Copaescu et al. World Allergy Organization Journal (2024) 17:101002
http://doi.org/10.1016/j.waojou.2024.101002

WORLD ALLERGY ORGANIZATION JOURNAL

BRIEF RESEARCH REPORT

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Exploring cytokine outputs for *ex vivo* diagnostics in drug reaction with eosinophilia and systemic symptoms (DRESS)

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ABSTRACT

Background: In an exploratory study to assess the potential to individualize T-cell diagnostics in antibiotic-associated severe T-cell mediated hypersensitivity, we focused on drug reaction with eosinophilia and systemic symptoms (DRESS) and the related cytokine outputs IL-4 and IL-5.

Methods: Patients with well-phenotyped RegiSCAR $\geq$4 DRESS, positive intradermal skin testing, and a previous negative IFN-γ Enzyme-Linked ImmunoSpot (ELISpot) assay were prospectively recruited. We specifically performed an ELISpot assay with IL-4 and IL-5 cytokine outputs. As comparative controls, these cytokine outputs were performed simultaneously in patients with a positive *ex vivo* IFN-γ release ELISpot result.

Results: Four antibiotic-associated DRESS cases were included. The IL-4 and IL-5 output ELISpot assay demonstrated various results among these patients, with at least 1 cytokine present in all the cases. As for the 2 controls with known positive IFN-γ release, compared to the IFN-γ secretion, the cytokine output using IL-4 and IL-5 showed an increased positivity.

Conclusion: In patients where the early response has suggested a TH2 response such as DRESS, IL-4 and IL-5 cytokine outputs could present an investigational advantage, including when IFN-γ is negative. In the future, larger prospective studies are required to understand the role of varied cytokine outputs in T-cell-mediated hypersensitivities.

Keywords: Antibiotic hypersensitivity, T-Lymphocytes, Drug reaction with eosinophilia and systemic symptoms, Enzyme-linked immunoSpot, Interferon-gamma

Ex vivo and *in vitro* diagnostics such as the enzyme-linked immunoSpot (ELISpot) assay have been increasingly investigated as diagnostic tools in presumed antibiotic-associated delayed immune-mediated adverse drug reactions (IM-ADRs). Currently, the most experience is with interferon-γ

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http://doi.org/10.1016/j.waojou.2024.101002

Received 7 May 2024; Received in revised from 10 October 2024; Accepted 2 November 2024
Online publication date xxx

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ID	Patient	Phenotype	Systemic steroids[a]	Culprit Drug	Positive IDT	Blood Sample Latency[b]	IFN-γ		IL-4		IL-5	
1	M 44	DRESS RegiSCAR 7	Yes	Piperacillin-tazobactam	PipTazo Amoxicillin Flucloxacillin Benzylpen	20	PipTazo 150 μg/ml	PipTazo 1500 μg/ml	PipTazo 150 μg/ml	PipTazo 1500 μg/ml	PipTazo 150 μg/ml	PipTazo 1500 μg/ml
							0 SFU	15 SFU	35 SFU	**70 SFU**	**150 SFU**	**105 SFU**
2	M 40	DRESS RegiSCAR 6	No	Amoxicillin	Flucloxacillin	23	Amox 200 μg/ml	Amox 2000 μg/ml	Amox 200 μg/ml	Amox 2000 μg/ml	Amox 200 μg/ml	Amox 2000 μg/ml
							0 SFU	6 SFU	0 SFU	**490 SFU**	**785 SFU**	0 SFU
3	M 52	DRESS RegiSCAR 5	Yes	Ceftriaxone	Ceftriaxone	77	Ceftri 200 μg/ml	Ceftri 2000 μg/ml	Ceftri 200 μg/ml	Ceftri 2000 μg/ml	Ceftri 200 μg/ml	Ceftri 2.000 μg/ml
							9 SFU	14 SFU	25 SFU	0 SFU	0 SFU	0 SFU
4	M 40	DRESS RegiSCAR 4	Yes	Amoxicillin	Amoxicillin Flucloxacillin Benzylpen	6	Amox 200 μg/ml	Amox 2000 μg/ml	Amox 200 μg/ml	Amox 2000 μg/ml	Amox 200 μg/ml	Amox 2000 μg/ml
							10 SFU	2 SFU	33 SFU	0 SFU	**70 SFU**	13 SFU

Table 1. Cytokine Output Results for the included cohort (N = 4). ***Abbreviations:*** *Amox, Amoxicillin; Benzylpen, Benzylpenicillin; Ceftri, Ceftriaxone; DRESS, drug reaction with eosinophilia and systemic symptoms; F, female; Fluc, Flucloxacillin; IDT, intradermal testing; M, male; n/a, non-applicable; PenG, Penicillin G; Pip, Piperacillin; PipTazo, piperacillin-tazobactam; RegiSCAR, Registry of Severe Cutaneous Adverse Reactions.* ***Note 1:*** *The value next to the drug name represents the tested concentration used in microgram/milliliter (μg/ml).* ***Note 2:*** *The number indicates the number of spot forming units (SFU) determined by using the enzyme-linked immunospot (ELISpot) assay. A positive response was defined as equal to or greater than 50 spot-forming units (SFU)/million cells after background (unstimulated control) removal, as per previously published definitions.[8,9] These values are bolded in the table. [a]The column indicates if patients were taking systemic steroids at the time of blood collection. [b]Latency is defined in days and represents the time between the index date of the reaction (first DRESS features) and the blood sample.*

(IFN-γ) as the output in the ELISpot release assay platform for delayed IM-ADRs.[1] This modality has primarily been used in research-only settings, and its sensitivity (52–91%) has limited its routine use.[2] This exploratory study aimed to identify new ELISpot cytokine targets beyond IFN-γ, which could be used to improve diagnostic sensitivity based on IM-ADR-specific immunopathogenesis. This study focuses on a specific antibiotic-associated T-cell mediated IM-ADR, drug reaction with eosinophilia and systemic symptoms (DRESS) and the related cytokine outputs of IL-4 and IL-5.[3]

Patients with well-phenotyped RegiSCAR ≥4 DRESS associated with an identified culprit drug, positive intradermal skin testing, and a previous negative IFN-γ ELISpot (as defined by < 50 spot forming units (SFU)/million) were prospectively recruited from two tertiary referral drug allergy testing centers (Supplement Material - Methods**)**. We specifically targeted an ELISpot assay with IL-4 and IL-5 cytokine outputs. As comparative controls, these cytokine outputs were also performed in patients with a severe cutaneous adverse reaction (SCAR) with a positive *ex vivo* IFN-γ release ELISpot result and increased IL-4 or IL-5 plasma cytokine. (Supplement Table 1). Descriptive results are presented as frequency (percentage) and median (interquartile range [IQR]). The study was approved by the local Research Ethics Committee, and the investigators obtained written informed consent from the participants.

Four DRESS cases met these enrollment criteria and were included (Table 1). The culprit drugs and the positive intradermal testing results for the four patients can be visualized in Table 1. Regarding the cases with the IL-4 and IL-5 output ELISpot assay, patients #1 and #2 showed ≥50 SFU/million. Patient 4 showed ≥50 SFU/million cells for IL-5 and ≥30 SFU/million cells for IL-4. Patient 3 showed 25 SFU/million cells for IL-4 but no positivity with IL-5.

The results for the ELISpot assay using IL-4 and IL-5 cytokine outputs in patients with known positive IFN-γ release (controls) can be visualized in Table 2. Compared to the IFN-γ secretion, the cytokine output using IL-4 and IL-5 showed an increased number of SFU for these 2 patients. The specific SCAR phenotype, the culprit drugs, the

	Patient	Phenotype	Systemic steroids[a]	Culprit Drugs	Latency[b]	Positive IDT	IFN-γ		IL-4		IL-5	
							Vanc 50 µg/ml	Vanc 500 µg/ml	Vanc 50 µg/ml	Vanc 500 µg/ml	Vanc 50 µg/ml	Vanc 500 µg/ml
C1	F 26	DRESS	No	Vancomycin Ceftazidime	45	n/a						
							40 SFU	**60 SFU**	**117 SFU**	**230 SFU**	0 SFU	**83 SFU**
C2	F 31	AGEP	No	Amoxicillin	3324	Amoxicillin Flucloxacillin Penicillin G						
							Amp 200 µg/ml	Amp 2000 µg/ml	Amp 200 µg/ml	Amp 2000 µg/ml	Amp 200 µg/ml	Amp 2000 µg/ml
							45 SFU	**133 SFU**	**183 SFU**	**135 SFU**	0 SFU	**76 SFU**

Table 2. Cytokine Output Results for positive IFN-γ ELISpot assay controls (N = 2). ***Abbreviations:*** *AGEP, acute generalized exanthematous pustulosis; Amp, ampicillin; DRESS, drug reaction with eosinophilia and systemic symptoms; F, female; IDT, intradermal testing; M, male; n/a, non-applicable; Vanc, vancomycin.* ***Note 1:*** *The value next to the drug name represents the tested concentration used in microgram/milliliter (µg/ml).* ***Note 2:*** *The number indicates the number of spot forming units (SFU) determined by using the enzyme-linked immunospot (ELISpot) assay. A positive response was defined as equal to or greater than 50 spot forming units (SFU)/million cells after background (unstimulated control) removal as per previously published definitions (see references below). These values are bolded in the table.Ref: Keane NM, Roberts SG, Almeida CA, Krishnan T, Chopra A, Demaine E, et al. High-avidity, high-IFNgamma-producing CD8 T-cell responses following immune selection during HIV-1 infection. Immunol Cell Biol. 2012;90(2):224-34.Keane NM, Pavlos RK, McKinnon E, Lucas A, Rive C, Blyth CC, et al. HLA Class I restricted $CD8^+$ and Class II restricted $CD4^+$ T cells are implicated in the pathogenesis of nevirapine hypersensitivity. AIDS. 2014;28(13):1891-901.* [a]*The column indicates if patients were taking systemic steroids at the time of blood collection.* [b]*Latency is defined in days and represents the time between the reaction and the blood sample.*

latency period, and the results for the specific cytokines can be visualized in Supplement Table 1.

The heterogeneity of cytokines and chemokines described across the spectrum of T-cell-mediated hypersensitivity phenotypes is well-described. Cytokines are secreted by key effector cells, including T cells, in the SCAR setting and play a crucial role in the mechanisms and clinical manifestations.[4] By understanding the role of key cytokines in the immunopathogenesis of SCAR, we can tailor diagnostics specific to disease immunophenotypes and mediators. Elevated levels of IL-4 and IL-5 have been well described in DRESS.[5] By performing the ELISpot using other cytokines on known IFN-γ ELISpot negative patients with DRESS, we demonstrated that these cytokines offered a potential advantage over the traditional IFN-γ output.

Our study is limited by the small number of patients who presented an antibiotic-associated reaction. The data was collected prospectively using data collection forms that can limit the available information regarding specific cases (eg, available information regarding the prescription of systemic corticosteroids but no information regarding the specific dose or duration). Further, the selection criteria also focused on positive IDT, which can be a limitation in recruiting DRESS patients. From a laboratory technique perspective, the used assays require further validation and the inclusion of several types of controls, such as patients who tolerated antibiotics.

Although IFN-γ cytokine output remains an important and established investigational method for the ELISpot assay targeting T-cell mediated processes,[6,7] in patients where the acute reaction suggests a TH2 response such as DRESS, IL-4 and IL-5 cytokine outputs could present an investigational advantage for *ex vivo* testing when the IFN-γ ELISpot cannot provide diagnostic causality. In the future, larger prospective studies are required to understand the role of varied cytokine outputs in T-cell-mediated hypersensitivities.

Abbreviations
DRESS, drug reaction with eosinophilia and systemic symptoms, IFN-γ, interferon-γ, IL, interleukin, IM-ADRs, immune-mediated adverse drug reactions, IQR, interquartile range, ELISpot, Enzyme-Linked ImmunoSpot, SCAR, severe cutaneous adverse reaction, SFU, spot forming units.

Data availability statement
The data that support the findings of this study are available on request from the corresponding author.

Author contributions statement
AMC was responsible for conception and design, performed the literature review, and wrote the manuscript text under the supervision of JAT and EP. FJ and MG were responsible for patient recruitment and data acquisition. AC and EM performed the laboratory assays as described in the methods section. All authors reviewed the manuscript and made a substantial, direct, and intellectual contribution. All authors approved the final version of the manuscript for publication.

Ethics approval
The study was approved by the Austin Health Human Research Ethics Committee (HREC/15/AUSTIN/75 and HREC/50791/Austin-19), and the investigators obtained written informed consent from the participants.

Consent for publication
All authors have approved the submission of this manuscript. This original research has not been previously published and is not being considered for publication in another journal.

Funding
There is no funding directly related to this work.
A.M.C. receives support from the Montreal General Hospital Foundation and Research Institute of the McGill University Health Centre (RI-MUHC) and was awarded the University of Melbourne Research Scholarship, the Anna Maria Solinas Laroche Career Award in Immunology and the Anita Garbarino Girard/Anna Maria Solinas/Dr. Phil Gold Award of Distinction.

Declaration of competing interest
The authors declare no conflict of interest in relation to this work.

Acknowledgments
We thank the clinical nurse specialists, Wendy Stevenson, Belinda Lambros and Rebecca Hall for their assistance in performing *in vivo* testing.

J.A.T was supported by the Austin Medical Research Foundation (AMRF) and by a National Health and Medical Research Council (NHMRC) postgraduate scholarship (GNT 1139902).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.waojou.2024.101002.

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