjects are needed to validate the findings. Moreover, showing clinical tolerance to cephalexin based on *in vitro* results would have validated the utility of this test in predicting drug tolerability. Gene expression changes in cytokines from drug-challenged PBMCs may be a useful assay to analyze T-cell-mediated hypersensitivity reactions and find safe alternative medications, and warrants further investigation as a diagnostic tool.

^aDivision of Pulmonary, Allergy, and Critical Care Medicine, Department of Medicine, Pennsylvania State University Milton S. Hershey Medical Center, Hershey, Pa

^bDepartment of Biochemistry and Molecular Biology, The Pennsylvania State University College of Medicine, Hershey, Pa

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Corresponding author: Faoud T. Ishmael, MD, PhD, The Pennsylvania State University College of Medicine, 500 University Drive, H171, Hershey, PA 17033. E-mail: fishmael@hmc.psu.edu.

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