

Analysis of HLA-B Allelic Variation and IFN- γ ELISpot Responses in Patients with Severe Cutaneous Adverse Reactions Associated with Drugs



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What is already known about this topic? There are no reliable tools to prevent severe cutaneous adverse reactions and identify the culprit drugs.

What does this article add to our knowledge? Less than a quarter of drug-induced severe cutaneous adverse reactions in Thailand were preventable by HLA-B screening, whereas drug-specific IFN- γ -releasing cells were detectable in almost half of these patients.

How does this study impact current management guidelines? New biomarkers are required to prevent severe drug allergy. The measurement of IFN- γ responses may have a role in identifying the culprit drugs.

BACKGROUND: The prevention and confirmation of drug-induced severe cutaneous adverse reactions (SCARs) are difficult. **OBJECTIVE:** To determine the benefit of HLA-B allele pre-screening and the measurement of drug-specific IFN- γ -releasing cells in the prevention and identification of the culprit drug in patients with SCARs.

METHODS: A total of 160 patients with SCARs were recruited from 6 university hospitals in Thailand over a 3-year period. HLA-B alleles were genotypically analyzed. The frequencies of drug-specific IFN- γ -releasing cells in patients with SCARs were also measured.

RESULTS: The drugs commonly responsible for SCARs were anticonvulsants, allopurinol, beta-lactams, antituberculosis agents, and sulfonamides. If culprit drugs had been withheld in patients carrying known HLA-B alleles at risk, it would have prevented 21.2% of SCAR cases, mainly allopurinol- and carbamazepine-related SCARs. Culprit drug-specific IFN- γ -releasing cells could be identified in 45.7% (53 of 116) of patients with SCARs caused by 5 major drug groups, particularly in patients diagnosed with drug reactions with eosinophilia and systemic symptoms (DRESS) (50.0%), followed by Stevens-Johnson syndrome/toxic epidermal necrolysis (46.0%),

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This study has been supported by The Skin and Allergy Research Unit, Chulalongkorn University and the National Research University Project, Office of Higher Education Commission (WCU-002-HR-57), and the Thailand Research Fund (RSA5880041).

Conflicts of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication March 29, 2017; revised May 2, 2018; accepted for publication May 3, 2018.

Available online May 22, 2018.

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2213-2198

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<https://doi.org/10.1016/j.jaip.2018.05.004>

Abbreviations used

AGEP- Acute generalized exanthematous pustulosis
 DRESS- Drug reaction with eosinophilia and systemic symptoms
 ELISpot- Enzyme-linked immunospot assay
 LTT- Lymphocyte transformation test
 PBMC- Peripheral blood mononuclear cell
 SCAR- Severe cutaneous adverse reaction
 SJS- Stevens-Johnson syndrome
 TEN- Toxic epidermal necrolysis

and acute generalized exanthematous pustulosis (31.3%). According to our study, high frequencies of drug-specific IFN- γ -releasing cells were significantly demonstrated in patients who suffered from DRESS phenotype, having anticonvulsants or the drugs belonging to the “probable” category based on the Naranjo algorithm scale, as the culprit drugs.

CONCLUSIONS: HLA-B prescreening would succeed in preventing only a minority of SCAR victims. Drug-specific IFN- γ -releasing cells are detectable in almost half of patients. Better strategies are required for better SCAR prevention and culprit drug confirmation. © 2019 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2019;7:219-27)

Key words: Drug hypersensitivity; Enzyme-linked immunospot assay; HLA-B genotyping; IFN- γ ; Severe cutaneous adverse reactions

Severe cutaneous adverse reactions (SCARs), comprising acute generalized exanthematous pustulosis (AGEP), drug reactions with eosinophilia and systemic symptoms (DRESS), Stevens-Johnson syndrome (SJS), and toxic epidermal necrolysis (TEN), are life-threatening conditions mostly triggered by drugs. The prevention of drug-induced SCARs is challenging, and confirming the culprit drug is difficult because a drug provocation test in SCARs is contraindicated.¹

Current knowledge of pharmacogenomics demonstrates that screening susceptible HLAs may be helpful in reducing the SCAR burden caused by drugs. Several reports indicate that certain HLA-B alleles are strongly associated with the increased risk of drug-induced adverse reactions in various ethnicities: for example, *HLA-B*1502* and carbamazepine/oxcarbazepine-induced SJS/TEN, *HLA-B*5801* and allopurinol-induced SJS/TEN in Han Chinese and Southeast Asian populations,²⁻⁴ *HLA-B*1301* and dapsone-induced hypersensitivity syndrome in Chinese, *HLA-B*5701* and abacavir-induced hypersensitivity syndrome in Caucasians, and to a lesser degree, *HLA-B*3505* in HIV-infected Thai patients who have experienced nevirapine-induced rash.⁵ Recent data suggest that *HLA-B*5801* may increase the risk of allopurinol-induced DRESS in the Thai population as well.⁶ Associations of *HLA-B*1502* with phenytoin- and lamotrigine-induced SCARs were also found in Asian ethnics.⁷⁻⁹

Altogether, these data suggest that prescreening HLA-B genotyping might be beneficial in the prevention of drug-induced SCARs in high-risk subjects who carry susceptible HLA-B alleles. In fact, multicenter studies in Taiwan have concluded that prospective screening of *HLA-B*1502* and *HLA-B*5801* significantly reduced the incidence of carbamazepine- and

allopurinol-associated SCARs, respectively, in Han Chinese.^{10,11}

In contrast, HLA-B screening may not be a worthwhile strategy for reducing drug-induced SJS/TEN in patients of European ancestry because HLA-B alleles at risk are reportedly associated with only a few drugs and their allele frequencies in European populations are low.¹² It is worth noting that the overall benefit of HLA-B prescreening before drug prescription in populations carrying high-risk alleles has never been described. The allele frequencies of *HLA-B*5801*, *HLA-B*1502*, and *HLA-B*1301* in the Thai population are 8.62%, 8.16%, and 6.95%, respectively.¹³ It would be interesting to know the extent to which universal HLA-B screening could potentially reduce the SCAR burden if it was implemented in a high-risk population like that in Thailand.

Culprit drug confirmation is another important issue besides SCAR avoidance. Identifying the drug responsible for a SCAR helps patients to avoid not only the culprit drug but also cross-reactive drugs for future use. The currently available tools for culprit drug confirmation—the skin patch test and lymphocyte transformation test (LTT)—are recommended for patients after remission from an acute drug allergic episode.^{14,15} Delayed diagnosis of a drug allergy confirmation could lead to deleterious consequences in certain circumstances: for example, in patients who develop a SCAR while taking multiple antibiotics, readministration of the safe antibiotic is required to combat serious infections. Confirmation of the culprit drug would be helpful in patients who develop SCARs while taking multiple drugs because avoidance of all suspected drugs could have deleterious implications for patient management.

Drug-specific circulating T cells can be detectable in patients with drug allergy with frequencies as low as 1:30,000 circulating leukocytes.¹⁶ By using the enzyme-linked immunospot (ELISpot) assay, drug-specific IFN- γ -releasing cells were demonstrated in patients presenting with maculopapular exanthema or anaphylaxis from amoxicillin and cephalosporins.^{16,17} The measurement of drug-specific cytokine-releasing cells by using the ELISpot assay can be performed during the acute stage of drug allergy.¹⁸ Moreover, this technique showed higher sensitivity than LTT and a positive response even in patients who were taking immunosuppressive drugs.^{18,19} It would be interesting to know whether this approach would be helpful in confirming the culprit drug in different SCAR phenotypes, especially in patients for whom culprit drug identification is urgently needed.

OBJECTIVES

The purposes of this study were to evaluate the potential impact of HLA-B allele screening in SCAR prevention if implemented, and to determine whether the detection of drug-specific IFN- γ -releasing cells during the acute drug allergic phase in SCARs could be a possible tool for confirming the causative drugs in patients diagnosed with drug-induced SCARs in Thailand.

METHODS

Patient recruitment

A total of 164 drug-induced patients with SCARs were initially recruited by dermatologists for this study from 6 university hospitals between November 2013 and July 2016. All cases were confirmed as probable or definite AGEP, DRESS, or SJS/TEN according to the

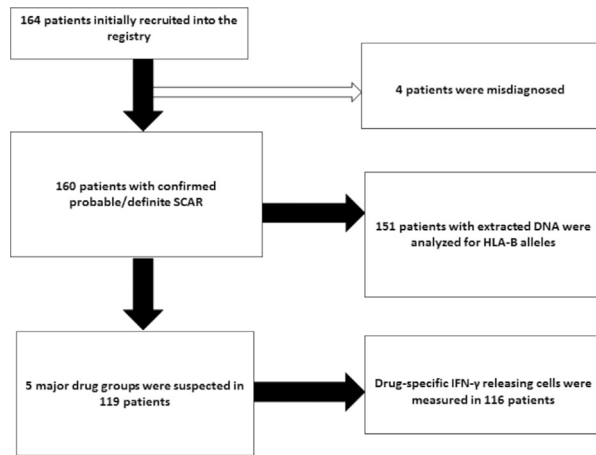


FIGURE 1. Schematic diagram of patient selection for this study. A total of 160 patients with confirmed probable or definite SCARs were recruited in the registry between 2013 and 2016. HLA-B genotyping was performed in 151 patients who had genomic DNA extracted. Drug-specific IFN- γ -releasing cells were measured in 116 patients with SCARs who suffered from 5 major drug groups (anticonvulsants, allopurinol, sulfonamides/sulfones, antituberculosis agents, and beta-lactams). *SCAR*, Severe cutaneous adverse reaction.

RegiSCAR diagnostic criteria.²⁰⁻²² Four patients were later excluded after the correct diagnosis (pustular psoriasis [2 cases], angioimmunoblastic T-cell lymphoma [1 case], and acrodermatitis enteropathica [1 case]) was made in patients initially diagnosed with AGEP (2 cases), DRESS (1 case), and SJS (1 case), respectively. The identification of culprit drugs was performed by dermatologists and pharmacists using the Naranjo adverse drug reaction probability scale. The suspected drugs could not be identified in 3 patients. HLA-B genotyping was performed in 151 patients whose extracted DNA could be obtained, and drug-specific IFN- γ -releasing cells were measured in patients allergic to the 5 most common groups of suspected drugs (anticonvulsants, allopurinol, sulfonamides/sulfones, antituberculosis agents, and beta-lactams) (Figure 1).

Genomic DNA extraction and analysis of HLA-A, -B, and -C alleles

Patients' DNA was isolated from the buffy coat using the MagNA Pure automated extraction system (Roche Diagnostics, Indianapolis, Ind) based on magnetic-bead technology. All the DNA was aliquotted and stored at -20°C before analysis.

The HLA-A, -B, and -C alleles were genotyped using Luminex multiplex technology (Luminex IS100) based on the polymerase chain reaction followed by sequence-specific oligonucleotide probe principles with a commercial kit (LABType SSO HLA Typing Kit; One Lambda Inc, CA). Data analyses were performed with HLA fusion 2.0 software.

The measurement of IFN- γ -releasing cells after stimulation of peripheral blood mononuclear cells with the suspected culprit drugs

The frequencies of drug-induced IFN- γ -releasing cells/ 10^6 peripheral blood mononuclear cells (PBMCs) after stimulating PBMCs with the suspected drugs were analyzed in patients who

developed SCARs after taking the 5 most common groups of eliciting drugs whose cryopreserved PBMCs were available ($N = 116$). The number of IFN- γ -releasing cells was measured using ELISpot assay kits (Mabtech, Stockholm, Sweden). Briefly, 96-well polyvinylidene fluoride membrane plates were coated for 16 hours at 4°C with the $5\text{ }\mu\text{g/mL}$ anti-IFN- γ antibody provided in the kit, and blocked with R10 medium for 1 hour at room temperature. PBMCs (2.5×10^5 in $100\text{ }\mu\text{L}$) were incubated for 40 hours at 37°C in 5% CO_2 with the suspected culprit drugs.

The plates were washed 6 times with phosphate-buffered saline/Tween 0.05%, incubated for 1.5 hours at 37°C with a biotinylated anti-IFN- γ antibody, and then washed extensively. Spot-forming cells were developed using streptavidin-alkaline phosphatase, incubated for 1 hour at 37°C , and washed extensively before adding the substrate. The results were expressed as the highest frequencies of IFN- γ -releasing cells/ 10^6 PBMCs on stimulation with 2 concentrations of the tested culprit drugs (as shown in Table E1, available in this article's Online Repository at www.jaci-inpractice.org) or nonallergic control drugs, after subtracting the value obtained from PBMCs cultured without drugs.

The average frequencies of IFN- γ -releasing cells on PBMC stimulation with 67 irrelevant nonculprit drug panels in 62 patients with SCARs who developed SCARs from other different drugs were used as negative control values. Basically, most patients were tested with 1 suspected culprit drug and 1 irrelevant nonculprit drug, except for 5 subjects who were tested with 1 suspected culprit drug along with 2 different nonallergic control drugs as shown in Table E2 (available in this article's Online Repository at www.jaci-inpractice.org). Values greater than the upper limit of the 95% confidence interval for mean IFN- γ -releasing cells stimulated by irrelevant drugs in these patients with SCARs were considered positive for the IFN- γ ELISpot assay. Oxypurinol-specific IFN- γ -releasing cells were measured for the evaluation of allopurinol hypersensitivity because they yielded higher immunogenic response.²³ The drug concentrations used in this study were generally similar to those used for the LTT or in the same range as therapeutic serum concentrations after being tested for having no immunosuppressive effect (PHA-induced proliferation inhibited by less than 15%). Pure drug substances for which intravenous preparation was not available were purchased from Sigma-Aldrich (St Louis, Mo).

Statistical analysis

Student's *t*-test and 1-way analysis of variance with Bonferroni correction were used for quantitative analysis in patients diagnosed with a SCAR after stimulating PBMCs with culprit drugs and controlled drugs. The average frequencies of drug-specific IFN- γ -releasing cells were expressed as means and 95% confidence interval. All statistical calculations were analyzed using SPSS 21 (IBM, Armonk, NY). *P* values $< .05$ were considered statistically significant.

Ethics

PBMCs employed in this experiment were cryopreserved specimens from patients enrolled in the Thailand Severe Cutaneous Adverse Reactions registry. PBMC isolation was performed within 14 days of rash onset. The registry was approved by the Ethics and Research Committee of the Faculty of Medicine, Chulalongkorn University, and informed consent was obtained from all participants. The ThaiSCAR study was registered at ClinicalTrials.gov (NCT02574988).

TABLE I. Baseline characteristics of patients diagnosed with drug-induced severe cutaneous adverse reactions at 6 university hospitals in Thailand

SCAR phenotypes (N)	All SCARs (160)	AGEP† (22)	DRESS (61)	SJS/TEN (77)
Gender (M/F)	58/102	4/18	23/38	31/46
Age (y)	52.1 ± 17.8	59.4 ± 19.9*	53.3 ± 18.1	49.1 ± 16.5
Latency period (d)	17.5 ± 15.8	2.5 ± 1.7**	24.0 ± 15.4	16.1 ± 15.2
Suspected culprit drugs (N= 162)				
Possible/probable	62/100	13/11	21/40	28/49
Anticonvulsants	35	—	19	16
Allopurinol	31	—	16	15
Beta-lactams	25	17	3	5
Sulfonamides/sulfones	16	—	6	10
Anti-tuberculosis agents‡	15	—	8	7
Non-beta-lactam antibiotics§	13	2	1	8
Analgesic drugs	11	2	1	8
Antiretroviral drugs	4	—	3	1
Miscellaneous drugs	12	3	3	5
Unidentified drugs	3	—	1	2
ELISpot assay latency (d)	7.0 ± 4.5	6.6 ± 6.5	7.3 ± 4.1	6.9 ± 4.3
Systemic steroid usage when blood drawn (N, %)	60/116 (51.7%)	2/16 (12.5%)**	31/50 (62.0%)	27/50 (54.0%)

AGEP, Acute generalized exanthematous pustulosis; DRESS, drug reaction with eosinophilia and systemic symptoms; ELISpot, enzyme-linked immunospot; SCAR, severe cutaneous adverse reaction; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

* $P < .05$ compared with SJS/TEN; ** $P < .01$ compared with SJS/TEN and DRESS.

†24 AGEP occurrences in 22 patients.

‡Antituberculosis agents: the combination of isoniazid, pyrazinamide, ethambutol, and rifampicin was considered a single drug in this study.

§Non-beta-lactam antibiotics: any antibacterial and antifungal drugs excluding beta-lactams, sulfonamides/sulfones, and antituberculosis agents.

RESULTS

Baseline characteristics of patients diagnosed with drug-induced SCARs in Thailand

A total of 160 confirmed cases of drug-induced SCARs were prospectively recruited from 6 university hospitals over a 3-year period, as shown in Table I. A preponderance of females was demonstrated (the female:male ratio was 1.8:1), particularly in AGEP. Almost half of the patients (48.1%) were diagnosed with SJS/TEN, followed by DRESS (38.1%) and AGEP (13.8%), respectively. Patients diagnosed with AGEP were significantly older than patients diagnosed with SJS/TEN (59.4 ± 19.9 years vs 49.1 ± 16.5 years, P value $< .05$) while having a shorter onset after drug exposure than patients diagnosed with DRESS and SJS/TEN (2.5 ± 1.7 days vs 24.0 ± 15.4 and 16.1 ± 15.2 days, P values $< .01$).

The 5 most common drug groups (anticonvulsants 21.6%, allopurinol 19.1%, beta-lactams 15.4%, antituberculosis agents 9.3%, and sulfa drugs 8.0%) were the suspected culprits in 73.5% of patients with drug-causing SCARs. Beta-lactam antibiotics were the main culprit drugs in AGEP, whereas anticonvulsants and allopurinol were the leading causes of DRESS and SJS/TEN.

Distribution of HLA class I haplotypes in Thai patients diagnosed with a drug-induced SCAR

HLA class I allele genotyping was determined in 151 patients with SCARs (18 AGEP, 59 DRESS, and 74 SJS/TEN) whose genotypic DNA could be obtained (Figure 2). The 3 most common HLA class I haplotypes in Thai patients diagnosed with drug-induced SCARs were $A*0207-B*4601-C*0102$ (6.42%); $A*1101-B*1502-C*0801$ (5.82%); and $A*1101-B*1301-C*0304$ (4.30%). The most frequent HLA class I haplotypes observed in

the AGEP, DRESS, and SJS/TEN groups were $A*1101-B*4002-C*0304$ (9.22%); $A*1101-B*1301-C*0304$ (5.56%); and $A*0207-B*4601-C*0102$ (10.19%), respectively.

Potential effect of HLA-B screening in drug-induced SCAR prevention

The potential effect of HLA-B screening in preventing drug-induced SCARs was analyzed (Table II). Of 151 patients who underwent genotyping, 27.2% developed SCARs after taking drugs with currently known HLA-B at risk ($HLA-B*1301$, $HLA-B*1502$, $HLA-B*3505$, $HLA-B*5701$, and $HLA-B*5801$ for dapsone, carbamazepine/oxcarbazepine, nevirapine, abacavir, and allopurinol hypersensitivity, respectively). Seventy-eight percent of these patients (32 of 41 cases) would have been protected from SCAR development if drugs potentially causing allergic reaction had been withheld in patients carrying susceptible HLA-B. Nevertheless, universal HLA-B prescreening could not save 78.8% of patients with SCARs in this cohort. The protective rate of HLA-B screening for SJS/TEN and DRESS prevention would be 24.3% (18 of 74 cases) and 23.7% (14 of 59 cases), respectively, whereas no preventive effect was observed for AGEP. According to our study, only 5 more patients would be saved from SJS/TEN and DRESS of 24 subjects if phenytoin and lamotrigine were also withheld in patients carrying $HLA-B*1502$.

Frequencies of drug-specific IFN- γ -releasing cells during the acute allergic phase of different SCAR phenotypes

Drug-specific T-cell responses after PBMC stimulation with the 5 most common groups of suspected culprit drugs (anticonvulsants, allopurinol, beta-lactams, antituberculosis

Most common HLA class I haplotypes in patients diagnosed with drug-induced severe cutaneous adverse reaction

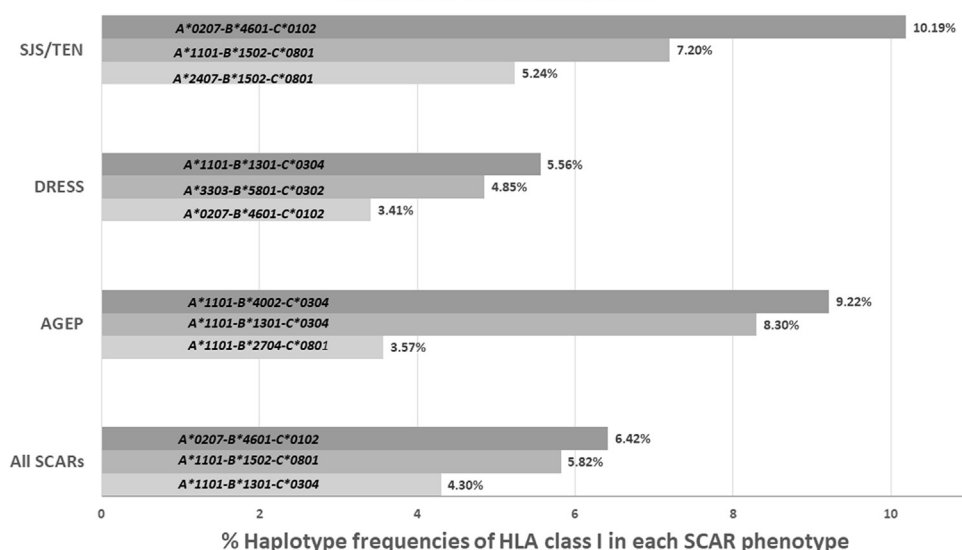


FIGURE 2. Allele frequency of the top 3 HLA class I haplotypes in patients diagnosed with each phenotype of drug-induced severe cutaneous adverse reaction (N = 151). *A*0207-B*4601-C*0102*, *A*1101-B*1502-C*0801*, and *A*1101-B*1301-C*0304* were the most common HLA class I haplotypes in 151 patients diagnosed with drug-induced SCARs in Thailand. The most frequent HLA class I haplotypes observed in the AGEF, DRESS, and SJS/TEN groups were *A*1101-B*4002-C*0304*, *A*1101-B*1301-C*0304*, and *A*0207-B*4601-C*0102*, respectively. AGEF, Acute generalized exanthematous pustulosis; DRESS, drug reaction with eosinophilia and systemic symptoms; SCAR, severe cutaneous adverse reaction; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

TABLE II. Potential effect of HLA-B screening in drug-induced SCAR prevention (N = 151)

Culprit drug and HLA-B at risk	All SCARs (N = 151)		DRESS (N = 59)		SJS/TEN (N = 74)	
	All cases	Positive risk allele	All cases	Positive risk allele	All cases	Positive risk allele
Allopurinol <i>HLA-B*5801</i>	30	25	15	12	15	13
Carbamazepine <i>HLA-B*1502</i>	7	5	2	0	5	5
Lamotrigine* <i>HLA-B*1502</i>	1	0	0	0	1	0
Phenytoin* <i>HLA-B*1502</i>	23	5	13	1	10	4
Dapsone <i>HLA-B*1301</i>	2	1	1	1	1	0
Nevirapine <i>HLA-B*3505</i>	2	1	2	1	0	0
Total	65	37	33	15	32	22

Numbers of patients who developed SCAR after taking drugs with reported HLA-B risk alleles are demonstrated along with numbers of these patients who actually carry HLA-B risk alleles. Nearly a quarter of patients in the ThaiSCAR registry would have been saved from developing severe cutaneous adverse reactions, mainly in allopurinol- and anticonvulsant-related SJS/TEN and DRESS, if susceptible HLA-B alleles had been screened.

DRESS, Drug reaction with eosinophilia and systemic symptoms; SCAR, severe cutaneous adverse reaction; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

*Lamotrigine and phenytoin were analyzed for an association with the *HLA-B*1502* allele.

agents, and sulfa drugs) in different SCAR phenotypes were comparatively measured (Figure 3). Culprit drug-specific IFN- γ -releasing cells were detectable in 45.7% of 116 patients with SCARs after stimulating PBMCs with the suspected culprit drug (31.3%, 50.0%, and 46.0% of patients diagnosed with drug-induced AGEF, DRESS, and SJS/TEN, respectively), whereas IFN- γ -releasing cells were detectable at low levels in only 9.0%

of patients with SCARs after stimulating PBMCs with nonallergic control drugs (P value < .01).

Sixty-seven drug panels (13 isoniazid/rifampicin/pyrazinamide/ethambutol [IRZE], 16 anticonvulsants, 11 oxypurinol, 18 cotrimoxazole, and 9 beta-lactams) were incubated with PBMCs harvested during the acute allergic phase from 62 patients with SCARs (28 DRESS, 28 SJS/TEN, and 6 AGEF)

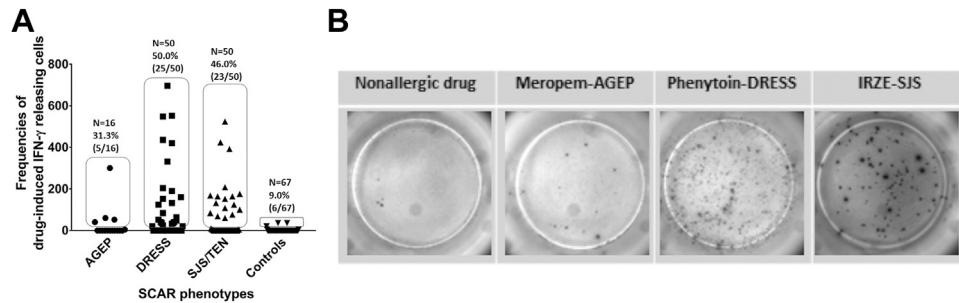


FIGURE 3. Quantification of drug-specific IFN- γ -releasing cells in different SCAR phenotypes during the acute allergic phase. **A**, A scatter dot plot of frequencies of drug-specific IFN- γ -releasing cells/ 10^6 PBMCs in 116 patients diagnosed with different phenotypes of severe cutaneous adverse reactions (16 AGEF, 50 DRESS, and 50 SJS/TEN) after stimulating PBMCs with the culprit drug compared with those in the nonallergic control group (nonallergic control group: frequencies of IFN- γ -releasing cells/ 10^6 PBMCs after incubating 67 drug panels [13 IRZE, 16 anticonvulsants, 11 oxypurinol, 18 cotrimoxazole, and 9 beta-lactams] with PBMCs harvested during the acute allergic phase from 62 patients with SCARs [28 DRESS, 28 SJS/TEN, and 6 AGEF] who developed SCARs from other different drugs). Each dot represents an individual patient. Groups of dots in circles are patients with detectable drug-specific IFN- γ -releasing cells (outliers not shown). **B**, Representative figures of drug-specific IFN- γ -releasing cells as demonstrated by the enzyme-linked immunospot assay in different SCAR phenotypes. (1) nonallergic irrelevant drug, (2) meropenem-induced AGEF, (3) phenytoin-induced DRESS, (4) antituberculosis agents (IRZE)-induced SJS. *AGEF*, Acute generalized exanthematous pustulosis; *DRESS*, drug reaction with eosinophilia and systemic symptoms; *PBMC*, peripheral blood mononuclear cell; *SCAR*, severe cutaneous adverse reaction; *SJS*, Stevens-Johnson syndrome; *TEN*, toxic epidermal necrolysis.

TABLE III. Factors influencing the detection of drug-specific IFN- γ -releasing cells during the acute allergic phase in SCARs

Influencing factors	Frequencies of drug-specific IFN- γ -releasing cells/ 10^6 PBMCs (mean, 95% confidence interval)	Percentage of positive IFN- γ ELISPOT assay†
SCAR phenotypes*		
AGEF	28.5 (–11.6 to 68.6)	25.0 (4/16)
DRESS	123.3 (42.5 to 204.2)*	38.0 (19/50)
SJS/TEN	79.6 (34.5 to 124.6)	36.0 (18/50)
Groups of culprit drugs*		
Anticonvulsants	170.8 (54.7 to 287.0)**	48.5 (16/33)
Allopurinol	45.0 (14.8 to 75.3)	34.5 (10/29)
Sulfonamides/sulfones	112.3 (–14.3–238.8)	31.3 (5/16)
Antituberculosis agents	91.9 (–15.9 to 199.6)	50.0 (7/14)
Beta-lactams	24.0 (–4.9 to 52.9)	16.7 (4/24)
Drug causality assessment based on Naranjo algorithm**		
Probable	119.3 (61.4 to 177.2)**	38.5 (30/78)
Possible	35.1 (14.3 to 55.9)	28.9 (11/38)
Total culprit drugs**	91.4 (51.5 to 131.3)**	35.3 (41/116)
Nonallergic control drugs	14.0 (–10.9 to 38.9)	1.5 (1/67)

AGEF, Acute generalized exanthematous pustulosis; *DRESS*, drug reaction with eosinophilia and systemic symptoms; *ELISPOT*, enzyme-linked immunospot; *PBMC*, peripheral blood mononuclear cell; *SCAR*, severe cutaneous adverse reaction; *SJS*, Stevens-Johnson syndrome; *TEN*, toxic epidermal necrolysis.

P* values < .05; *P* values < .01 compared with nonallergic control groups.

†Values greater than the upper limit of the 95% confidence interval for the mean frequencies of IFN- γ -releasing cells/ 10^6 PBMCs in patients with SCARs incubated with nonallergic control drugs.

who developed SCARs from other different drugs. The average frequencies of nonculprit drug-stimulated IFN- γ -releasing cells in these patients were 14.0 cells/ 10^6 PBMCs (95% confidence interval, –10.9 to 38.9).

Factors influencing the detection of drug-induced IFN- γ -releasing cells in SCARs

According to our study, high frequencies of drug-specific IFN- γ -releasing cells in patients with SCARs during the acute

symptomatic phase were significantly demonstrated in patients who suffered from DRESS phenotype, having anticonvulsants or the drugs belonging to the “probable” category based on the Naranjo algorithm scale, as the culprit drugs (Table III). Drug-induced DRESS yielded the highest positive IFN- γ responses, whereas drug-induced AGEF yielded the lowest. The average frequencies of drug-specific IFN- γ -releasing cells ranged from 24.0 to 170.8 cells/ 10^6 PBMCs depending on the culprit drug groups. The frequencies of drug-specific IFN- γ -releasing cells in

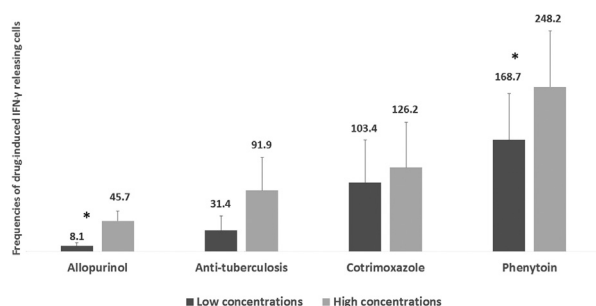


FIGURE 4. Dose-response of drug-specific IFN- γ -releasing cells for selected culprit drugs. Average frequencies of drug-specific IFN- γ -releasing cells/ 10^6 PBMCs at different concentrations of selected culprit drugs are shown. IFN- γ ELISpot responses were significantly higher on stimulation with high concentrations compared with low concentrations for allopurinol and phenytoin (P values $< .05$). *ELISpot*, Enzyme-linked immunospot; *PBMC*, peripheral blood mononuclear cell.

suspected culprit drugs belonging to the “probable” category were much higher than those that belonged to the “possible” category based on the Naranjo algorithm scale. Among the 4 most common drugs causing SCARs in our cohort, a dose-response relationship was observed in the IFN- γ ELISpot results for allopurinol and phenytoin, as shown in Figure 4 (P values $< .05$). The figure illustrating the relationship between the HLA-B risk alleles and frequencies of IFN- γ -releasing cells of allopurinol, carbamazepine, and phenytoin is included in this article’s Online Repository at www.jaci-inpractice.org (Figure E1).

DISCUSSION

In clinical practice, further use of any suspected drug-induced SCARs is not recommended because drug rechallenging might lead to fatal consequences. In a real-world situation, however, the avoidance of all suspected causative agents that patients used before SCAR development is not always possible. In seriously ill patients who have limited alternative drugs, identification of the causative agents may urgently be required before considering drug rechallenge: for instance, in epilepsy patients who developed SCARs while on multiple anticonvulsants, or in sepsis patients who developed SCARs while taking multiple antibiotics.

Pharmacogenomic screening and culprit drug identification are strategies for preventing drug-induced SCARs in a high-risk population and for reducing the risk of future reactions in SCAR victims. Several HLA-Bs at risk have been identified and screening of susceptible HLAs has been proven useful in reducing the prevalence of SCARs associated with certain drugs. However, data on the extent to which universal screening of currently known susceptible HLA-Bs could potentially help to reduce the SCAR burden in the real world are still lacking. This study showed that *HLA-B*1502* and *HLA-B*5801* were the most frequent HLA-B alleles in Thai SCAR subjects. The high prevalence of HLA alleles at risk of allopurinol and carbamazepine hypersensitivity in the Thai population, the accessibility of high-risk drugs as over-the-counter medicines, the high prevalence of tuberculosis, and the use of sulfonamides to prevent opportunistic infections in HIV-infected patients probably contributed to the high SCAR burden in the country.

Our study cohort confirmed previous reports that *HLA-B*1502* and *HLA-B*5801* screening could reduce carbamazepine- and allopurinol-related SCARs in high-risk populations. Cost-effective studies of HLA risk alleles have shown varying results depending on the proportion of HLA risk alleles and the set-forth quality-adjusted life years in each analytical model. In fact, there has been an economic evaluation of the cost utility of providing *HLA-B*1502* screening for all patients in the Thai setting, concluding that universal *HLA-B*1502* screening for preventing SJS/TEN is cost-effective in carbamazepine-treated patients with neuropathic pain in Thailand.²⁴ *HLA-B*5801* genotyping seems cost-effective in gout patients with renal insufficiency according to a study performed in South Korea,²⁵ but may not be cost-effective in gout patients before allopurinol prescription in Singapore.²⁶

Interestingly, not only most of the drug-related SJS/TEN cases, but about half of the drug-related DRESS cases with known HLA-B at risk could also have been avoided. Unfortunately, none of the drug-related AGEP cases were preventable. Ironically, although prescreening HLA-B has beneficial effects in reducing the SCAR burden from certain drugs, the results showed that the majority of SCAR victims could not be saved. The study pointed out that even in the Thai population, who carry high frequencies of high-risk HLA-B alleles, the implication of HLA-B screening in all subjects before drug administration could prevent only a minority of drug-related SCARs because the types of drug-inducing SCARs are too diverse. Altogether, less than a quarter of patients with SCARs would have been saved if HLA-B prescreenings had been performed in Thailand.

In reality, the diversity of SCAR phenotypes and suspected culprit drugs limits the ability of HLA-B screening to prevent SCARs. To combat this disadvantage, additional genetic markers to screen other drugs potentially causing SCARs such as sulfonamides, beta-lactams, antituberculosis, and analgesic drugs are urgently needed. At the current time, the screening of the HLA-B alleles other than those that are standard of care (with strong reproduced associations) is not recommended. Several genetic factors other than HLA-B also play a role in the pathogenesis of drug hypersensitivity reactions and are yet to be explored.²⁷ HLA class I haplotype diversity was observed in drug-induced patients with SCARs, but the immunopathogenetic relevance needs further exploration. A comprehensive analysis of other HLA class I and class II alleles, HLA haplotypes, and drug-metabolizing enzymes could yield additional valuable information to predict the risk of SCAR development. New pharmacogenomic markers with higher specificity are required because positive predictive values of currently available genetic markers are low, resulting in unnecessary drug avoidance in most subjects carrying those alleles at risk. To reduce the SCAR burden, novel biomarkers for predicting severe reactions from any drugs in general should also be explored. It is worth noting that prescreened pharmacogenomics may not be practical in urgent clinical conditions: for instance, before antibiotic administration in sepsis patients.

According to our study, drug-specific T cells were largely demonstrated when patients’ PBMCs were incubating with the suspected culprit drugs. There were only a few patients in whom control drugs could induce IFN- γ responses, and in those cases, the frequencies of IFN- γ -releasing cells were small whereas the frequencies of culprit drug-stimulated IFN- γ -releasing cells were much higher, indicating that IFN- γ -releasing cell responses were specific to culprit drug stimulation. Frequencies of drug-specific

IFN- γ -releasing cells were high in cases where the culprit drugs were anticonvulsants, sulfonamides, or antituberculosis agents. However, these results are based on the concentrations used in this study, so it is possible that different drug concentrations may not yield similar responses. Determining the accurate cutoff value for each particular drug group needs further analysis. Our *in vitro* data indicate that antituberculosis agents could be ones of the most common drugs-induced SCARs in tuberculosis endemic areas.

The results of this study suggest the possibility of employing the detection of drug-specific IFN- γ -releasing cells as a useful test to identify the culprit drug in subjects who developed SCARs after taking multiple suspected causative agents or as a guidance to select the safe drug for future use. However, diagnostic cutoff values yielding optimal sensitivity and specificity need to be clinically validated before implementing in routine allergy practice. Interestingly, higher detectable rates and frequencies of drug-induced IFN- γ release cells in patients with DRESS and SJS/TEN than in patients with AGEP were observed. False positive IFN- γ response in culprit drug identification could be due to enhanced activation of drug-specific T cells and lower threshold of T-cell reactivity to drugs in some patients with severe drug hypersensitivity syndrome during the acute drug allergic period.²⁸ The measurement of drug-specific IFN- γ -releasing cells in patients with SCARs later on during the recovery phase may reduce nonspecific IFN- γ response. Cytokines other than IFN- γ should also be explored as possible markers of drug-induced SCARs, especially in AGEP to increase sensitivity of the test.

To the best of our knowledge, this is the first study to demonstrate the potential values of HLA-B screening and the measurement of drug-specific IFN- γ -releasing cells for SCAR prevention and culprit drug identification in a prospective cohort of drug-induced SCAR subjects. However, the generalizability of the results in our study needs to be confirmed in patients of different genetic backgrounds such as in African and European populations who might have different distribution of causative drugs. More susceptible genes for drug allergy screening are required for better preventive coverage and diagnostic criteria to confirm the culprit drug by using the IFN- γ ELISpot assay needed to be established.

CONCLUSIONS

Anticonvulsants, allopurinol, and beta-lactams are the most common causes of drug-induced SCARs in Thailand. The majority of SCAR cases still cannot be avoided by HLA-B screening. The detection of drug-specific IFN- γ -releasing cells may have a role in confirming the culprit drug in problematic cases.

Acknowledgments

This study has been supported by The Skin and Allergy Research Unit, Chulalongkorn University and the National Research University Project, Office of Higher Education Commission (WCU-002-HR-57), and the Thailand Research Fund (RSA5880041). Study data were collected and managed using Research Electronic Data Capture (REDCap) hosted at HIV Netherlands Australia Thailand Research Collaboration.

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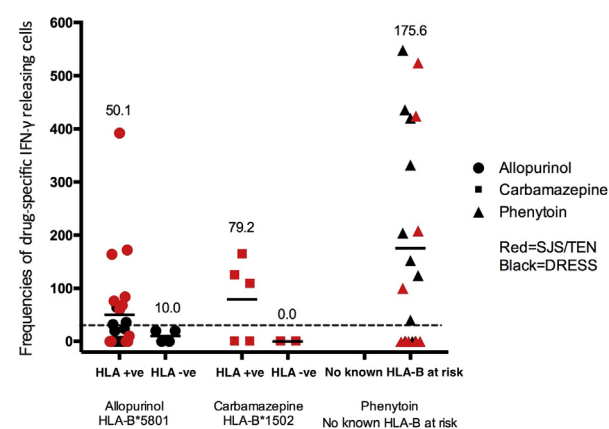


FIGURE E1. Frequencies of drug-specific IFN- γ -releasing cells/ 10^6 PBMCs of allopurinol, carbamazepine, and phenytoin are categorized by HLA-B risk alleles. Values above the dashed line indicate the positive IFN- γ ELISpot assay. There were trends that the absence of HLA-B risk alleles in patients with drug-induced SCARs was associated with low frequencies of drug-specific IFN- γ -releasing cells. Large-scale studies are needed to confirm our preliminary results. *ELISpot*, Enzyme-linked immunospot; *PBMC*, peripheral blood mononuclear cell; *SCAR*, severe cutaneous adverse reaction.

TABLE E1. Drug concentrations for the IFN- γ enzyme-linked immunospot assay

Drugs	Concentrations (µg/mL)		Remarks
Anticonvulsants			
Carbamazepine	20	100	
Lamotrigine	20	50	
Phenobarbital	20	100	
Phenytoin	20	100	
Valproate sodium	1	10	
Antituberculosis agents			
Isoniazid, rifampicin, pyrazinamide, and ethambutol combined	10	100	Concentrations of each drug
Beta-lactams			
Amoxicillin	100	500	
Amoxicillin/clavulanate	100	500	Amoxicillin concentrations
Cefazolin	40	200	
Cefoperazone/sulbactam	40	200	Cefoperazone concentrations
Ceftazidime	40	200	
Ceftriaxone	40	200	
Imipenem/cilastatin	40	200	Imipenem concentrations
Meropenem	40	200	
Penicillin G	100	500	
Piperacillin/tazobactam	40	200	Piperacillin concentrations
Allopurinol			
Oxypurinol	10	100	
Sulfonamides and sulfones			
Cotrimoxazole	40	200	Sulfamethoxazole concentrations
Dapsone	0.2	2	
Sulfasalazine	20	100	

TABLE E2. The frequencies of drug-induced IFN- γ -releasing cells on PBMC stimulation with suspected culprit drugs and irrelevant drugs in 62 patients with severe cutaneous adverse reactions

Patient no.	Diagnosis	Gender	Age (y)	Culprit drug tested	Culprit drug frequencies*	Irrelevant drug tested	Irrelevant drug frequencies*
1	DRESS	M	32	Oxypurinol	0	Phenytoin	0
2	SJS	M	68	Oxypurinol	132	Ceftriaxone	0
3	SJS/TEN	F	75	Cefazolin	0	IRZE	0
4	SJS	M	64	Carbamazepine	0	Cotrimoxazole	0
5	SJS	M	56	Meropenem	0	IRZE	0
6	DRESS	F	21	Phenobarbital	0	IRZE	0
7	SJS	F	33	Phenytoin	100	IRZE	36
8	SJS	M	67	Oxypurinol	392	Amoxicillin	0
9	SJS	F	40	Lamotrigine	0	Ceftriaxone, cotrimoxazole	0
10	DRESS	M	64	Phenytoin	1724	Oxypurinol	0
11	AGEP	F	76	Amoxicillin	300	Oxypurinol	0
12	SJS	F	30	Cotrimoxazole	0	Meropenem	0
13	SJS	F	40	Carbamazepine	124	Cotrimoxazole	0
14	SJS	M	52	Cotrimoxazole	152	IRZE	0
15	DRESS	M	65	Oxypurinol	0	Phenytoin	0
16	DRESS	F	31	IRZE	0	Oxypurinol	0
17	TEN	F	47	Cefoperazone/sulbactam	0	Oxypurinol	0
18	TEN	M	60	Phenytoin	424	IRZE	0
19	SJS	M	60	IRZE	168	Cotrimoxazole	0
20	DRESS	F	60	IRZE	190	Ceftriaxone, meropenem	0
21	DRESS	M	18	IRZE	0	Phenytoin	0
22	DRESS	F	66	Phenytoin	420	Cotrimoxazole	0
23	DRESS	F	62	Oxypurinol	0	Phenytoin	0
24	DRESS	F	73	Phenytoin	0	Cotrimoxazole	0
25	SJS	F	51	Carbamazepine	164	Phenytoin	0
26	DRESS	F	74	Phenytoin	548	Cotrimoxazole	0
27	SJS	F	32	Carbamazepine	108	Ceftriaxone	0
28	DRESS	F	54	Phenytoin	436	Cotrimoxazole	0
29	DRESS	F	54	IRZE	48	Phenytoin, oxypurinol	0
30	SJS	F	76	Oxypurinol	0	IRZE	0
31	SJS	F	73	Oxypurinol	0	Cefazolin	0
32	AGEP	F	35	Ceftriaxone	0	IRZE	836
33	DRESS	F	48	Valproate	0	IRZE	0
34	DRESS	F	37	Phenytoin	332	Cotrimoxazole	0
35	DRESS	M	93	Carbamazepine	0	Cotrimoxazole	0
36	DRESS	M	41	Cotrimoxazole	0	Oxypurinol	0
37	SJS	F	65	Cotrimoxazole	0	Oxypurinol	0
38	SJS	F	59	Phenytoin	0	Ceftriaxone	0
39	DRESS	F	45	Cotrimoxazole	552	IRZE	4
40	AGEP	F	89	Meropenem	0	Phenytoin	0
41	SJS/TEN	M	27	IRZE	0	Ceftriaxone	0
42	DRESS	F	19	Cotrimoxazole	0	Phenytoin	4
43	SJS/TEN	F	53	Oxypurinol	0	Cotrimoxazole	0
44	AGEP	M	61	Piperacillin/tazobactam	0	Cotrimoxazole	0
45	DRESS	F	38	Carbamazepine	0	IRZE	0
46	DRESS	F	57	IRZE	132	Phenytoin	0
47	DRESS	M	86	Phenytoin	40	IRZE, valproate	0
48	DRESS	F	54	Phenytoin	204	Cotrimoxazole	0
49	AGEP	F	34	Cefazolin	0	Phenytoin	0
50	AGEP	F	77	Piperacillin/tazobactam	0	Ceftriaxone	0
51	DRESS	F	46	Phenytoin	124	Valproate	0
52	DRESS	M	64	Dapsone	0	Oxypurinol	0

(continued)

TABLE E2. (Continued)

Patient no.	Diagnosis	Gender	Age (y)	Culprit drug tested	Culprit drug frequencies*	Irrelevant drug tested	Irrelevant drug frequencies*
53	DRESS	F	58	Phenytoin	0	Cotrimoxazole	0
54	SJS	F	31	Ceftriaxone	0	IRZE, phenytoin	0
55	SJS	F	48	Meropenem	0	Oxypurinol	0
56	DRESS	F	35	Phenobarbital	0	Oxypurinol	0
57	SJS	F	66	Amoxicillin	0	Phenytoin	0
58	SJS	F	73	Phenytoin	524	Cotrimoxazole	0
59	DRESS	F	37	Phenytoin	152	Oxypurinol	20
60	SJS	M	48	Cotrimoxazole	0	Phenytoin	0
61	SJS	M	30	Phenytoin	0	Cotrimoxazole	36
62	SJS	F	76	IRZE	0	Phenytoin	0

AGEP, Acute generalized exanthematous pustulosis; DRESS, drug reaction with eosinophilia and systemic symptoms; PBMC, peripheral blood mononuclear cell; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

*The frequencies of drug-induced IFN- γ -releasing cells/ 10^6 PBMCs.