



Letter

Peripheral blood mononuclear cell gene expression testing and the heterogeneity of T-cell–mediated drug reaction with eosinophilia and systemic symptoms syndrome

Drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome is a rare but potentially life-threatening adverse drug hypersensitivity reaction.^{1,2} Not uncommonly, the reaction occurs in hospitalized patients who may have been placed on multiple new medications, making identification of the offending agent extremely difficult. In these instances, a physician can avoid all new medications or those most likely to have caused the reaction. This is problematic because a drug challenge with even a small amount of the true offending agent has the potential to induce a severe relapse of DRESS syndrome. Thus, a tool to safely evaluate and characterize immune response to medications in vitro is highly desirable, and we have used cytokine assay release effectively in this scenario before.³

Excessive T-cell activation plays a major role in DRESS syndrome. Expansion of activated CD4⁺ and CD8⁺ T cells occurs in the acute phase of the disease.^{1,2} More specifically, the tissue destruction and systemic symptoms observed in DRESS syndrome can be attributed to particular proinflammatory cytokines produced by activated T-cells.² However, DRESS syndrome has a heterogeneous presentation with different reactions and severities. Little is known about the specific patterns of cytokine expression seen in DRESS syndrome. A previous report described a type IVb drug hypersensitivity pattern in DRESS syndrome with a granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL) 5, IL-12, and IL-2 predominance.³ We describe variability of cytokine response within the type IVb characterization in a 17-year-old boy with DRESS syndrome secondary to vancomycin; however, this patient had a predominately GM-CSF, CCL2 expression pattern.

Our patient, a 17-year-old, previously healthy boy, was initially admitted at our institution for methicillin-resistant *Staphylococcus aureus* bacteremia complicated with pneumonia after a left toenail paronychia and influenza B infection. Two days after completing a 14-day course of vancomycin and rifampin, he developed low-grade temperature elevation (37.5°C) followed by a maculopapular rash in the inguinal area that quickly progressed to cover all cutaneous surfaces, prompting readmission (there was no mucosal involvement). On admission, his creatinine (2.2 mg/dL) and C-reactive protein (>27 mg/dL) level were elevated compared with his values at discharge 2 weeks ago. His total white blood cell count (13,800/ μ L) was elevated with an absolute eosinophil count of 1,160/ μ L. Within 1 day of admission, his liver transaminase levels increased as well (alanine aminotransferase, 139 IU/L; aspartate aminotransferase, 81 IU/L), revealing systemic organ involvement, all consistent with DRESS syndrome. His eosinophil count continued to climb during the next 3 days to a peak of 3,420/ μ L. On

the basis of the scoring report by Kardaun et al,⁴ his DRESS score was 5, corresponding to a probable likelihood of DRESS syndrome.

Antibiotic treatment was stopped, and he was given systemic corticosteroids (he had not previously been on any form of corticosteroid therapy), with marked improvement during the next 48 hours. He was discharged with a prednisone taper (60–40–20–10 mg/d for 3 days per dose strength). After discharge, he had 1 episode of skin desquamation while his rash resolved, but he returned to his baseline health with no new symptoms or mucosal involvement. Six weeks later, his eosinophilia returned to normal (absolute eosinophil count, 140/ μ L).

To further characterize the reaction and determine which drug was the culprit, we performed an in vitro T-cell gene expression assay with the patient's peripheral blood mononuclear cells (PBMCs) mixed with vancomycin or rifampin. PBMCs were isolated from the patient and control subject by Ficoll gradient and placed in AIM-V media at a concentration of 1×10^6 cells/mL. Vancomycin (0.2 mg/mL), rifampin (0.12 mg/mL), or diluent were added to PBMCs and incubated for 12 hours. RNA was extracted from cells using TRIzol reagent (Life Technologies, Carlsbad, California), isolated using Direct-zol RNA columns (Zymo Research Corp, Irvine, California), and reverse transcribed as previously described.⁵ Real-time polymerase chain reaction was used to measure expression of IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-17a, GM-CSF, tumor necrosis factor α (TNF- α), CCL2, transforming growth factor β , interferon γ (IFN- γ), and Fas ligand (FasL). Data were normalized to glyceraldehyde 3-phosphate dehydrogenase, and the internal control and relative expression differences between drug-treated cells and diluent control were calculated by the delta delta cycle threshold (ddCt) method.

A normal baseline was established for PBMCs from healthy individuals ($n = 6$) incubated with vancomycin. Mean (SD) cytokine changes are shown in Figure 1. The combined cytokine change from baseline was 0.80-fold (0.32-fold), with a minimum of 0.17-fold change and a maximum of 1.60-fold change. These data were interpreted to define the baseline background variability; thus, we set a cutoff of a 2-fold change to be minimum detectable difference, and this value is consistent with previously published data reading what types of fold changes can be detected by the method, but larger studies are necessary to fully validate this technique. On the basis of this cutoff, the in vitro testing revealed increases after vancomycin incubation (but not rifampin) in IL-2, IL-4, IL-6, IL-8, TNF- α , IFN- γ , and FasL, with the greatest fold increases in GM-CSF and CCL2 (Fig 1). These results provided evidence that vancomycin was the causative agent. The expression pattern of GM-CSF, a potent eosinophil growth factor, and CCL2, an eosinophil chemoattractant, was consistent with a type IVb T-cell–mediated

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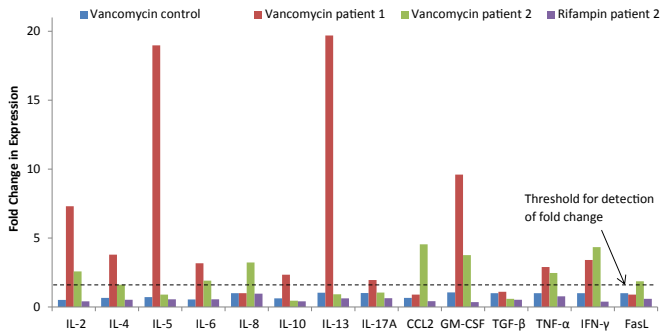


Figure 1. Lymphocyte cytokine expression profile. Relative expression of genes in peripheral blood mononuclear cells (PBMCs) from a patient incubated with vancomycin (vancomycin patient 2), a patient incubated with rifampin (rifampin patient 2), a different patient with drug reaction with eosinophilia and systemic symptoms syndrome (vancomycin patient 1), and a control receiving vancomycin (vancomycin control) shown as a fold change. FasL indicates Fas ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN- γ , interferon γ ; IL, interleukin; TGF- β , transforming growth factor β ; TNF- α , tumor necrosis factor α .

response, consistent with the patient's clinical presentation and eosinophilia. Interestingly, the pattern of cytokine expression differed from our previous report of a patient with vancomycin-reported DRESS syndrome, where the predominant cytokine increase was in IL-5 and IL-13 (Fig 1). These studies suggest that there may be heterogeneity within eosinophilic T-cell responses; however, the end result of eosinophil activation provides a similar clinical picture. Differential cytokine responses is not surprising because there is already described heterogeneity in the clinical and histological patterns of DRESS.^{6,7} Furthermore, it is possible that there could be features of T_H1 responses in DRESS syndrome, as evidenced by the increase in IFN- γ in our patient, and possibly a component of CD8⁺ T-cell stimulation as well, given the increase in FasL expression and the skin exfoliation observed clinically. Alternative explanations include the possibility of disease progression from DRESS syndrome to epidermal necrolysis reactions or an overlap syndrome between the two.⁸ Lastly, knowledge of cytokine profiles might help distinguish which patients would be candidates for emerging therapies, such as intravenous immunoglobulin.⁹

This case highlights the heterogeneity of T-cell-mediated drug hypersensitivity reactions and supports the utility of PBMC gene expression testing as a potentially useful tool to identify causative drugs and characterize the mechanism of drug reactions. Furthermore, the observation of vancomycin-induced DRESS syndrome in multiple patients raises the question of whether this drug may play a larger role in these drug hypersensitivity reactions than

previously appreciated. A review by Young et al¹⁰ in 2014 found only 19 cases of vancomycin-induced DRESS syndrome in the literature; however, 9 of these were reported after 2012. Included within this review was an 18-month review of DRESS syndrome cases at Massachusetts General Hospital, where 5 of the 6 cases of DRESS syndrome were attributable to vancomycin.¹¹ We believe these speak to vancomycin-induced DRESS syndrome as an emerging player in drug hypersensitivity reactions. As more cases are described, we believe there will continue to be heterogeneous presentations of the disorder, and perhaps the use of PBMC gene expression will help distinguish different phenotypes with DRESS that ultimately may help with prognosis and treatment for patients.

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