


รายงานวิจัยฉบับสมบูรณ์

โครงการการปรับปรุงการทดสอบทางห้องปฏิบัติการเพื่อใช้วินิจฉัยการแพ้ยาแบบไม่เฉียบพลันชนิดรุนแรงในประเทศไทย

The optimization of in vitro test for the diagnosis of drug-induced severe cutaneous adverse reactions in Thailand

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สาขาวิชาโรคภูมิแพ้และภูมิคุ้มกันทางคลินิก ภาควิชาอายุรศาสตร์
คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ธันวาคม 2561

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย
และจุฬาลงกรณ์มหาวิทยาลัย

กิตติกรรมประกาศ

ขอขอบคุณ แพทย์ผู้ช่วยวิจัย แพทย์ประจำบ้าน และแพทย์ประจำบ้านต่อยอดสาขาวิชาตจวิทยาและสาขาวิชาโรคภูมิแพ้และภูมิคุ้มกันทางคลินิกของทุกโรงพยาบาลที่ช่วยให้การดูแลผู้ป่วยและรวบรวมข้อมูลผู้ป่วยที่อยู่ในโครงการวิจัย

ขอขอบคุณเจ้าหน้าที่ พยาบาล เภสัชกร นักเทคนิคการแพทย์และนักวิทยาศาสตร์ผู้เกี่ยวข้องที่ให้ความช่วยเหลือในการทำวิจัย และขอขอบคุณผู้ป่วยทุกท่านที่ให้ความร่วมมือในการทำวิจัยด้วยความเต็มใจ

โครงการวิจัยนี้ได้รับทุนอุดหนุนจากสำนักงานกองทุนสนับสนุนการวิจัยและจุฬาลงกรณ์มหาวิทยาลัย ประจำปีงบประมาณ 2557

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บทคัดย่อ

วัตถุประสงค์: การแพ้ยาแบบไม่เฉียบพลันชนิดรุนแรงเป็นปัญหาการแพ้ยาที่พบได้บ่อยในประเทศไทยและอาจถึงแก่ชีวิตได้ ในปัจจุบันยังไม่มีกรรมวิธีมาตรฐานทางห้องปฏิบัติการสำหรับใช้ในการวินิจฉัยชนิดของยาที่เป็นสาเหตุในผู้ที่แพ้ยาหลังจากได้รับยาหลายชนิด การศึกษานี้มีวัตถุประสงค์เพื่อหาวิธีทางห้องปฏิบัติการเพื่อใช้วินิจฉัยยืนยันชนิดของยาที่เป็นสาเหตุของผื่นแพ้ยาแบบไม่เฉียบพลันชนิดรุนแรง

วิธีทดลอง: เม็ดเลือดขาวของผู้ป่วยผื่นแพ้ยาแบบไม่เฉียบพลัน 3 ประเภท ได้แก่ acute generalized exanthematous pustulosis (AGEP), drug reaction with eosinophilia and systemic symptoms (DRESS), และ Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) ถูกกระตุ้นด้วยยาที่สงสัยว่าเป็นสาเหตุเพื่อตรวจวัดระดับของไซโตไคน์ที่สูงขึ้นในน้ำยาเลี้ยงเซลล์ และเลือกไซโตไคน์ชนิดดังกล่าวมาตรวจหาปริมาณของเซลล์ที่หลั่งไซโตไคน์ชนิดนั้นๆออกมาหลังการกระตุ้นด้วยยาที่แพ้ด้วยวิธี enzyme-linked Immunospot (ELISpot) และเติมสารกระตุ้นภูมิคุ้มกันในหลอดทดลองเพื่อเพิ่มความไวในการวินิจฉัยสาเหตุของผื่นแพ้ยาชนิดรุนแรงประเภทต่างๆ

ผลการทดลอง: ไซโตไคน์ 22 ชนิดได้รับการตรวจวัดในน้ำยาเพาะเลี้ยงเซลล์หลังกระตุ้นเม็ดเลือดขาวของผู้ป่วยที่แพ้ยาด้วยยาที่เป็นสาเหตุโดยการใช้ multiplex immunoassay technique และพบว่าระดับของ interferon-gamma (IFN-γ), interleukin (IL)-12p70, granzyme B, perforin, granulysin, และIL-27 เพิ่มสูงขึ้นหลังกระตุ้นเม็ดเลือดขาวในผื่นแพ้ยาชนิดต่างๆกัน ผลการตรวจ ELISpot พบว่าเซลล์ที่หลั่ง granzyme-B, IFN-γ, และ IL-22 ตรวจพบได้บ่อยใน DRESS, SJS และ AGEP ตามลำดับ การเติม adjuvants สามารถเพิ่มความไวของการตรวจพบเซลล์ที่หลั่งไซโตไคน์ชนิดต่าง ๆ หลังกระตุ้นด้วยยาในผู้ป่วยผื่นแพ้ยารุนแรง การตรวจวัดระดับของเซลล์ที่หลั่ง granzyme-B และ IFN-γ หลังกระตุ้นด้วยยาพร้อมด้วยการเติม anti-TIM3 อาจช่วยในการวินิจฉัยยาที่เป็นสาเหตุของ DRESS และ SJS/TEN ในขณะที่การตรวจวัดระดับของเซลล์ที่หลั่ง IL-22 หลังกระตุ้นด้วยยาอาจช่วยในการวินิจฉัยยาที่เป็นสาเหตุของ AGEP

สรุปและวิจารณ์ผลการทดลอง: ไซโตไคน์ที่เกี่ยวข้องกับผื่นแพ้ยาชนิดรุนแรงแตกต่างกันตามประเภทของการแพ้ยา การตรวจวัดปริมาณของเซลล์ที่หลั่ง granzyme-B, interferon-gamma, และ interleukin-22 หลังการกระตุ้นด้วยยามีประโยชน์ในการวินิจฉัยชนิดของยาที่เป็นสาเหตุของผื่นแพ้ยาชนิดรุนแรงประเภทต่าง ๆกัน

ข้อเสนอแนะสำหรับงานวิจัยในอนาคต: ต้องการการศึกษาในผู้ป่วยจำนวนมากเพื่อยืนยันคุณค่าทางคลินิกในการวินิจฉัยยืนยันชนิดของยาที่เป็นสาเหตุของผื่นแพ้ยาชนิดรุนแรง

Abstract

Objectives: Drug-induced severe cutaneous adverse reactions (SCARs) are common in Thailand and potential life-threatening. At present, the standard in vitro test to identify the culprit drug in patients who develop SCAR after taking multiple drugs is not yet available. This study was to explore the potential in vitro tests to identify the culprit drug in different phenotypes of SCARs.

Methods: SCAR patient's peripheral blood mononuclear cells (PMBCs) were stimulated with the suspected culprit drug and screened for heightened levels of multiple cytokines in culture media. The potential cytokines involved in 3 SCAR phenotypes; acute generalized exanthematous pustulosis (AGEP), drug reaction with eosinophilia and systemic symptoms (DRESS), and Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) were then measured by using enzyme-linked Immunospot (ELISpot) assay. The frequencies of culprit drug-induced cytokines of interested were further enhanced by the supplementation of different adjuvants to maximize test sensitivity for culprit drug confirmation in various phenotypes of SCARs.

Results: Twenty-two cytokines were screened in supernatants after incubating PBMCs with the suspected culprit drugs by using multiplex immunoassay technique. Levels of interferon-gamma (IFN-γ), interleukin (IL)-12p70, granzyme B, perforin, granulysin, and IL-27 were increased upon PBMC stimulation in different phenotypes of SCARs. According to ELISpot results, drug-induced granzyme-B, IFN-γ, and IL-22 releasing cells were predominantly detectable in DRESS, SJS, and AGEP, respectively. The supplementation of various adjuvants could enhance the detection of drug-induced cytokine releasing cells in SCARs. The measurement of drug-induced granzyme-B and IFN-γ releasing cells in the presence of anti-TIM3 supplementation could be helpful to confirm the diagnosis of drug-induced DRESS and SJS/TEN while the detection of drug-induced IL-22 releasing cells could identify the culprit drug in AGEP subjects.

Conclusions & Discussion: Predominant cytokines in various SCAR phenotypes are different. The measurement of drug-induced granzyme-B, interferon-gamma, and interleukin-22 releasing cells are beneficial to identify the culprit drugs in different phenotypes of drug-induced SCARs.

Further suggestions: Large-scale studies are required to determine the clinical diagnostic values for culprit drug identification in SCARs.

Keywords

Drug hypersensitivity, Enzyme-linked Immunospot, In vitro diagnostic test, Severe cutaneous adverse reactions,

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บทนำ

Drug-induced severe cutaneous adverse reactions (SCARs) are ones of the most serious side effects from drugs comprised of acute generalized exanthematous pustulosis (AGEP), drug rash with eosinophilia and systemic symptoms (DRESS), and Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN).(1)(2)(3) The prevalence of SCARs in Southeast Asia including Thailand is among the highest in the world.(4)(5) The reason behind this is unclear, probably from increased genetics at risk to certain drugs in our population and more importantly, widespread abuse of drug in the countries.

The problem of drug allergy diagnosis in Thailand is that patients often take multiple drugs simultaneously. As a result, it is sometimes difficult to identify the culprit drug and to point out drugs whose patients can still safely use in the future. Since drug provocation test is contraindicated in SCAR patients (6)(7) and the yield of available tests such as skin test is rather low, in vitro tests have a potential role to identify the culprit drug and guide the prescription of safe drugs in these patients.

Lymphocyte transformation test (LTT) is a conventional test for drug allergy diagnosis. However, sensitivity of this test is not yet satisfactory.(8)(9)(10) Enzyme-linked Immunospot Assay (ELISPOT) has recently been introduced to diagnose drug-induced non-immediate reactions and found to have better sensitivity than LTT.(11) Unfortunately, this test is not yet customized to diagnose SCARs since each SCAR phenotype has different immune pathogenesis. The purpose of this study is to analyze immunological responses of peripheral blood mononuclear cells upon stimulation with the culprit drug and explore potential markers to develop laboratory diagnosis for SCARs and to enhance immunological responses in vitro by customization of ELISPOT technique to increase sensitivity of the tests for identification of culprit drug in various SCAR types.

Literature review

Severe cutaneous adverse reactions (SCARs) are the most serious allergic reactions from drug administration in clinical practice. Common manifestations of SCARs in Thailand according to data from Food and Drug Administration Thailand (Thai FDA) are Stevens - Johnson syndrome (SJS)/Toxic Epidermal Necrolysis (TEN), Drug Rash with Eosinophilia and Systemic Symptoms (DRESS), and Acute Generalized Exanthematous Pustulosis (AGEP), respectively.(12) The prevalence of SCARs in Thailand is 2.2% of spontaneous adverse drug

reaction reports, which is among the highest in the world leading to high morbidity and mortality. The problem of avoidance potential allergenic drugs causing SCARs is that they are the treatment of choice in common diseases such as allopurinol (for hyperuricemia and gouty arthritis), aromatic anticonvulsants: phenytoin, carbamazepine, and phenobarbital (for epilepsy and seizure prevention), co-trimoxazole or trimethoprim/sulfamethoxazole (for pneumocystis carinii pneumonia prophylaxis and treatment), and beta-lactam antibiotics: ceftriaxone, amoxicillin, amoxicillin/clavulanate (for treatment of bacterial infections). As a result, it is difficult to completely avoid these drugs in clinical practice. Current knowledge on pharmacogenomics allows us to reduce risk of allergic reactions to certain drugs in Thai population by screening for HLA-B*1502 allele for carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis, HLA-B*5801 allele for allopurinol-induced Stevens-Johnson syndrome and toxic epidermal necrolysis, HLA-B*3505 allele or CCHCR1 allele for nevirapine-induced skin rash, and HLA-B*5701 allele for hypersensitivity to Abacavir.(13) However, the purpose of using these tests is to reduce patient's risk by screening subjects carrying the susceptible genes but they were not designed to identify the culprit drug in patients who already develop allergic reactions. The problem of drug allergy diagnosis in clinical practice is that clinical history is largely not reliable, skin test has low sensitivity and can aggravate allergic drug reaction in some cases, and drug provocation test, which is the gold standard for drug allergy diagnosis, is contraindicated in SCARs. (6) Therefore, in vitro diagnostic tests need to be developed to figure out the culprit drugs in patients whom severe cutaneous adverse reaction erupt while taking multiple potential allergenic drugs.

Traditionally, lymphocyte transformation test (LTT), a proliferation-based assay detecting drug-specific proliferation of sensitized T cells, has been used to evaluate non-immediate reactions to the culprit drugs for decades.(14)(15) The principle of LTT is to measure stimulation index (SI) of peripheral blood mono nuclear cells (PBMCs) after incubation with the suspected drugs. Drug causing allergic reaction will induce PBMC proliferation, which can be measured by using 3H-Thymidine incorporation assay.(16) The problem of LTT method lies to its low sensitivity in certain types of drug reactions. Sensitivity of LTT in toxic epidermal necrolysis is usually very low (<10%).(9) Besides, it is a time-consuming process as PBMC culture takes 5 days prior to 3H-Thymidine incorporation assay can be analyzed. Since this procedure requires working with radioisotopes, newer diagnostic modalities are gearing towards non-radioactive cell proliferation assay to reduce radio-hazard risk of working personnel, for example, using carboxyfluorescein succinimidyl ester (CFSE), bromodeoxyuridine (BrdU) labeling or some other new techniques.(17)(18)(19)(20)

The limitation of cell proliferation based-assay is very low frequencies of drug-specific T cells in peripheral blood. For instance, patients with a remote drug allergy history or having minor drug reaction, proliferation assay often yield negative results due to low numbers of drug-specific T cells in these patients. Furthermore, in some types of severe cutaneous adverse reactions such as Stevens-Johnson syndrome or toxic epidermal necrolysis, LTT can give a negative result (stimulation index is less than 2) since immune pathogenesis of SJS/TEN is related to cell-mediated cytotoxicity and should be measured for cell death markers, not those for cell proliferation.(21) Newer techniques targeting cytokine release that more promising to detect drug-specific T cells responses in peripheral blood than proliferation-based assay should be investigated.

Drug-induced SCARs is a major threat in Thailand since the prevalence is very high. It is well-known that SCAR patients who develop allergic reactions while taking multiple drugs suffer from psychological, medical, and economical problems if the culprit drug could not be identified. These patients have to avoid all drugs used prior to symptom development and need to take alternative drugs, which are not the first drug of choice, often less effective, almost always more expensive, while may give poorer therapeutic outcome.

Several factors need to be considered regarding drug allergy diagnostic test development, particularly for SCARs.

1. Multiple cytokine involvement and diverse phenotypes in drug-induced severe cutaneous adverse reactions
2. Simplicity, flexibility of the test and turn-around time for clinical use application
3. Sensitivity of the test: low frequency of drug-specific cytokine-releasing cells, drug allergenicity, and suppressor activity of regulatory T cells

Different levels of cytokine production from peripheral blood mononuclear cells (PBMCs) between patients with drug-allergic and drug-tolerant individuals were demonstrated upon stimulation with the responsible drug. A study demonstrated that levels of interleukin-2 (IL-2), IL-5, IL-13 and interferon-gamma (IFN-γ) secretion in response to the culprit drugs were significantly increased in patients with drug allergy compared to healthy controls.(22) As a result, the measurement of cytokine production from PBMCs incubated with the suspected drug by sensitive laboratory methods would have a potential to distinguish patients with actual drug allergy from drug-tolerant subjects.

However, it is noteworthy that severe cutaneous adverse reactions are comprised of 3 main phenotypes; drug rash with eosinophilia and systemic symptoms (DRESS), Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN), and acute generalized

exanthematous pustulosis (AGEP). Different histopathologies have been demonstrated in each SCAR phenotypes.(23) Other than lymphocytic infiltration, eosinophil infiltration in the dermis is a prominent feature in DRESS, which is may be secondary to IL-5 release from drug-specific T cells.(24)(25) Pustular eruptions in patients with AGEP are often found in sub/intracorneal (41%), intraepidermal (20%), or combined (38%) of cases along with dermal neutrophils and/or eosinophils.(26). The prominent finding of neutrophil infiltration suggests that cytokines modulating neutrophil responses, such as IL-8, IL-17, and IL-22, might play a role in AGEP.(27)(28) Stevens-Johnson syndrome and toxic epidermal necrolysis, on the other hand, demonstrate extensive epidermal cell death in addition to cellular infiltration. Interestingly, granulysin was found to be a key mediator responsible for keratinocyte death in SJS/TEN.(29)(30) Our group and our collaborators in Korea recently observed the elevation of intracellular granulysin in natural killer cells after incubating PBMCs with lamotrigine for 4 days in patients who developed lamotrigine-induced lamotrigine (data in press). This collective data indicate that several mediators and cytokines from different cell types contribute to heterogeneous SCAR manifestations. Therefore, different panels of multiple cytokines may be required to increase sensitivity of the test in diverse phenotypes of SCAR patients. Future comprehensive studies are mandatory to assess which combination of cytokines is the most appropriate for drug allergy diagnosis.

Several laboratory techniques can be employed to detect heterogeneous cytokine responses. Each technique, however, has its own limitation. Although the detection of cytokines release in supernatant after stimulation with the suspected drugs by using enzyme-linked immunosorbent assay (ELISA) is possible, this technique is not suitable for the urgent use to identify the culprit drug in SCARs. Since these patients come to hospital one-by-one and urgently need to discontinue the culprit drug and switch to the safe alternative drug, they cannot wait for collecting multiple specimens before analysis. The suitable technique for this purpose, therefore, needs to be individually processed and customized based on SCAR phenotype in each patient. Other than ELISA, flow cytometric analysis or fluorescence activating cell sorting (FACS), particularly intracellular staining technique is another tool capable of detecting intracellular cytokine production and has ability to identify antigen-specific T lymphocytes at the single cell level. The ability to detect intracellular cytokine expression following antigen-specific stimuli could provide subset analysis of low frequency drug-specific T cells and give new insight into the immune pathomechanisms of allergic drug responses. Phenotypic analysis of cytokine-releasing cells can easily be determined by the staining of specific surface markers without the need to purify the cell types of interest.(31) The advantage of flow cytometry over ELISA is that it can be performed on an individual basis if needed, however, well-trained personals are

required to operate the machine and skill in color compensation strongly needed for proper data interpretation. Moreover, sensitivity of FACS may be not sufficient to identify the culprit drug in some cases since the frequency of drug-specific T cells is usually very low (less than 0.01%).(32)

Keystone Studies of Enzyme-Linked Immunospot (ELISPOT) Assay in Drug Allergy Diagnosis

The enzyme-linked immunospot (ELISPOT) assay is one of the most sensitive ex vivo techniques to analyze low-frequency antigen-specific, cytokine-producing cells in peripheral blood. Prior to the application of ELISPOT to diagnose drug reaction, there were reports that numbers of IFN-γ releasing cells detected with ELISPOT after incubating nickel sulfate or chromium chloride with PBMCs were much higher in patients who allergic to nickel or chromium compared to non-allergic subjects.(33)(34) There were evidence that a frequency of penicillin-specific $CD4^+$ T lymphocytes in healthy donors was very low (0.29 cells per million of CD4+ T cells).(35) In contrast, amoxycillin-specific circulating T cells were detected by the measurement of IFN-γ releasing cells with ELISPOT in patients who developed amoxicillin-induced maculopapular exanthems with frequencies ranging from 1: 8000 to 1: 30,000 circulating leucocytes (11) This study was an evidence that the measurement of IFN-γ producing cells with ELISPOT has higher sensitivity than LTT and is very helpful to diagnose amoxicillin allergy. By using technique ELISPOT, it was shown that drug-specific cytokine-releasing cells can be observed for many years after strict avoidance of the culprit drug.(35) Following this study, our group applied ELISPOT technique to diagnose patients with cephalosporin-induced maculopapular rash by measuring IFN-gamma (IFN-γ), interleukin 5 (IL-5), and interleukin 10 (IL-10) after stimulating PBMCs with culprit drugs and found that the combination of IFN-γ and IL-5 increased the sensitivity of the test. The frequencies of drug-specific circulating leukocytes in patients with positive ELISPOT in our study ranged from 26 to 86 SFC/10^6 PBMC for IFN-γ-secreting cells and 2 to 194 SFC/10^6 PBMC for IL-5-secreting cells and we demonstrated that ELISPOT assay has much better sensitivity than skin tests to diagnose cephalosporin allergy.(32) Besides simple drug allergy, it might be possible to extend ELISPOT use to diagnose drug-induced severe cutaneous reactions as well. There was a study shown that granzyme B ELISPOT, along with other in vitro tests, could be used to detect drug-specific cytotoxic cells in various types of drug-induced skin diseases (35). By analyzing with LTT, IFN-γ/IL-13 ELISPOT, and drug-specific T cell clones, no cross-reactivity among beta-lactams was observed.(36) Recent collective data demonstrated that ELISPOT can be applied to identify the

culprit drugs other than beta-lactams such as carbamazepine and abacavir hypersensitivity as well.(37)(38)

The ELISPOT technique is currently used to characterize T cell responses to drugs.(39) Besides mild maculopapular exanthems, recent papers reported the possibility of using ELISPOT to diagnose drug-induced severe cutaneous adverse reactions such as DRESS, SJS, and TEN.(40)(41) It was evidence that drug-specific IFN-γ response could be sustained over several years and suggest that patients should avoid causal drug re-exposure after the recovery of TEN and SJS.(41) Several case reports and case series confirmed positive ELISPOT response in SCAR patients leading to the possibility of using this technique to diagnose culprit drugs in SCAR. At present, however, no study systematically analyzes the diagnostic values of this test (drug-specific cytokine-releasing cells measured by ELISPOT) in SCAR diagnosis. Low prevalence of SCAR in other countries may result in not enough cases to pursue the study and most wealthy countries prefer to avoid all possible culprit drugs rather than identify the exact cause. In Thailand, the prevalence of SCARs is very high and the access to expensive alternative drugs is limited making the investigation of the culprit drug for SCAR more possible and cost-effective. We recently published a case report demonstrated that IFN-γ measurement with ELISPOT was beneficial to confirm diagnosis of sulfasalazine-induced hypersensitivity syndrome (sulfasalazine-induced DRESS).(42) Similar to flow cytometry, ELISPOT procedure is less time-consuming that LTT and can be performed on an individual basis if result is urgently needed while demanding less skill than FACS in terms of data interpretation. All together leads us to explore the possibility of using this technique to identify the culprit drugs in all types of SCARs in Thai patients.

Previous works on drug allergy by using ELISPOT were largely focused on drugs-induced mild non-immediate reactions. A few available works on drug-induced severe cutaneous adverse reactions were mostly in the form of case reports. Due to the rarity of SCAR patients worldwide, the possibility of using this technique for the diagnosis of SCARs is currently limited as the differences of drug-specific cytokine-releasing cells between drug-tolerant subjects and drug-allergic subjects have not been established. Since the prevalence of SCARs is very high in Thailand and this problem creates an enormous impact on the country's public health and patient's quality of life, this project will systematically study the frequencies of drug-specific cytokine-releasing cells in SCAR patients and drug-tolerant subjects based on ELISPOT technique and try to enhance diagnostic values of the test in order to identify the culprit drug in SCAR patients.

This project will evaluate the most appropriate cytokine panel to diagnose 3 major SCAR phenotypes in Thailand (AGEP, DRESS, and SJS/TEN) with ELISPOT assay, and

enhance sensitivity of the test by suppressing activity of regulatory T cells in PBMC culture system

Suppressive activity of regulatory T cells and allergenicity of the drug can influent sensitivity of the test. It is also possible that numbers of drug-specific Treg cells may be different between drug allergic and drug tolerant individuals. Not only in patients with a remote history of drug allergy history whose numbers of drug-specific T cells are reasonably low, in acute phase of allergic drug reaction, there is evidence that some DRESS patients have an enhanced activity of regulatory T cells (Treg) resulting in the difficulty in the detection of drug-specific effector cell responses.(43)(44) Procedures to counteract Treg activity may be useful to enhance cytokine response of drug-specific T cells. A few studies have demonstrated a beneficial effect of suppressing T reg function in vitro by adding antibodies to Treg co-stimulatory molecules [anti-CD134 (OX40), anti-PD-L1, anti-PD-L2 & anti-CTLA4] in order to boost immunological responses.(45)(46)(47)(48)(49) This approach is more promising for clinical use than Treg depletion with magnetic beads since it is less time-consuming and more practical for routine laboratory service.

As the prevalence of SCARs in Thailand is very high, the development of in vitro system to evaluate the cause of drug-induced severe cutaneous adverse reactions will be beneficial to identify the culprit drug in patients whose symptoms develop while taking multiple drugs, to guide physicians to select safe alternative drug for further use, and reduce economical expense to patients and the country from avoiding all drugs and switching to more expensive drug without any confirmation. The purpose of our study is to develop in vitro system for drug allergy diagnosis based on ELISPOT technique. Appropriate cytokines for each SCAR phenotypes will be selected, the supplementation of blocking antibodies to Treg will be optimized to see whether this approach can improve sensitivity of the test by enhancing drug allergenicity.

Preliminary data on in vitro diagnosis for drug allergy conducted by our group.

Our group has been working on diagnostic tests to identify the culprit drug and confirm diagnosis in patients with a history of drug-induced immediate and non-immediate reactions. In drug-induced immediate reactions, we reported that minor determinants of penicillin play a major role in patients with a history of penicillin allergy in Thailand.(50) We also demonstrated that basophil activation test is a useful test to confirm diagnosis of NSAID allergy (51) and allergic reaction to radiocontrast media.(52) However, drug-induced non-immediate reactions are the main clinical manifestation in Thailand according to THAI FDA report and the most severe forms of drug-induced non-immediate reactions in THAILAND are SCARS (AGEP,

DRESS, and SJS/TEN). Since drug re-challenge in these patients can be fatal and contraindicated, in vitro diagnostic tests are needed to confirm drug allergy diagnosis and identify the culprit drug in these patients. The problems of in vitro test diagnosis for drug allergy is that numbers of drug-specific T cells are very small, therefore, available tests at that time were not sensitive enough to detect that small amounts of drug-specific T cells until recently. At present, enzyme-linked immunospot assay (ELISPOT) is a sensitive technique capable to detect small numbers of antigen-specific T cells in peripheral blood, as a result, we aim to improvise ELISPOT as a tool to study drug allergy. Our study demonstrated that frequency of cephalosporins-induced maculopapular exanthems (MPE) were about cells/million peripheral blood mononuclear cells and IFN-gamma and IL-5 ELISPOT are beneficial to diagnose cephalosporins-induce MPE.(32) We also reported the advantage of using ELISPOT to identify the culprit drug in patient who developed drug hypersensitivity syndrome while taking multiple drugs.(42) We and our Korean collaborators recently demonstrated the roles of granulysin and IFN-gamma measurement in confirm diagnosis of drug-induced SCAR (TEN).(53) Our data focusing on the improvement of in vitro test for diagnosis of allopurinol-induced severe cutaneous adverse reactions will be presented in The European Academy of Allergy and Clinical Immunology Congress 2015 in Barcelona this coming June (Appendix I). The measurement of drug-specific interferon-gamma releasing cells are now part of our allergy consultation in King Chulalongkorn Memorial Hospital and our retrospective review on the clinical values of interferon-gamma ELISPOT for management of antibiotic hypersensitivity in hospitalized patients has been submitted for presenting in World Allergy Congress 2015 at Seoul, South Korea this October 2015 (Appendix II). We have started collecting blood specimens from SCAR patients from six major university hospitals in Thailand for a year and able to detect a significant number of drug-specific cytokine-releasing cells in these SCAR patients, while they are almost undetectable in patients who can safety receive the same drug. Therefore, we are confidence that the measurement of drug-specific cytokine-releasing cells in patients is helpful to distinguish drug-allergic subjects from drug-tolerant subjects, which will be very beneficial to apply this knowledge for better management of patients suffer from drug-induced severe cutaneous adverse reactions in Thailand in the near future.

Objectives

The objectives of this study are three folds:

1) To analyze the alteration of in vitro immunological responses after stimulating peripheral blood mononuclear cells with drugs commonly caused severe non-immediate hypersensitivity reactions in Thailand compared to baseline in drug allergic subjects
2) To identify potential in vitro parameters and enhance immune responses after stimulating peripheral blood mononuclear cells with the culprit drugs
3) To explore the appropriate immunological markers and optimization of in vitro techniques to identify the culprit drug in patients with drug-induced severe non-immediate reactions and to guide the prescription of safe alternative drug

วิธีการทดลอง

Methodology

Study design: Experimental study

Target population

Patients diagnosed with drug-induced severe cutaneous adverse reactions [SJS/TEN, DRESS, and AGEP] in Thailand

Sampled population

1. Study population: Patients diagnosed with SCAR who are registered in Thailand severe cutaneous adverse reactions (THAISCAR) project, recruiting from 6 university hospitals [King Chulalongkorn Memorial Hospital, Siriraj Hospital, Ramathibodi Hospital, Thammasat Hospital, Chiangmai University Hospital, and Phamongkutklao Hospital).
2. Control population: Subjects who can receive drugs of interests without adverse reactions are patients admitted in King Chulalongkorn Memorial Hospital and show no adverse reaction after taking drugs of interests will be asked to be negative control subjects. They will have their blood drawn to harvest PBMCs after giving informed consent.

Specimens selected for this study

For the purpose to develop in vitro diagnosis of drug allergy: specimens selected for this study will be limited to peripheral blood mononuclear cells collected from verified patients recruited from THAISCAR registry who are allergic to four most common groups of drugs responsible for SCARS in Thailand based on REGISCAR criteria [allopurinol, aromatic anticonvulsants (phenytoin, phenobarbital, carbamazepine), co-trimoxazole, and beta-lactams (amoxicillin, amoxicillin/clavulanate, ceftriaxone)/NSAIDs] according to data from Thai FDA (2008 - 2012) as follow;

SJS/TEN: anticonvulsants (carbamazepine/phenytoin/ phenobarbital), allopurinol, co-trimoxazole
DRESS: anticonvulsants (carbamazepine/phenytoin/ phenobarbital), allopurinol, co-trimoxazole
AGEP: beta-lactams (ceftriaxone, amoxicillin/clavulanate, amoxicillin, sulperazone, meropenem), NSAIDs (ibuprofen)

Cell preparation and stimulation

Peripheral blood mononuclear cells (PBMCs) will be prepared from 20 ml of ACD-blood drawn by density gradient separation with Isoprep. Cells from the interface will be washed in RPMI1640 medium containing 2 mM L-Glutamine and resuspended at a final concentration of $2.5*10^6$ cells/ml in RPMI1640 supplemented with 100 U/ml of penicillin, 100 U/ml of streptomycin and 10% heat-inactivated fetal bovine serum. PBMCs will be counted and checked for viability before further incubation with the study drugs (culprit drug or drug that patient can tolerate).

Biomarker analysis in cell culture supernatant with multiplex immunoassay system

PBMCs ($2.5*10^5$ in 100 µl) will be incubated at 37ºC in 5% CO2 in the presence of allergic drug or tolerated drug, respectively. After 3 days, 50 microliters of each cell culture supernatant sample will be analyzed using a Multiplex immunoassay (human Th1/Th2/Th9/ Th17/Th22/Treg Cytokine Panel in combination with ProcartaPlex Human Granzyme B, Perforin, and Granulysin simplexes from Affymetrix; eBioscience, San Diego, California). Cytokines in test samples and recombinant standards bound to capture beads will be detected for GM-CSF, IFN γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-17A, IL-18, IL-21, IL-22, IL-23, IL-27, TNFα, Granzyme B, Perforin, and Granulysin and will be measured with Luminex® 200™ (Luminexcorp, Austin, Texus) before analysis of the data with ProcartaPlex Analyst 1.0 software.

Quantitative analysis of numbers of drug-specific regulatory T cells in SCAR subjects

PBMCs from patients with drug-induced SCAR will be co-cultured with the study drug for 2 days. At baseline and on day 2 after incubation, flow cytometric measurement of surface and intracellular staining in PBMCs will be performed according to our previous study.(54) Anti-CD4 FITC, and anti-CD25 APC will be purchased from BD Bioscience (San Jose, CA, USA). Anti-FOXP3 PE and PE-conjugated Rat IgG2a isotype control will be purchased from

eBioscience (San Diego, CA, USA). In brief, 1×10^6 PBMCs will be stained for the surface markers, anti-CD4 FITC/anti-CD8-PerCP/anti-CD25-APC, and incubated for 30 min at 4 °C in the dark and washed in a cold staining buffer. Cell permeabilization will then be performed for 45 min with fixation/permeabilization buffer (eBioscience, San Diego, CA, USA) at 4 °C in the dark. After washing twice with the permeabilization buffer, the cells will be stained with PE-conjugated anti-human Foxp3 (10 μl/test) for 30 min at 4 °C. Finally, the cells will be washed once with a permeabilization buffer and resuspended in 1 % paraformaldehyde before being analyzed on a FACSCalibur flow cytometer with CellQuestPro software (Becton–Dickinson, San Jose, CA, USA) for the percentages of $CD4^+$, and $CD4^+CD25^+FoxP3^+$ cells.

Detection of distinctive biomarkers in each SCAR phenotype with ELISPOT

Cytokines that significantly increase in SJS/TEN, DRESS, and AGEP will be further tested with ELISPOT whether the differences will be clearly seen between stimulation with the culprit drug and stimulation with one of the other drugs mentioned above that patients can tolerate. For example, PBMCs from patient who develop SCAR from allopurinol will be stimulated with allopurinol and may be stimulated phenytoin (n case patient can tolerate phenytoin) while PBMCs from patient who develop SCAR from co-trimoxazole will be stimulated with co-trimoxazole and may also be stimulated with allopurinol (in case patient can tolerate allopurinol). Types of tolerated drugs for PBMC stimulation in each patient will be selected to make allopurinol, aromatic anticonvulsants, co-trimoxazole, and beta-lactams evenly distributed in all patients as control drugs.

The predicted suitable combined biomarkers for each phenotype of SCAR (for now) are IFN-γ and IL-5 for DRESS, IFN-γ and IL-17 for AGEP, and IFN-γ and granulysin for SJS/TEN, respectively. The numbers of IFN-γ-, IL-5-, IL-17-, and granulysin- releasing cells will subsequently be determined using ELISPOT assay. Briefly, 96-well nitrocellulose membrane plates will be coated for 16 hours at 4ºC with 5 μg/ml of anti-IFN-γ antibody, (or anti-IL-5 antibody, or anti-IL-17 antibody, or anti-granulysin antibody) and blocked with R10 for1 hour at room temperature. PBMCs ($2.5*10^5$ in 100 μl) will be incubated for 48 hours at 37ºC in 5% CO2 in the presence of culprit drug or tolerable drug, respectively. Plates will be washed six times with PBS/Tween 0.05% and incubated for 1.5 hours at 37ºC with a biotinylated anti-IFN-γ antibody (or anti-IL-5 antibody, or anti-IL-17 antibody, or anti-granulysin antibody) and then extensively washed. IFN-γ-, IL-5-, IL-17- , and granulysin spot forming cells (SFCs) will be developed using streptavidin-alkaline phosphatase, incubated for 1 hour at 37ºC, and extensively washed before adding the substrate (5-bromo-4-chloro-3-indolylphosphate and nitro blue tetrazolium). Phytohaemagglutinin (PHA) 10 μg/ml will be used as a positive control in all

samples. The numbers of IFN-γ-, IL-5-, IL-10-, and granulysin SFCs present in each well will be counted using the ELISPOT reader. Results of the IFN-γ, IL-5, IL-17, and granulysin ELISPOT assay will be expressed as the numbers of IFN-γ, IL-5, IL-17, and granulysin SFC/10^6 PBMC cultured with the drug subtracted by the nonspecific background values obtained from PBMC cultured without the drug.

However, a final set of cytokines actually selected as distinctive biomarkers for ELISPOT development will be based on the result of multiple immunoassay mentioned above and the availability of ELISPOT cytokine kits.

Improvement of ELISPOT response by adding antibodies to Treg co-stimulatory molecules

Sets of biomarkers potentially used to identify the culprit drug from the ELISPOT experiments mentioned above will be selected to test immunological enhancement in vitro by adding anti-PD-L1 (10 mcg/ml), anti-Tim-3 (7.5 mcg/ml), anti-CTLA4 (10 mcg/ml), or alpha-GalCer (100 ng/ml) along with the culprit drug and PBMCs while performing ELISPOT. Types of antibodies added into the system will be tested to reach maximum ELISPOT response compared to PBMCs incubation with the culprit drug alone.

The experiment results will be reported as appropriate set of biomarker panels and blocking antibodies for the identification of drug-induced SCARs whether they can maximize in vitro immune response to the culprit drug. The results will be categorized according to different SCAR phenotypes (DRESS, SJS/TEN, and AGEP).

Outcomes after the end of the project and the application to patient management in clinical practice

In summary, novel information obtained from this study will be the comparatively analyzed data on the frequencies of drug-specific cytokine-releasing cells in drug-induced SCAR patients versus drug-tolerant patients. The optimization of ELISPOT technique will be performed to set drug-allergic-and drug-tolerant subjects apart.

After optimization of ELISPOT technique, this project will help clinicians (allergists, dermatologists, pharmacists) for the management of patients suffer from severe cutaneous adverse reactions in three main aspects.

1). To confirm drug-induced SCAR in patients presenting with clinical SCAR but other causes of SCAR other than drug in origin (such as infectious-related SCAR) could not be excluded.

2). To identify the culprit drug in patients who develop SCAR while taking multiple drugs

3). To use the frequencies of drug-specific cytokine-releasing cells as a guidance to minimize recurrent SCAR risk before prescribing potentially cross-reactive drugs in patients who already develops drug-induced SCAR

ผลการทดลอง

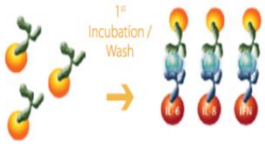
Table 1. SCAR patients (AGEP, DRESS, SJS/TEN) screening for cytokines released upon PBMC stimulation with the suspected culprit drugs and non-allergic control drugs

Phenotypes	Sex	Age	Culprit drugs	Culprit drugs	Control drugs
AGEP	F	44	Amoxicillin	Amoxicillin	NA
AGEP	F	26	Ibuprofen	Ibuprofen	NA
AGEP	M	65	Meropenem	Meropenem	NA
AGEP	F	97	Sulperazone	Sulperazone	NA
AGEP	F	68	Meropenem	Meropenem	NA
DRESS	F	27	Phenobarbital	Phenobarbital	Oxypurinol
DRESS	F	47	Phenytoin	Phenytoin	Oxypurinol
DRESS	F	68	Phenytoin	Phenytoin	Oxypurinol
DRESS	F	43	Allopurinol	Oxypurinol	Phenytoin
DRESS	F	74	Allopurinol	Oxypurinol	Phenytoin
SJS	F	42	Phenytoin	Phenytoin	Oxypurinol
TEN	F	72	Allopurinol	Oxypurinol	Phenytoin
SJS	M	35	Carbamazepine	Carbamazepine	Oxypurinol
SJS	F	58	Allopurinol	Oxypurinol	Phenytoin
SJS	M	67	Allopurinol	Oxypurinol	Phenytoin

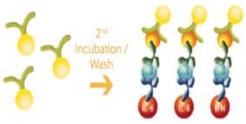
Figure 1. Cytokine measurement after stimulating PBMCs with the culprit drugs and non-allergic control drugs for 72 hours by using multiplex immunoassay (human Th1/Th2/Th9/ Th17/Th22/Treg Cytokine Panel and ProcartaPlex Human Granzyme B, Perforin, and Granulysin simplexes)

1. Incubate Antibody coated beads

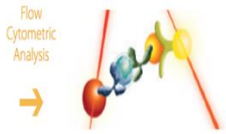
2. Beads bind with specific cytokines



3. Add biotin with cytokine-specific Ab



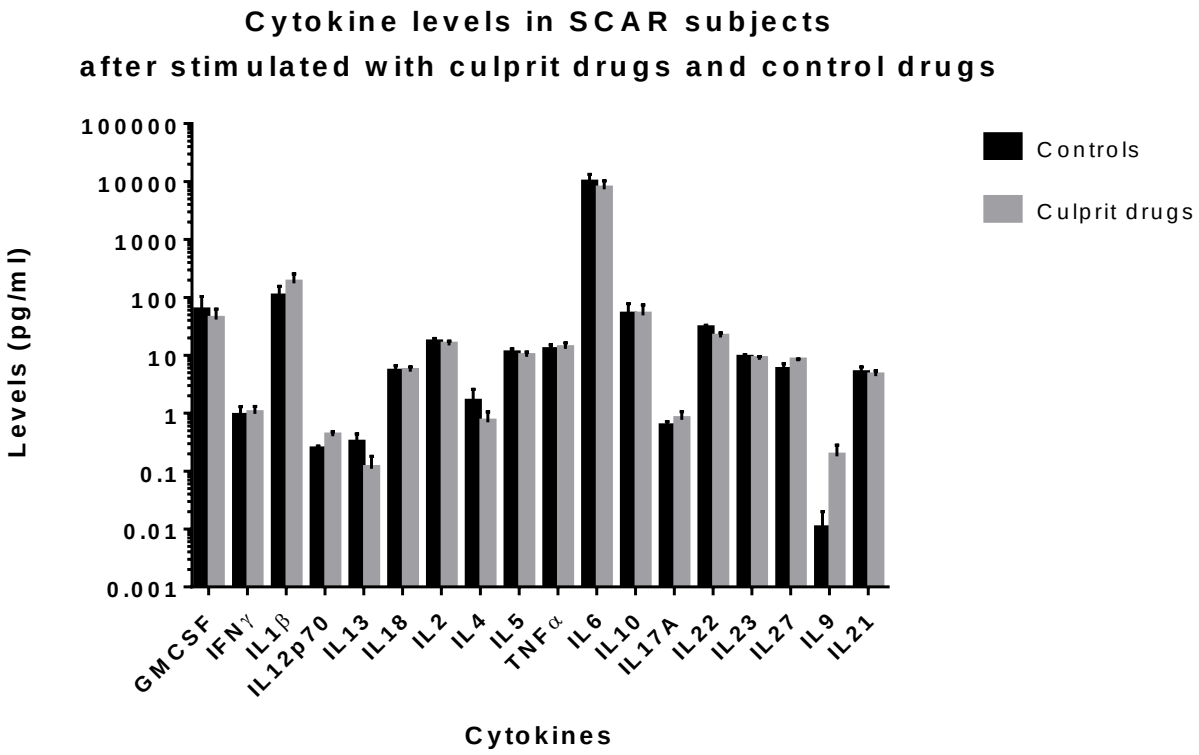
4. Add streptavidin labelled with PE fluorescent



5. Analyze with flow cytometer

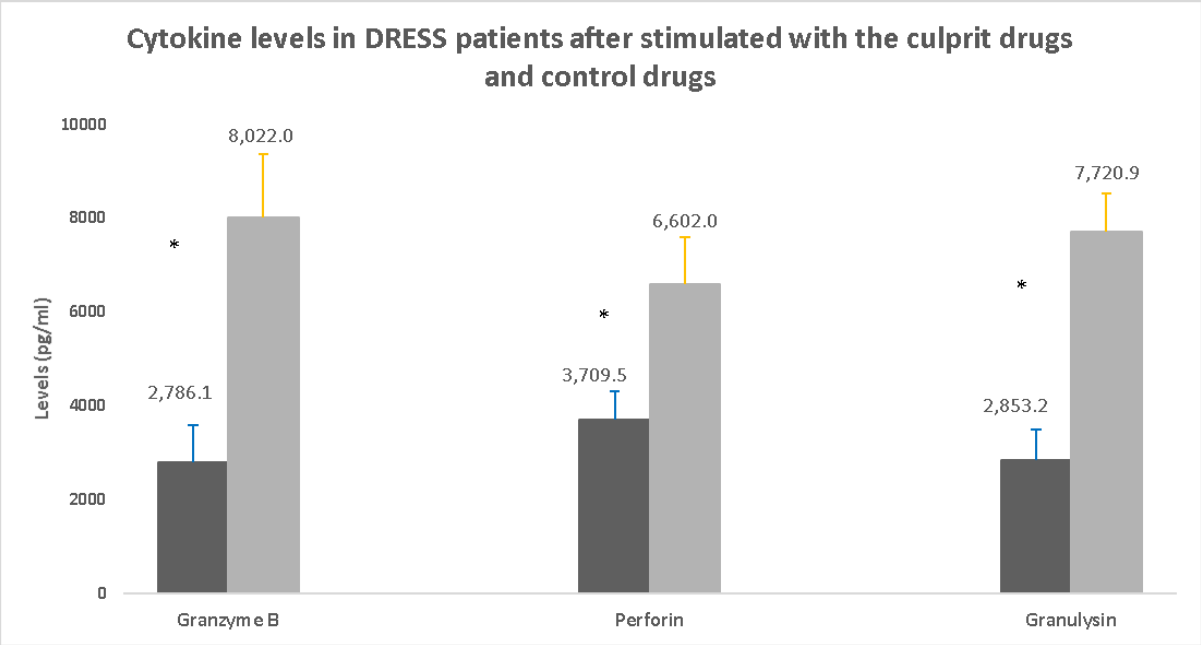
Figure 2. Cytokines released from PBMCs in culture media after stimulation with culprit drugs compared to control drugs in SCAR subjects

Figure 2A. Average cytokine levels in all SCAR subjects measured with multiplex immunoassay (human Th1/Th2/Th9/ Th17/Th22/Treg Cytokine Panel)



- Most of released cytokine levels in culture media were found comparable upon PBMC stimulation with either the culprit drugs or control drugs based on the results of multiplex immunoassay.

Figure 2B. Average cytokine levels in DRESS subjects measured with ProcartaPlex Human Granzyme B, Perforin, and Granulysin simplexes



- Granzyme B, perforin, and granulysin levels were significantly higher in DRESS after stimulating PBMCs with the culprit drugs alone compared to stimulating PBMCs with control drugs.
- According to the screening cytokine results in culture media, it was potentially possible to identify the culprit drugs in patients who suffer from SCAR even during acute stage by measuring appropriate cytokines released from PBMCs upon stimulation with the suspected drugs, particularly the measurement of drug-inducing granzyme B in DRESS subjects.

Figure 3. Strategy gating of regulatory T cells by flow cytometric analysis

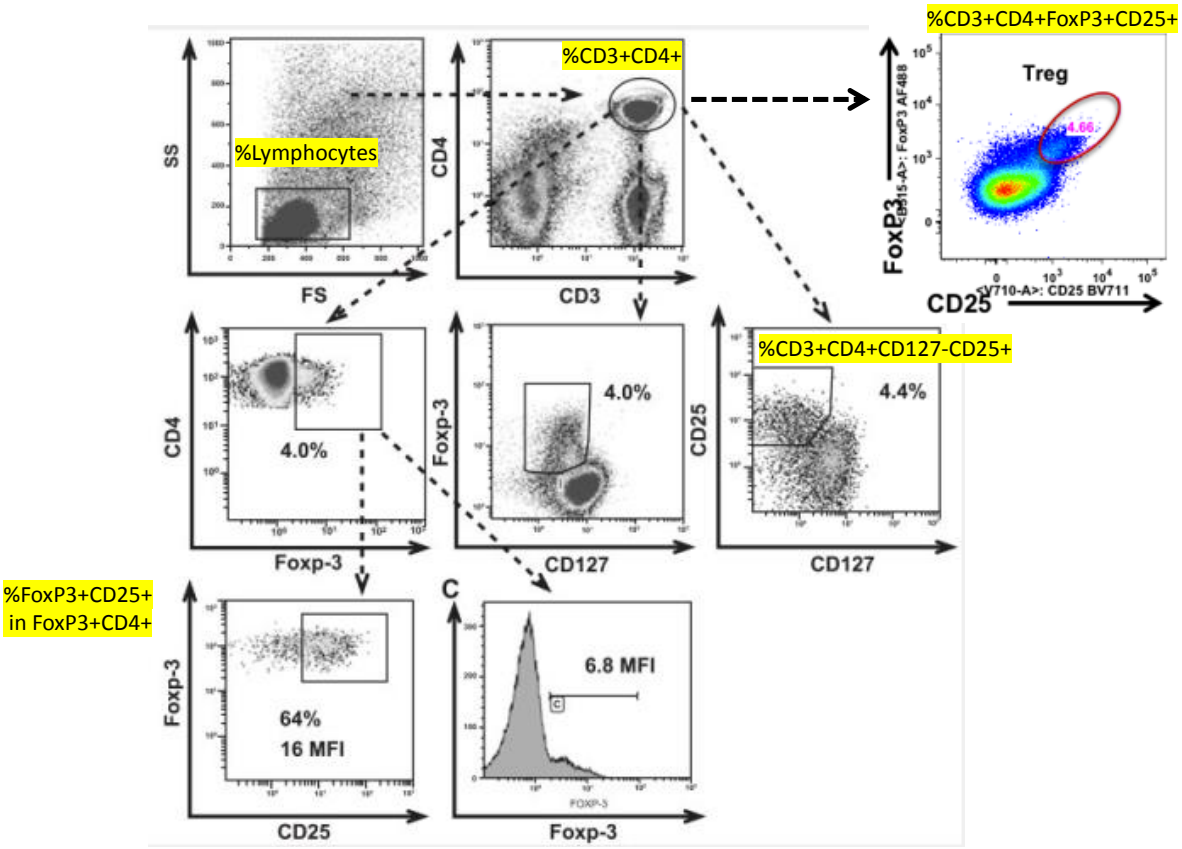


Figure 3.1 Regulatory T cells at baseline (Day 0) prior to culprit drug stimulation

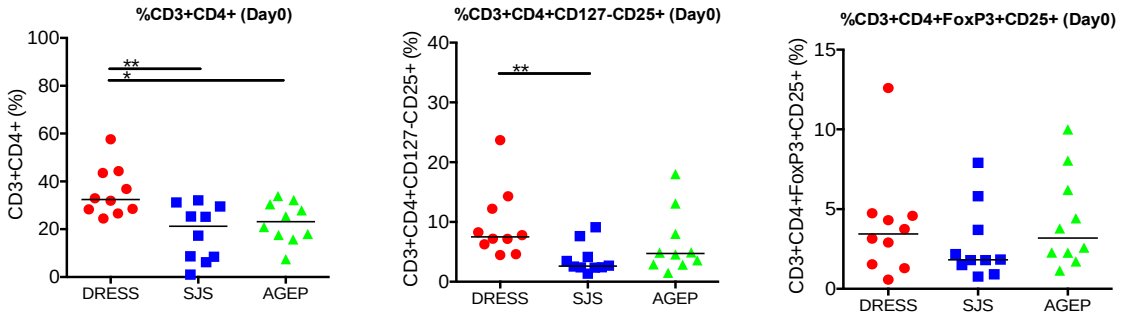


Figure 3.2 Regulatory t cells at Day 2 after culprit drug stimulation

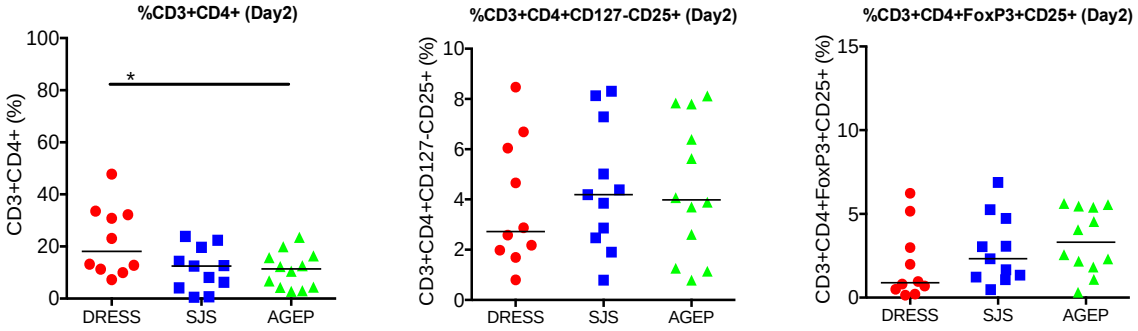
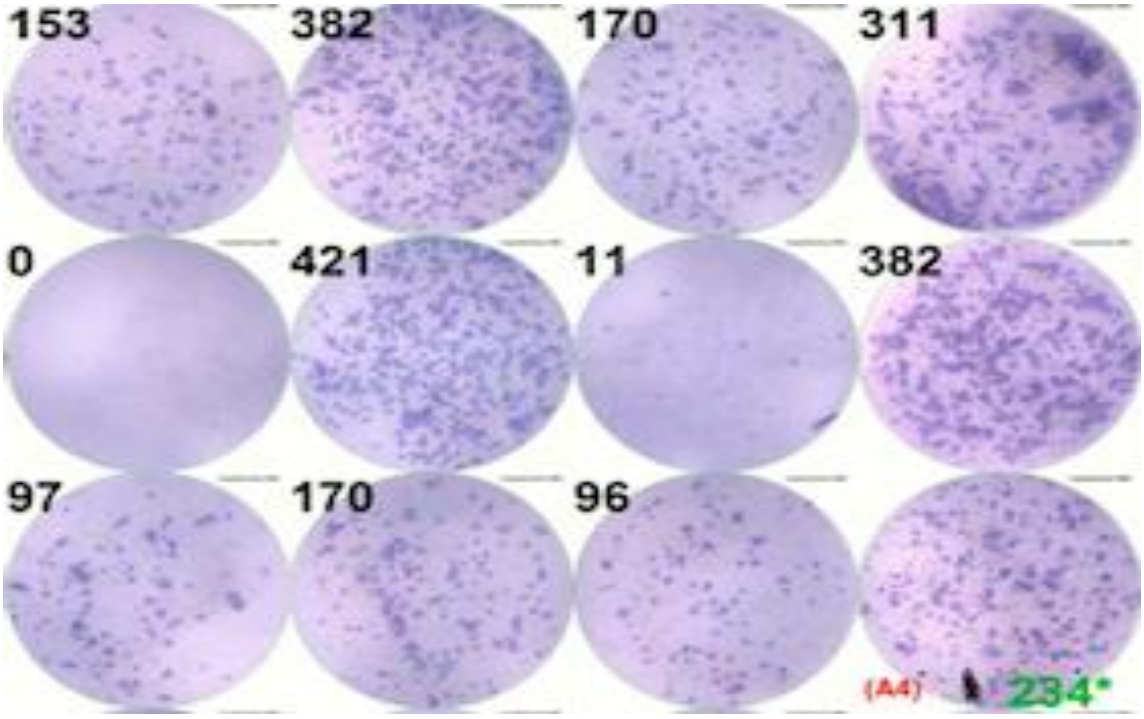


Table 2. Comparative proportions of regulatory T cells among different SCAR phenotypes

	% At baseline			% at 48 hours after drug stimulation		
Phenotypes	CD3CD4	CD127CD25	FoxP3CD25	CD3CD4	CD127CD25	FoxP3CD25
AGEP	22.9±3.6	7.4±2.2	4.7±1.2	13.6±2.9	4.9±0.9	3.7±0.6
DRESS	35.5±3.3**	9.6±1.8**	4.0±1.1	22.2±4.2*	3.8±0.8	2.0±0.7
SJS	21.3±4.0	3.9±1.0	3.0±0.9	12.1±2.5	5.2±0.9	3.3±0.7

The percentages of regulatory T cells (based on %CD3+CD4+CD127-CD25+ve cells and %CD3+CD4+FoxP+CD25+ve cells) were comparatively analyzed among 3 SCAR phenotypes. The proportions of CD4+ve T cells and CD3+CD4+CD127-CD25+ve regulatory T cells at baseline in DRESS were significantly higher than those in other phenotypes, particularly in SJS, but paradoxically lower after culprit drug stimulation for 48 hours. Our findings suggest that regulatory T cells may play a special role in DRESS compared to other SCAR phenotypes and worth further exploration.

Figure 4. ELISpot assay demonstrate the frequencies of drug-induced cytokine releasing cells upon stimulation with different stimuli.



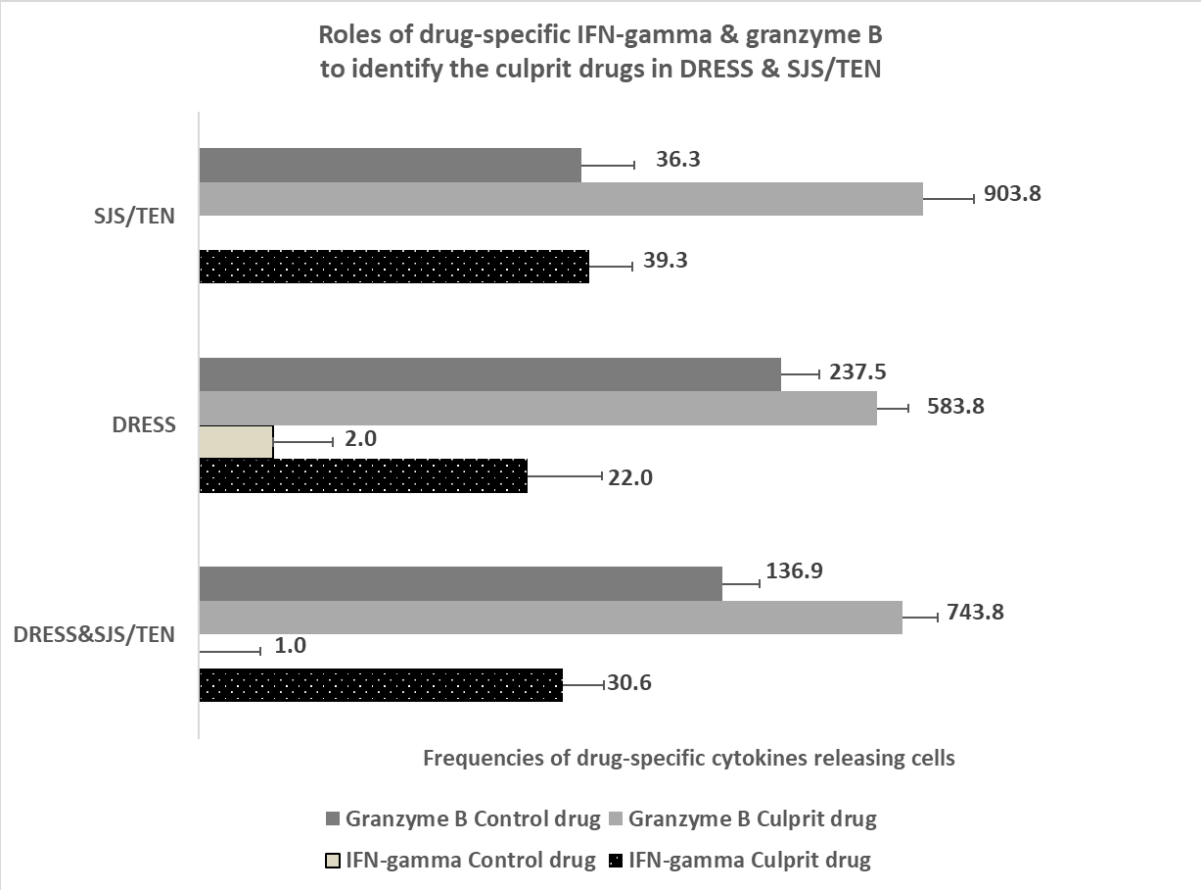
This figure illustrates how we measured frequencies of granzyme-B-releasing cells after stimulation with negative control (no stimuli), culprit drug, non-allergic drug, and positive control. The results demonstrated that frequencies of cytokine-releasing cells upon culprit drug stimulation were significantly higher than those upon stimulation with non-allergic tolerant drug (the final reports were numbers of spots in wells incubated with culprit drug after subtracted with numbers of spots in negative control wells (no stimuli background). Granzyme-B and some other cytokines were then selected to measure PBMC responses upon culprit drugs stimulation in SCAR subjects.

Table 3. Average frequencies of drug-induced cytokine releasing cells obtained from SCAR patients after PBMC stimulation with culprit drugs compared to control drug.

Measured cytokines	Frequencies of drug-induced cytokine releasing cells/10^6 PBMCs (*=p value < 0.05)
IFN-γ	
Culprit drugs	23.3±10.7*
Control drugs	0.8±0.6
IL-17	
Culprit drugs	1.3±1.3
Control drugs	undetectable
IL-5	
Culprit drugs	8.8±8.4*
Control drugs	undetectable
Granzyme B	
Culprit drugs	567.1±217.1*
Control drugs	104.3±42.8

The results of ELISpot assay confirm that frequencies of drug-induced granzyme B and IFN-γ releasing cells upon PBMC stimulation with the culprit drugs were significantly higher than those of the control drugs. The same trend was also observed for frequencies of drug-induced IL-5 and IL-17 releasing cells but the numbers were so low that they would be difficult to utilize in clinical practice. Interestingly, the frequencies of drug-induced granzyme B and IFN-γ releasing cells were mainly detectable in DRESS and SJS/TEN as shown in Figure 5 but not in AGEP subjects (data not shown). As a consequence, biomarkers to confirm diagnosis of drug-induced AGEP were further needed. Since IL-17 and IL-22 belong to the same cytokine family and play roles in neutrophil regulation, which are the predominant cells in AGEP, the frequencies of IL-22 releasing cells were later tested in the following experiments whether they could be detectable in this SCAR phenotype.

Figure 5. Frequencies of drug-induced granzyme-B and IFN-γ in DRESS and SJS/TEN



New set of ELISpot experiments revealed that drug-induced IL-22 releasing cells were detectable in AGEP subjects after incubating PBMCs with the culprit drugs as shown in Figure 6. However, the detectable numbers were much lower than those of granzyme B and IFN-γ in DRESS and SJS/TEN (see Figure 7). The average drug-induced IL-22, granzyme-B, and IFN-γ releasing cells upon stimulation with the suspected culprit drugs were 40.0, 18.0, and 20.0 cells/million PBMCs, respectively. The results indicated that different cytokines probably play a role in different SCAR phenotypes

Figure 6. Examples of drug-induced granzyme-B, IFN-γ, and interleukin-22 releasing cells in severe cutaneous adverse reactions

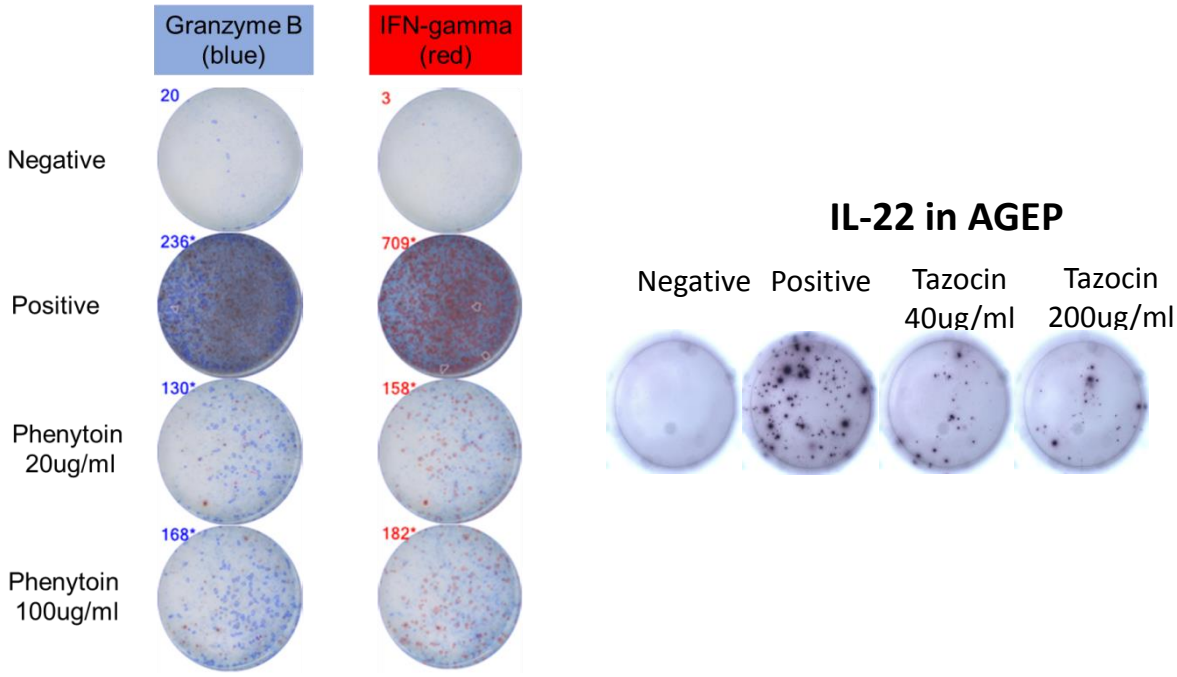


Figure 7. Average frequencies of drug-induced cytokine releasing cells in different SCAR phenotypes

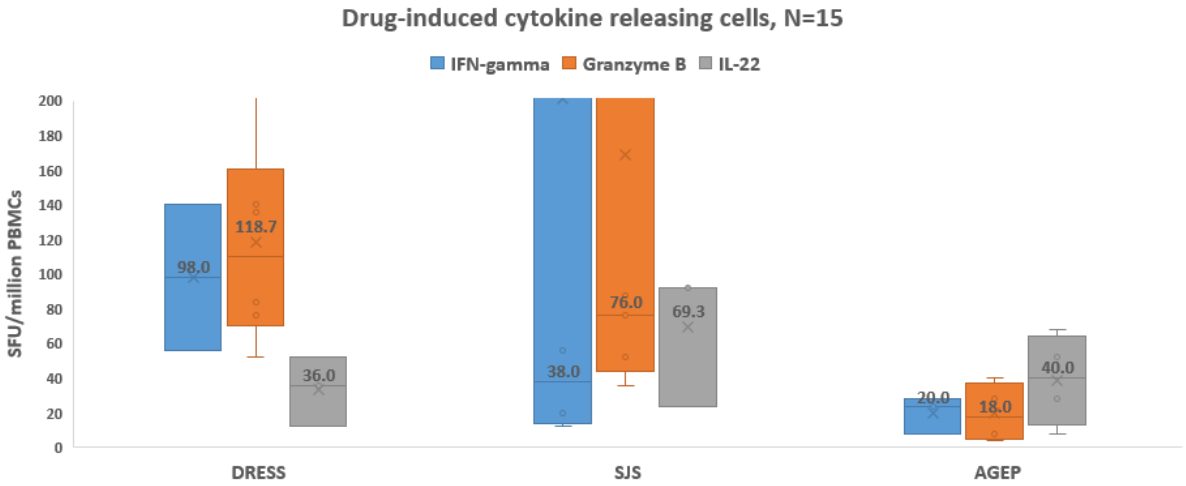
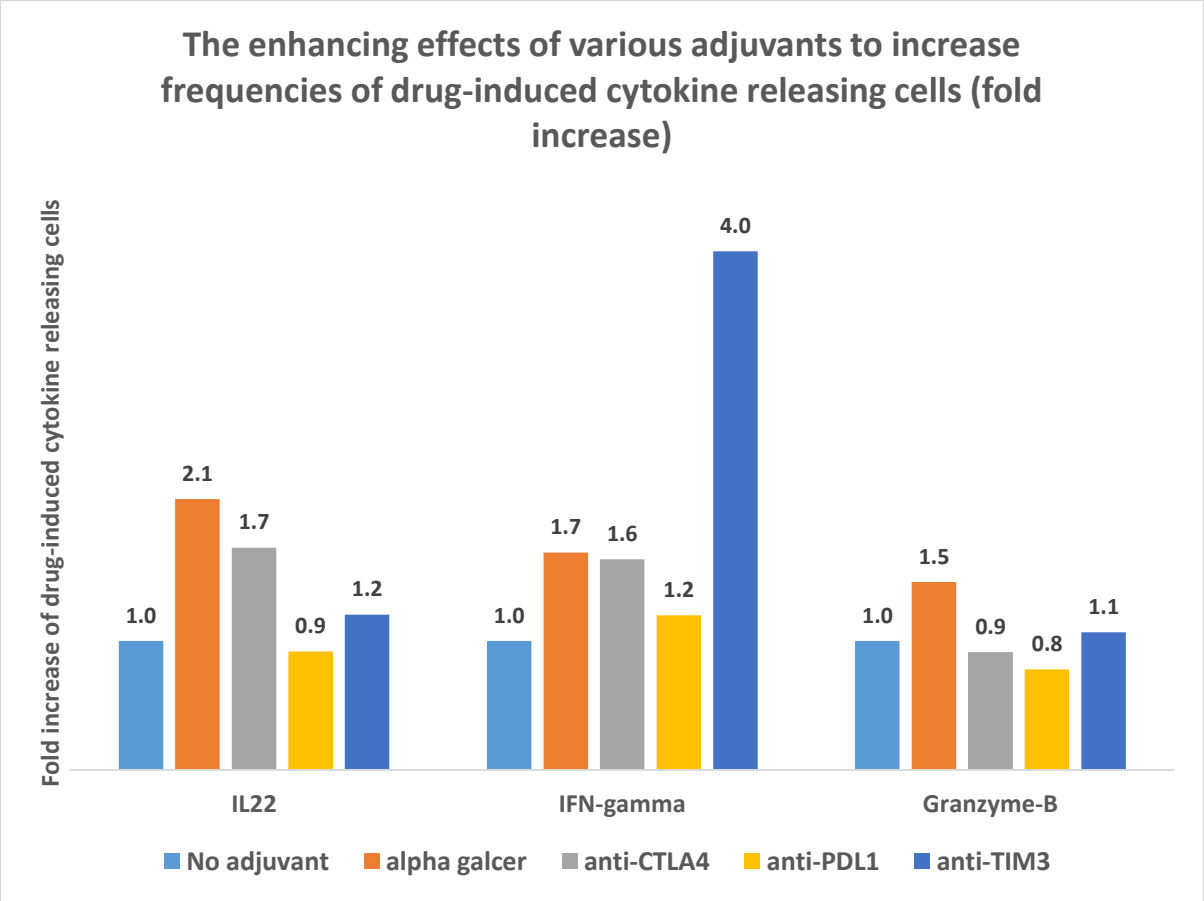
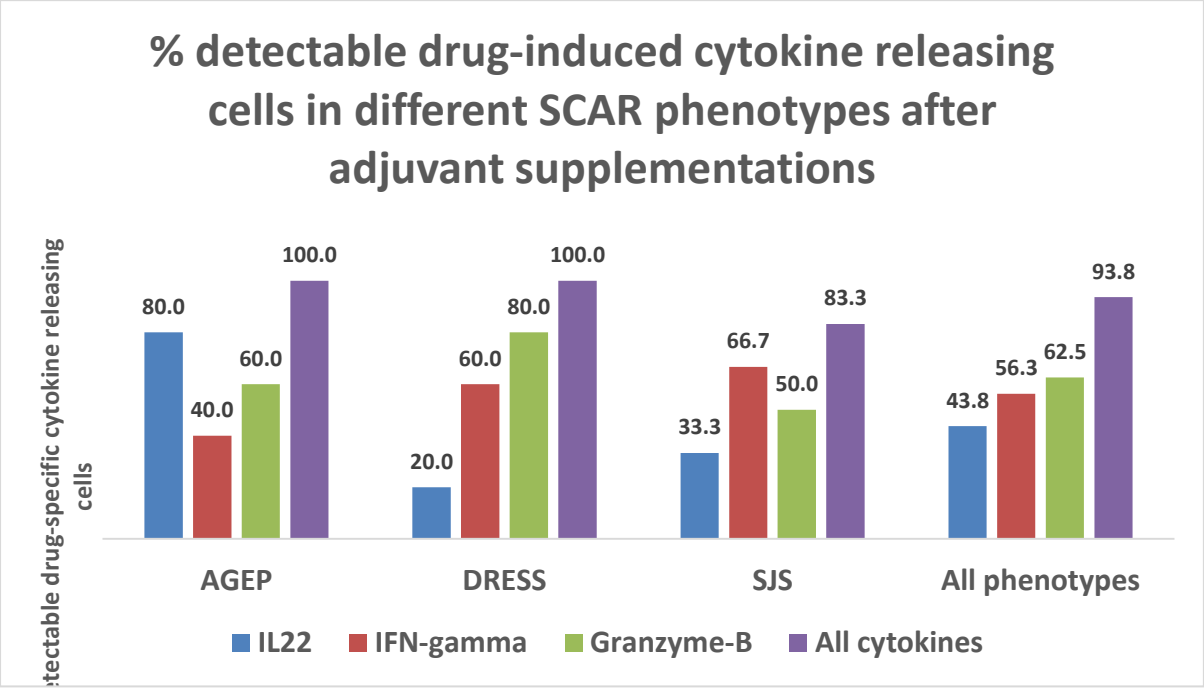


Figure 8. The enhancing effects of various in vitro adjuvants to increase frequencies of drug-specific cytokine releasing cells in SCAR subjects



Different in vitro adjuvants were shown to increase frequencies of drug-induced cytokine releasing cells after stimulating PBMCs with the suspected culprit drugs. The frequencies of IL-22 releasing cells increased 2.1 folds upon alpha-GalCer supplementation, while the frequencies of IFN-γ releasing cells increased 4.0 folds upon anti-TIM3 supplementation. The frequencies of granzyme-B releasing cells were not much affected with the supplementation of any adjuvants as shown in Figure 8.

Figure 9. The proportion of patients with detectable drug-specific cytokine releasing cells after adjuvant supplementations in different SCAR phenotypes



The measurement of drug-specific cytokine releasing cells after stimulation with the combinations of the suspected drugs and in vitro adjuvants revealed that drug-induced IL-22 releasing cells were mainly demonstrated in AGEP (80% of cases), while drug-induced IFN-γ and granzyme-B were mainly demonstrated in SJS/TEN (66.7% of cases) and DRESS (80.0% of cases), respectively. The measurement of all 3 cytokines (IL-22, IFN-γ, and granzyme-B) in the presence of adjuvants would be helpful to identify the culprit drugs in 93.8% of SCAR cases as shown in Figure 9.

บทวิจารณ์

Drug-induced severe cutaneous adverse reactions (SCARs) are life-threatening conditions and very common in Thailand and southeast Asian nations compared to the other regions worldwide. The accessibility to high risk drugs without prescription and high prevalence of susceptible genes at risk contribute to the disease's high burden. Other than the deleterious consequences in SCAR victims, the selection of further medications used in these patients are very limited, particularly in subjects who develop SCAR after taking multiple drugs simultaneously. The identification of the actual culprit drug from other concurrent drugs in these patients would be helpful to select safe alternative drugs in the future. Culprit drug confirmation is also beneficial in some patients whom medical history was not reliable or clinical diagnosis of SCAR remains uncertain.

The results of cytokine screening demonstrated that several cytokines were detectable in culture media when incubating PBMCs collected from SCAR subjects with the culprit drugs and non-allergic control drugs. Since PBMCs were harvested during the active phase of SCARs, it was possible that the majority of increased cytokines might be related to acute inflammatory response in SCARs rather than the specific response to drugs. However, different levels of certain cytokines were consistently observed upon PBMC stimulation between culprit drugs and concurrent tolerated drugs, for examples, IFN-γ, Granzyme B, perforin, and granulysin. Therefore, we speculated that the measurement of drug-induced cytokine releasing cells on this cytokine panel may have a potential role to identify the culprit drugs in SCAR subjects.

We also observed the expansion of regulatory T cells in patients diagnosed with DRESS in relation to other SCAR phenotypes (particularly when compared to SJS/TEN). However, the paradoxical reduction of regulatory T cells was evidence after incubation PBMCs with the culprit drug. The results support earlier findings in the literatures that the functions of regulatory T cells were heightened in DRESS while those may be defective in SJS/TEN. The difference of regulatory T cell functions between DRESS and SJS/TEN possibly leads to unique clinical manifestations in each phenotype even though the types of culprit drugs in both phenotypes are pretty similar. Roles of regulatory T cells in controlling and modulating immune response or tolerance to drugs deserve further exploration.

Our study indicates that the measurement of cytokines releasing cells upon stimulation PBMCs with the suspected culprit drug has a potential role to distinguish the actual culprit drug from the concurrent non-allergic drugs since the numbers of measurable drug-induced cytokine releasing cells after PBMC incubation with the culprit drugs and tolerated drugs were

significantly different. According to our study, the measurement of drug-induced cytokine releasing cells is beneficial to identify the culprit drugs in various SCAR phenotypes. Drug-specific granzyme-B and IFN-γ releasing cells were mainly detectable in DRESS and SJS/TEN while drug-specific IL-22 releasing cells were predominantly detectable in AGEP phenotype. The supplementation of in vitro adjuvants, such as anti-TIM3, to inhibit functions of regulatory T cells/reverse effector T cell exhaustion could enhance drug-specific T cell expression and increase sensitivity of the test to identify the culprit drugs in SCAR.

In summary, the results of this study suggest that the measurement of drug-induced granzyme-B and IFN-γ releasing cells in the presence of anti-TIM3 supplement would be helpful to confirm the diagnosis of drug-induced DRESS and SJS/TEN. However, large-scale studies on the customized cytokine panels and appropriate adjuvants are needed before this approach can be routinely recommended in clinical practice for drug allergy diagnosis.

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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

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2. การนำผลงานวิจัยไปใช้ประโยชน์

☐ ด้านนโยบาย ☑ ด้านสาธารณะ ☐ ด้านชุมชนและพื้นที่ ☐ ด้านพาณิชย์
☑ ด้านวิชาการ

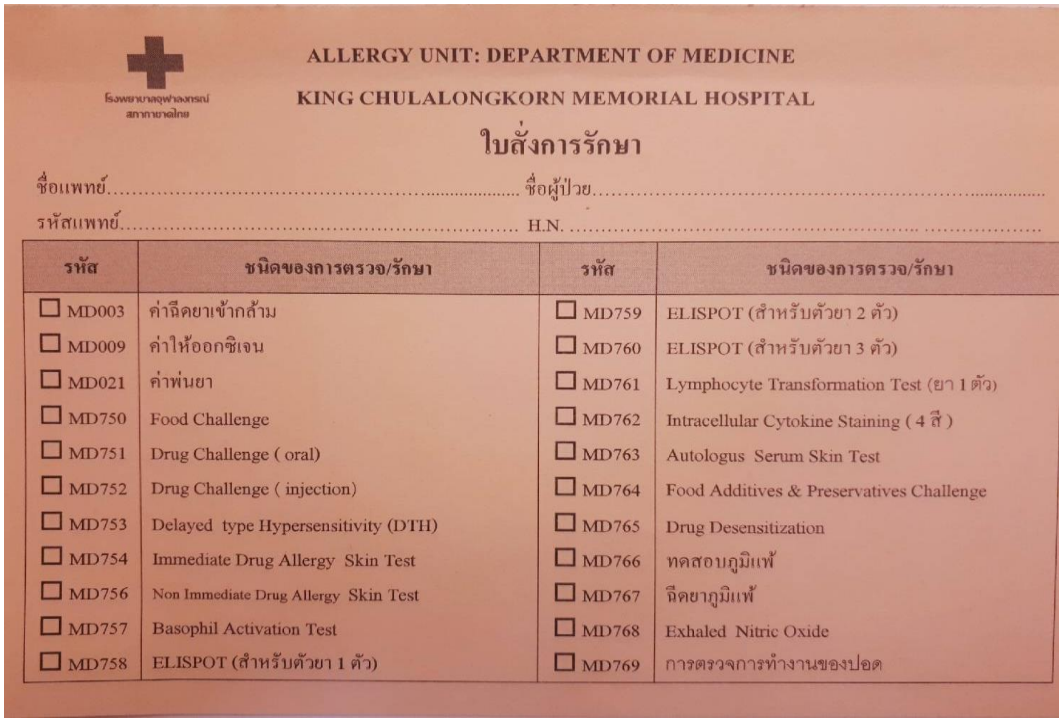
มีการนำผลงานวิจัยไปใช้ประโยชน์เชิงสาธารณะ

☑ หน่วยงาน (ภาครัฐ/~~เอกชน~~) ☐ สถาบันการศึกษา ☐ ผู้ประกอบการ ☐ เกษตรกร
☐ อื่น ๆ

โรงพยาบาลจุฬาลงกรณ์ สภากาชาดไทย

การนำไปใช้

เปิดรับตรวจทางห้องปฏิบัติการเพื่อวินิจฉัยการแพ้ยาด้วยการใช้เทคนิค ELISpot เพื่อตรวจหา drug-induced cytokine releasing cells เพื่อบริการผู้ป่วยแพ้ยาในทางเวชปฏิบัติแล้ว



ALLERGY UNIT: DEPARTMENT OF MEDICINE
KING CHULALONGKORN MEMORIAL HOSPITAL
ใบสั่งการรักษา

ชื่อแพทย์..... ชื่อผู้ป่วย.....
รหัสแพทย์..... H.N. .....

รหัส	ชนิดของการตรวจ/รักษา	รหัส	ชนิดของการตรวจ/รักษา
☐ MD003	ค่าฉีดยาเข้ากล้าม	☐ MD759	ELISPOT (สำหรับตัวยา 2 ตัว)
☐ MD009	ค่าให้ออกซิเจน	☐ MD760	ELISPOT (สำหรับตัวยา 3 ตัว)
☐ MD021	ค่าพ่นยา	☐ MD761	Lymphocyte Transformation Test (ยา 1 ตัว)
☐ MD750	Food Challenge	☐ MD762	Intracellular Cytokine Staining (4 สี)
☐ MD751	Drug Challenge (oral)	☐ MD763	Autologus Serum Skin Test
☐ MD752	Drug Challenge (injection)	☐ MD764	Food Additives & Preservatives Challenge
☐ MD753	Delayed type Hypersensitivity (DTH)	☐ MD765	Drug Desensitization
☐ MD754	Immediate Drug Allergy Skin Test	☐ MD766	ทดสอบภูมิแพ้
☐ MD756	Non Immediate Drug Allergy Skin Test	☐ MD767	ฉีดยาภูมิแพ้
☐ MD757	Basophil Activation Test	☐ MD768	Exhaled Nitric Oxide
☐ MD758	ELISPOT (สำหรับตัวยา 1 ตัว)	☐ MD769	การตรวจการทำงานของปอด

มีการนำผลงานวิจัยไปใช้ประโยชน์เชิงวิชาการ
โดย คณะวิศวกรรมศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

การนำไปใช้ดำเนินการวิจัยต่อยอดจากโครงการวิจัยนี้ โดยกำลังพัฒนาอุปกรณ์ต้นแบบสำหรับทดสอบการแพ้ยาแบบพกพาด้วยงบประมาณจากกองทุนรัชดาภิเษกสมโภช จุฬาลงกรณ์มหาวิทยาลัย ประจำปี 2561 เพื่อสร้างอุปกรณ์สำหรับวินิจฉัยการแพ้ยาสำหรับให้โรงพยาบาลขนาดเล็กสามารถใช้ในการวินิจฉัยการแพ้ยาให้ผู้ป่วยได้แพร่หลายยิ่งขึ้น

3. อื่น ๆ

การเสนอผลงานในที่ประชุมวิชาการ

International speaker

1. Clinical experience on ELISpot assay for drug allergy management. Klaewsongkram J. The 10th International Congress on Cutaneous Adverse Drug Reactions, Matsue city, Shimane prefecture, Japan, November10, 2018

International presentations

Oral presentations

1. Combination of in-vivo and ex-vivo tests for drug causality assignment in severe cutaneous adverse drug reactions. Rerknimitr P, Sittiwattanawong P, Hirankarn N, Klaewsongkram J. The 10th International Congress on Cutaneous Adverse Drug Reactions, Matsue city, Shimane prefecture, Japan, November11, 2018

2. Clinical diagnostic values of drug-specific IFN-gamma releasing cells measurement confirmed by drug provocation tests. Kantachatvanich T, Chongpison Y, Thantiworasit P, Suthumchai N, Buranapraditkun S, Rerknimitr P, Sittiwattanawong P, Klaewsongkram J. the European Academy of Allergy and Clinical Immunology Annual Congress 2018, Munich, Germany, May29, 2018

3. The reliability of ALDEN scores for the prediction of drug-specific T cells in Stevens-Johnson syndrome. Prasertcharoensuk A, Chongpison Y, Thantiworasit P, Suthumchai N, Buranapraditkun S, Rerknimitr P, Klaewsongkram J. the European Academy of Allergy and Clinical Immunology Annual Congress 2018, Munich, Germany, May28, 2018

Poster presentations

1. In vitro detection of drug-induced granzyme B, interferon-gamma, and interleukin-22 releasing cells in different phenotypes of severe cutaneous adverse reactions. Klaewsongkram J, Buranapraditkun S, Thantiworasit P, Suthumchai N, Rerknimitr P, Tuchinda P, Chularojanamontri L, Rerkpattanapipat T, Chanprapaph K, Disphanurat W, Chakkavittumrong P, Tovanabutra N, Sukasem C, Srinoulprasert Y, Srisuttiyakorn C. The 2019 American Academy of Allergy Asthma and Immunology Annual Meeting, San Francisco, California, USA, February24, 2019

2. The appropriate cut-off value of interferon-gamma ELISpot assay for drug hypersensitivity diagnosis in clinical practice. Kantachatvanich T, Chongpison Y, Thantiworasit P, Suthumcha N, Buranapraditkun S, Rerknimitr P, Sittiwattanawong P, Chantaphakul H, Klaewsongkram J. The 2019 American Academy of Allergy Asthma and Immunology Annual Meeting, San Francisco, California, USA, February24, 2019

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1. Reliability and validity of the Thai Drug Hypersensitivity Quality of Life Questionnaire: a multi-center study.
2. Analysis of HLA-B allelic variation and interferon-gamma ELISpot responses in patients with severe cutaneous adverse reactions associated with drugs.
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Article

Reliability and validity of the Thai Drug Hypersensitivity Quality of Life Questionnaire: a multi-center study

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Abstract

Objective: To adapted the Drug Hypersensitivity Quality of Life (DrHy-Q) Questionnaire from Italian into Thai and assessed its validity and reliability.

Design: Prospectively recruited during January 2012–May 2017.

Setting: Multicenter; six Thai tertiary university hospitals.

Study Participants: Total of 306 patients with physician-diagnosed drug hypersensitivity.

Interventions: Internal consistency and test–retest reliability were evaluated among 68 participants using Cronbach's α and intra-class correlation coefficient (ICC). The validity of Thai DrHy-Q was assessed among 306 participants who completed World Health Organization Quality of Life-BREF (WHOQOL-BREF-THAI). Construct and divergent validities were assessed for Thai DrHy-Q. Known-groups validity assessing discriminating ability was conducted in Thai DrHy-Q and WHOQOL-BREF-THAI.

Main outcome measures: Validity; reliability; single vs. multiple drug allergy; non-severe cutaneous adverse reactions (SCAR) vs. SCAR.

Results: Thai DrHy-Q showed good reliability (Cronbach's $\alpha = 0.94$ and ICC = 0.8). Unidimensional factor structure was established by confirmatory factor analysis (CFI&TLI = 0.999, RMSEA = 0.02). Divergent validity was confirmed by weak correlation between Thai DrHy-Q and WHOQOL-BREF-THAI domains (Pearson's $r = -0.41$ to -0.19). Known-groups validity of Thai DrHy-Q was confirmed with significant difference between patients with and without life-threatening SCAR ($P = 0.02$) and patients with multiple implicated drug classes vs. those with one class ($P < 0.01$); while WHOQOL-BREF-THAI could differentiate presence of life-threatening SCAR ($P < 0.01$) but not multiple-drug allergy.

Conclusions: Thai DrHy-Q was reliable and valid in evaluating quality of life among patients with drug hypersensitivity. Thai DrHy-Q was able to discriminate serious drug allergy phenotypes from non-serious manifestations in clinical practice and capture more specific drug-hypersensitivity aspects than WHOQOL-BREF-THAI.

Key words: drug hypersensitivity, quality of life, validity and reliability, adverse drug reaction

Introduction

Drug hypersensitivity in Thailand has been a prominent problem among Thai population [1–3]. Drug hypersensitivity, by its nature, is dynamic with some symptoms subsiding quickly, and patients recovering in a short time without any sequelae; whereas, other patients have life-threatening symptoms with long-term sequelae (e.g. developing new diseases and ocular or pulmonary sequelae [4, 5]). An encounter with drug hypersensitivity reaction may leave patients with vivid memories that could trigger anxiety, fear and concerns about the future, and, consequently, may influence patient's quality of life (QOL).

Issues related to quality of care and health-related QOL among patients with drug hypersensitivity are scarcely addressed. Tools eliciting health-related QOL have been translated and validated among non-English speaking countries, and associations between quality of care and health-related QOL among patients with various conditions have been evaluated [6–8]. However, the lack of a valid and reliable questionnaire makes it difficult for addressing the correlation between quality of care and drug-hypersensitivity related QOL. To our knowledge, despite questionnaires addressing QOL among patients with allergy diseases being available [9–11], to date no validated questionnaire for assessing QOL among patients with drug hypersensitivity exists in the Thai language.

Baiardini *et al.* [12] developed Drug Hypersensitivity Quality of Life Questionnaire (DrHy-Q), which was shown to be suitable for evaluating QOL related to having drug hypersensitivity. Subsequently, cross-cultural validation studies of DrHy-Q questionnaire were conducted in Spain, Turkey and the Netherlands [13–16]. This tool could potentially be used among Thai population.

The aims of study were to translate the original Italian version of the DrHy-Q into Thai, assess its validity and reliability for use in Thai patients, and compare known-groups validity of Thai version of DrHy-Q (Thai DrHy-Q) to that of the Thai version of the World Health Organization Quality of Life-BREF (WHOQOL-BREF-THAI). Hypotheses were that the translated questionnaire would be valid and reliable to use in Thai patients with drug hypersensitivity and that patients with severe drug-hypersensitivity symptoms would have poorer QOL that those with mild allergic symptoms.

Methods

Study population

Patients with suspected drug hypersensitivities referred to allergy and clinical immunology clinics at six tertiary university teaching hospitals in Bangkok and Chiang Mai, Thailand were recruited during January 2012 to May 2017. Hospital centers included King Chulalongkorn Memorial Hospital, Ramathibodi Hospital, Siriraj Hospital, Pramongkutklao Hospital, Thammasart University Hospital and Maharaj Nakorn Chiang Mai Hospital. Inclusion criteria were patients with at least 15 years of age, having history of suspected drug allergy or symptoms related to drug hypersensitivity, and had been reviewed by allergists and/or dermatologists. Informed consent was obtained from all patients by completion of the

questionnaire. Clinical presentations (e.g. life-threatening severe-cutaneous adverse reaction (SCAR)), and drug-hypersensitivity characteristics including onset of drug hypersensitivity reaction were obtained.

Measures

The original 15-item DrHy-Q was in Italian, and contained five-point Likert response scale [12]. The items address any burden, such as physical and psychological aspects, of having drug hypersensitivity. Higher scores signify worse QOL.

The Thai version of the WHOQOL-BREF-THAI consists of 26 items, two generic items and 24 domain-specific items, with a 5-point Likert scale response [17, 18]. The two generic items address overall QOL and general health status. Four domains assessed are physical health (7 items), psychological health (6 items), social relationships (3 items) and environmental health (8 items). Each domain scores range from four to 20 points, and scores from two global items range from one to five. Higher WHOQOL-BREF-THAI scores indicate better QOL.

Translation

To ensure the meaning of original questionnaire has not been lost, the forward- and back-translations method proposed by World Health Organization [19] was conducted on the original Italian version of the DrHy-Q.

The Italian version was translated into Thai by two independent translators who were proficient in both languages. The draft of Thai version of the DrHy-Q (Thai DrHy-Q) was back-translated by a bilingual native Italian. The back-translated version was then reviewed by the developer group and tested for comprehension of the questions. An additional question about comprehensibility of the items with a 10-point Likert scale (ranging from 1—most difficult to 10—very easy) was added to assess face validity.

Reliability

Internal consistency was evaluated using Cronbach's *a*. We noted that, for group comparison, values of 0.7–0.8 was adequate. Cronbach's *a* of 0.9–0.95 was needed for clinical application [20].

Test-retest reliability was conducted among 68 subjects who were asked to complete the Thai DrHy-Q twice. To minimize patients' ability to recall the questions and answers, the second administration of the Thai DrHy-Q occurred at least 1 week apart after the initial administration. Prior to completing to the questionnaire second time, participants were asked if there was any significant change in life including their health. Participants with any substantial change during the 1 week period were excluded. The intra-class correlation coefficient (ICC 3,1: two-way mixed effect model) were used to assess the test–retest reliability [21, 22]. Criteria for ICC values were <0.4 suggesting poor correlation, 0.4–0.59 as fair, 0.6–0.74 as good and >0.75 as excellent reliability [23].

The Bland–Altman plot was also generated to visualize the agreement between the questionnaire responses at the two time points [24].

Validity

Construct validity, divergent validity and known-groups validity were performed to assess the validity of the Thai DrHy-Q.

Confirmatory factor analysis to evaluate the fit of the unidimensional model acquired from the original Italian version to our data. As the items were measured using ordinal scales, we employed using diagonally weighted least squares robust estimation to fit the DrHy-Q measurement model. Goodness-of-fit indices indicating how well the model fits the sample were Comparative Fit Index (CFI), Tucker–Lewis Index (TLI) and root mean square error of approximation (RMSEA). The CFI and TLI of >0.90 and >0.95 suggested acceptable and excellent fit, respectively, and RMSEA of <0.08 suggested reasonable fit [25]. Chi-square statistics, a conventional index for measurement of model fit, was also included. Any item with factor loading below 0.40 would be excluded.

In absence of a 'gold standard' for assessing health-related QOL in drug hypersensitivity, divergent validity was evaluated with Pearson's correlation coefficients to measure the association between the Thai DrHr-Q and the WHOQOL-BREF-THAI. Divergent validity was considered to be confirmed if the absolute value of correlations are <0.40 [26, 27].

Known-groups validity establishes the instrument ability to distinguish between different subgroups based on clinical outcomes or characteristics [28]. Known-groups validity of both Thai DrHy-Q and WHOQOL-BREF-THAI was evaluated. The WHOQOL-BREF-THAI has been used in assessing QOL among various clinical populations [29–31]; however, its ability to differentiate between subgroups of patients with drug hypersensitivity is unknown. Subgroups were determined based on clinical presentations and drug-hypersensitivity characteristics. Clinical presentations included present or absence of anaphylaxis or life-threatening SCAR. Drug-hypersensitivity characteristics were onset of drug-hypersensitivity reaction (immediate: <2 h after the exposure to an implicated drug and non-immediate: >2 h), number of implicated pharmaceutical classes (one vs. multiple classes), non-steroidal anti-inflammatory drugs (NSAIDs) allergy and/or beta-lactam antibiotics allergy. The analyses were performed using Student's *t* test (for the two group case) or analysis of variance (ANOVA) with Tukey's post-hoc multiple comparison (for the multiple group case), with the significance level of $P < 0.05$. Where a statistical difference was established, receiving operating characteristic (ROC) curves were generated to assess the discriminating ability on criteria such as present or absence of life-threatening SCAR.

We determined sample size for all analyses. We aimed to recruit 300 subjects which were suitable for confirmatory factor analysis [32]. With this number of subjects, we would have sufficient power to conduct all other analyses for validation and reliability. For comparison between clinical groups, 40 subjects for each group was needed such that we would have 80% power to detect a clinical significant difference of 8 points on the DrHy-Q scale at significance level of 0.05. All validity and reliability analyses were conducted on subjects with complete values of Thai DrHy-Q and The R version 3.4.1 for Windows was used [33]. R libraries used in the study were sem, lavaan, nFactors, BlandAltmanLeh, psych, stats, Epi and dplyr. The COSMIN checklist was followed to ensure a good methodological quality of validation procedure [34]. This study was registered in ClinicalTrials.gov (NCT01666470).

Results

Pre-test and study population: The Thai DrHy-Q was initially tested among a group of physicians and patients with drug-hypersensitivity experience at King Chulalongkorn Memorial Hospital. The questions were considered by these participants to be acceptable, but a minor amendment was suggested on one item. A phrase was added to help ensure an item carrying the meaning of the original item. The item was then back-translated to Italian and was shown to have the same meaning. No further modifications were suggested. The final version was used in this study (Appendix 1).

Table 1 Characteristics of 306 participants with drug hypersensitivity experience

Characteristics	Mean (SD)
Age (years)	46.3 (15.9)
Time to complete questionnaire (min)	7.44 (5.90)
Self-reported comprehensibility of questionnaire	7.57 (2.47)
	N (%)
Sex	
Females	225 (76.0)
Males	71 (24.0)
Pharmaceutical classes[a]	
Non-steroidal anti-inflammatory drugs and other pain reliefs	149 (34.9)
Beta-lactam antibiotics	129 (30.2)
Sulfonamide antibiotics	13 (3.0)
Other antibiotics[b]	71 (16.6)
Anti-epileptic drugs	11 (2.6)
Other drugs	54 (12.6)

[a]Each participant can be hypersensitive to more than one drug classes.
[b]Including antituberculosis drugs.

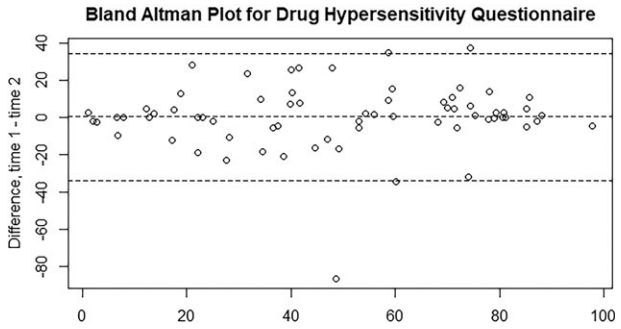

Figure 1 Bland Altman plot between responses of Thai DrHy-Q Questionnaire at time 1 and time 2.

A total of 306 participants with average age of 46.3 years (SD = 15.9) were included in this study. The average time to complete the Thai DrHy-Q was ~7.4 min and the average self-reported ease of comprehensibility was 7.57 points. Table 1 also shows the number of implicated drug classes where NSAIDs and beta-lactam antibiotics classes are the two largest groups.

Reliability

Internal consistency among 306 participants was shown to be quite strong with a Cronbach's α of 0.94 (95% confidence interval (95% CI): 0.93, 0.95). Test–retest reliability was performed on 68 participants with average age of 45.7 (SD = 16.1) and proportion of males was 25%. Average duration between first and subsequent tests was 22 days (SD = 16.4 days). The single measure of ICC was 0.80 (95% CI: 0.7, 0.87) indicating excellent test–retest reliability.

The Bland–Altman plot indicated no systematic bias (Figure 1). Test and retest scores were close to each other with mean difference of 0.35. Only one participant fell outside of the expected 95% limits of agreement (LOA: −33.87, 34.57).

Validity

Percentages of missing values for each Thai DrHy-Q items were quite low with values ranging from 0 to 1.2%. The confirmatory factor analysis showed that the unidimensional structure was confirmed for Thai DrHy-Q version with χ^2 of 101.25 (df = 90, P = 0.196). The CFI and TLI were both 0.999. RMSEA was 0.02 (95% CI: 0.00, 0.038, P = 0.999). Table 2 provides items' standardized factor loading for Thai DrHy-Q. None of the items had factor loading <0.40, and all were statistically different from zero ($P < 0.05$).

Table 2 Factor loading of each item in the Thai DrHy-Q questionnaire

Items	Standardized factor loading[a]
Since I am unable to take drugs every illness limits me more than other	0.683
I am afraid of being administered a drug during an emergency to which I am allergic	0.652
I feel frightened due to my problem of allergy reaction	0.817
The problem of adverse reaction to drugs affects my life	0.751
I would like the allergist's opinion before taking drugs prescribed by other specialists	0.635
Even a little discomfort for me is a problem	0.775
The fact that I cannot use medication safely made me feel different from others	0.784
I feel anxious due to my problem of allergy reaction	0.866
For each disease I would be confident that there is a drug that I can safely take	0.501
I am afraid I could not deal with the pain	0.698
I feel anguished due to my problem of allergy reaction	0.829
I worry every time I take a drug different from ones that cause my allergic reactions	0.809
I give up leisure activities (sport, vacations, trips) because of my problem	0.517
I'm in a bad mood due to my problem of allergy reaction	0.667
The idea of taking a medicine makes me feel anxious	0.803

Thai DrHy-Q = Thai version of Drug Hypersensitivity Quality of Life Questionnaire.
[a]All coefficients were significantly different from zero ($P < 0.05$).

Table 3 Pearson's correlation coefficients between WHOQOL-BREF-THAI domains and global items and Thai DrHy-Q scores

WHOQOL-BREF domains and global items	Pearson's correlation (95% CI, *P*-value)
Physical health	−0.41 (−0.50, −0.31, $P < 0.001$)
Psychological health	−0.39 (−0.48, −0.29, $P < 0.001$)
Social relationship	−0.19 (−0.30, −0.08, $P < 0.01$)
Environmental	−0.27 (−0.37, −0.16, $P < 0.001$)
General health	−0.29 (−0.39, −0.18, $P < 0.001$)
Overall quality of life	−0.26 (−0.36, −0.15, $P < 0.001$)

Divergent validity of Thai DrHy-Q was established by low levels of negative correlations with all WHOQOL-BREF-THAI domain scores, general health scores, and overall QOL scores (r ranging from −0.41 to −0.19, Table 3). The lowest level of correlation was between Thai DrHy-Q scores and WHOQOL-BREF-THAI social relationship domain scores.

Known-groups validity analysis for the Thai DrHy-Q confirmed its ability to distinguish patients with multiple implicated pharmaceutical classes from those with one drug class ($P < 0.01$), and patients with life-threatening SCAR from those without the condition (P = 0.02, Table 4). The ANOVA showed significant differences in means of the Thai DrHy-Q scores among subgroups of

Table 4 Comparison of Thai DrHy-Q scores between clinical presentations and drug-hypersensitivity characteristics

	N (%)	Thai DrHy-Q scores mean (95% CI)
Reaction time		
Immediate	124 (42.8)	46.03 (41.58, 50.49)
Non-immediate	149 (51.4)	43.14 (39.07, 47.20)
Unknown	17 (5.9)	48.43 (36.40, 60.45)
Number of suspected pharmaceuticals		
Single	189 (64.9)	41.48 (37.92, 45.05)
Multiple	102 (35.1)	50.67 (45.65, 55.69)*
NSAIDs vs. beta-lactam ABX allergy*		
NSAIDs	113 (46.7)	38.75 (34.14, 43.36)[a]
Beta-lactam ABX	93 (39.7)	46.12 (41.04, 51.21)[a]
Allergic to both groups	36 (13.6)	58.11 (49.94, 66.28)
Life threatening SCAR		
Yes	44 (15.4)	52.61 (45.63, 59.58)**
No	242 (84.6)	42.94 (39.73, 46.16)
Anaphylaxis		
Yes	13 (4.5)	43.11 (28.10, 58.12)
No	273 (95.5)	44.49 (41.47, 47.50)

95% CI = 95% confidence interval; ABX = antibiotics; NSAIDs = non-steroidal anti-inflammatory drugs.

*$P < 0.01$, **$P = 0.02$.

[a]Those groups sharing a letter did not differ significantly.

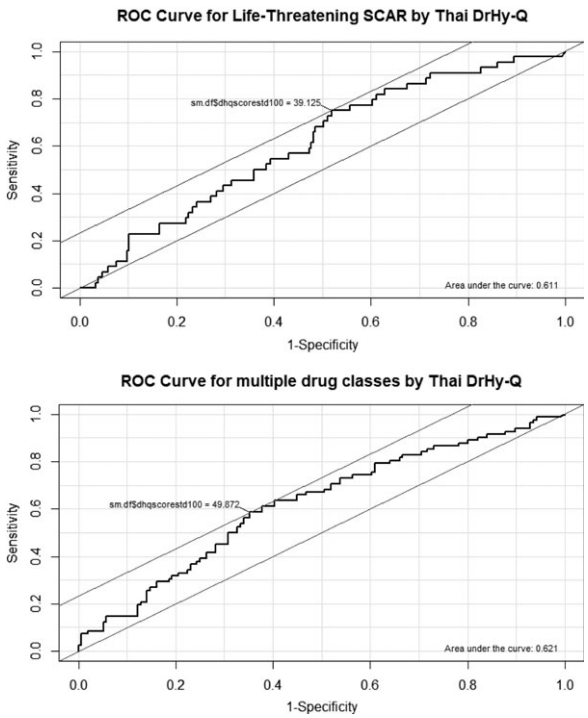

Figure 2 The receiving operating characteristic curves for life-threatening severe cutaneous adverse reaction and multiple numbers of implicated pharmaceutical classes by Thai DrHy-Q.

patients allergic to NSAIDs and/or beta-lactam antibiotics ($F = 8.51$, df = 2, $P < 0.001$). The Tukey's post hoc test showed patients allergic to NSAIDs only had lower DrHy-Q scores (i.e. better QOL) than either those with beta-lactam antibiotic allergy only or those who were allergic to both (Table 4).

The known-groups validity of WHOQOL-BREF-THAI was not confirmed for all domains and facets. Only physical health, psychological health and overall QOL showed ability to differentiate between patients with life-threatening SCAR and those without. Patients with life-threatening scar had poorer physical health (mean, $\bar{X} = 12.62$; 95% CI: 11.77, 13.53, $P < 0.001$), psychological health ($\bar{X} = 14.04$, 95% CI: 13.12, 14.96, $P = 0.002$), and overall QOL ($\bar{X} = 3.68$, 95% CI: 3.47, 3.90, $P = 0.007$) than those without the life-threatening condition (physical health: $\bar{X} = 14.44$, 95% CI: 14.11, 14.77; psychological health: $\bar{X} = 15.33$, 95% CI: 15.02, 15.64; overall QOL: $\bar{X} = 3.97$; 95% CI: 3.89, 4.05).

Areas under the ROC curves (AUCs) for predicting clinical criteria—multiple implicated drug classes and a life-threatening SCAR by Thai version of DrHy-Q, were 0.621 and 0.611, respectively (Figure 2). The physical health, psychological health, and overall QOL of WHOQOL-BREF-THAI poorly discriminated the life-threatening SCAR condition with AUCs ranging between 0.334 and 0.390.

Discussion

In this study, we describe the translation process and evaluate the psychometric properties for the Thai DrHy-Q. We found that the Thai version of DrHy-Q to be both reliable and valid for assessing health-related QOL among patients with drug hypersensitivity.

The finding shows that the internal consistency and test–retest reliability were good. To reduce recall of the questions and patient's answers, we had a wider time interval (22 days) between test and retest than other studies [12–14, 16]. This potentially suggested that an impact of drug-hypersensitivity experience on QOL may not change rapidly over time. The Bland–Altman plot suggested no systematic bias between the two time points, and slightly wide variation.

The correlation between DrHy-Q and WHOQOL-BREF was negative as expected. Higher WHOQOL-BREF domain scores referred to better QOL; whereas higher scores of Thai DrHy-Q suggested poorer QOL. The observed poor-to-fair correlations support the notion of adequate divergent validity. These results suggest that the Thai DrHy-Q not only measures specific drug-hypersensitivity burden, but also partially captures general QOL. Our findings differed from Italian DrHy-Q where very weak correlations was reported [12]. However, the magnitudes of reported correlations were similar to that of the Turkish and Dutch versions of DrHy-Q [13, 14]. Interestingly, the higher levels of correlations were observed between Thai DrHy-Q scores and two domains of WHOQOL-BREF-THAI (physical health and psychological health). This seems to reflect the wordings in the Thai DrHy-Q which were mainly emotional- and physical-related concerns.

Although, this is not the first study to validate DrHy-Q for another population, we employed a comparatively large and multicenter sample ($N = 306$). Four studies have sample size ranging from 30 to 736 subjects [12–14, 16]. However, only one study reported 6% (41/657) of patients with delayed cutaneous reaction, which includes mild cutaneous conditions and found no significant difference between two groups [14]. Our study not only have relatively large group of participants with life-threatening SCAR (15%), but also found significantly a poorer QOL in patients with life-threatening SCAR. In addition, our study evaluated different factors, specifically comparison between two specific drug classes (NSAIDs

vs. beta-lactam antibiotics) and number of implicated drug classes instead of a specific drug. This reflected current practice where patients are advised to avoid an entire pharmaceutical class.

The Thai DrHy-Q was able to distinguish patients with or without life-threatening SCAR, patients with allergy to either NSAIDs or beta-lactam antibiotics, or both, and participants with one or multiple implicated drug classes. We could not demonstrate that the DrHy-Q can discriminate between patients with and without anaphylaxis, a result similar to the Turkish and Dutch studies [13, 14]. However, the original Italian validation study and its subsequent study showed that significant difference in the DrHy-Q scores between participants with or without anaphylaxis reaction [12, 35]. Culturally different impression of anaphylaxis reaction could explain this departure. For Thais, the anaphylaxis reaction may not leave permanent physical and psychological damages such that a recall of anaphylaxis experience was not so tormenting to have a substantial impact on the QOL.

The Thai DrHy-Q with ability to discriminate three subgroups was superior to the WHOQOL-BREF-THAI which was able to only detect the difference between patients with or without life-threatening SCAR. The areas under the ROC curves supported this finding. The AUCs of Thai DrHy-Q for differentiating multiple drug classes and life-threatening SCAR condition were close to 0.70; while the AUCs of WHOQOL-BREF-THAI physical health, psychological health and overall QOL for discriminating life-threatening SCAR were substantially lower, and none exceeded 0.50.

The strengths of the study include having multi-center sample with large proportion of life-threatening SCAR and the use of drug classes as clinical subgroups, which provide us an adequate generalizability of our finding to other Thai patients with drug hypersensitivity. In addition to conventional approach in assessing known-groups validity, ROC analysis was performed and shown evidence supporting that the Thai DrHy-Q was suitable as a disease-specific instrument. However, our study did not investigate the responsiveness of the Thai DrHy-Q to interventions (e.g. oral provocation test or drug desensitization), the different underlying diseases, and the difference between patients experiencing drug hypersensitivity once and patients with recurrent events.

We demonstrate that the Thai version of DrHy-Q is both reliable and valid for evaluating patients with drug hypersensitivity among Thai population, and capture more specific drug-hypersensitivity aspects than the WHOQOL-BREF-THAI does. The instrument was non-invasive and low cost. The Thai DrHy-Q is suitable for use in the future studies in assessing QoL among patients with drug hypersensitivity. In this study, we observed poorer QoL among patients with allergic reactions to multiple drug classes or life-threatening SCAR. Future studies should include other factors, such as underlying diseases and number of drug-hypersensitivity reactions, investigate long-term impact of the drug hypersensitivity experience, and design a care approach such that patient's QoL is maintained.

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Review Boards of Faculty of Medicine Chulalongkorn University and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all patients included in the study.

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Appendix

Thai version of Drug Hypersensitivity Questionnaire.

เลขที่ผู้ป่วย _____ ผู้มีประวัติแพ้ยาเป็นผู้ให้ข้อมูล

อักษรย่อชื่อผู้ป่วย (Initial) ❑❑ วันที่เก็บข้อมูล (วัน/เดือน/ปี) ____/____/____

ปฏิกิริยาข้างเคียงจากการใช้ยาสามารถส่งผลกระทบต่อสุขภาพจิตของแต่ละบุคคล
กรุณาระบุความยากลำบากที่ท่านได้รับเนื่องจากปัญหานี้
(โปรดทำเครื่องหมาย ☒ ลงในช่องเพียงช่องเดียวที่ตรงกับคำตอบของคุณมากที่สุด)

	ไม่เลย ▼	เล็กน้อย ▼	ปานกลาง ▼	มาก ▼	มากอย่างยิ่ง ▼
1. เนื่องจากไม่สามารถใช้ยาได้ ฉันจึงมีข้อจำกัดเมื่อเป็นโรคต่างๆ มากกว่าผู้อื่น	□1	□2	□3	□4	□5
2. ฉันกลัวว่าในกรณีฉุกเฉิน ฉันอาจจะถูกจ่ายยาสัวที่ฉันแพ้	□1	□2	□3	□4	□5
3. ปัญหาเรื่องยาของฉันทำให้ฉันรู้สึกหวาดกลัว	□1	□2	□3	□4	□5
4. ปัญหาเรื่องปฏิกิริยาที่เกิดจากยามีผลกระทบต่อชีวิตฉัน	□1	□2	□3	□4	□5
5. ฉันต้องการความเห็นของแพทย์ผู้เชี่ยวชาญด้านโรคภูมิแพ้ก่อนที่จะใช้ยาที่สั่งจ่ายโดยผู้เชี่ยวชาญด้านอื่นๆ	□1	□2	□3	□4	□5
6. แม้ความรู้สึกไม่สบายเพียงเล็กน้อยก็กลายเป็นปัญหาสำหรับฉันได้	□1	□2	□3	□4	□5
7. ความจริงที่ว่าไม่สามารถใช้ยาได้อย่างปลอดภัย ทำให้ฉันรู้สึกแตกต่างจากผู้อื่น	□1	□2	□3	□4	□5
8. ปัญหาเรื่องปฏิกิริยาที่เกิดจากยาของฉันทำให้ฉันรู้สึกวิตกกังวล	□1	□2	□3	□4	□5
9. ฉันอยากมั่นใจว่า ในแต่ละโรคมียาที่ฉันสามารถใช้ได้อย่างปลอดภัย	□1	□2	□3	□4	□5
10. ฉันกลัวว่าจะไม่สามารถได้รับการรักษาเมื่อมีอาการเจ็บปวด	□1	□2	□3	□4	□5
11. ปัญหาการแพ้ยาทำให้ฉันรู้สึกกลัดกลุ้ม	□1	□2	□3	□4	□5
12. ฉันรู้สึกวิตกกังวลทุกครั้งที่ต้องใช้ยา ถึงแม้จะเป็นยาคนละชนิดกับที่ฉันแพ้	□1	□2	□3	□4	□5
13. ฉันทิ้งโอกาสทำกิจกรรมพักผ่อนหย่อนใจต่างๆ (เช่น กีฬา, พักร้อน, ท่องเที่ยว, ฯลฯ) เนื่องจากปัญหานี้ของฉัน	□1	□2	□3	□4	□5
14. เพราะปัญหาแพ้ยาของฉัน ทำให้ฉันรู้สึกหดหู่	□1	□2	□3	□4	□5
15. เพียงมีความคิดที่จะใช้ยา ก็ทำให้ฉันรู้สึกกังวลใจ	□1	□2	□3	□4	□5

ท่านคิดว่า ภาษาที่ใช้ในแบบสอบถาม เข้าใจยากง่ายมากน้อยเพียงใด (เข้าใจยากที่สุด = 1, เข้าใจง่ายที่สุด = 10)

□1 □2 □3 □4 □5 □6 □7 □8 □9 □10

เวลาที่ใช้ในการตอบแบบสอบถาม _____ นาที

Original Article

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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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.

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

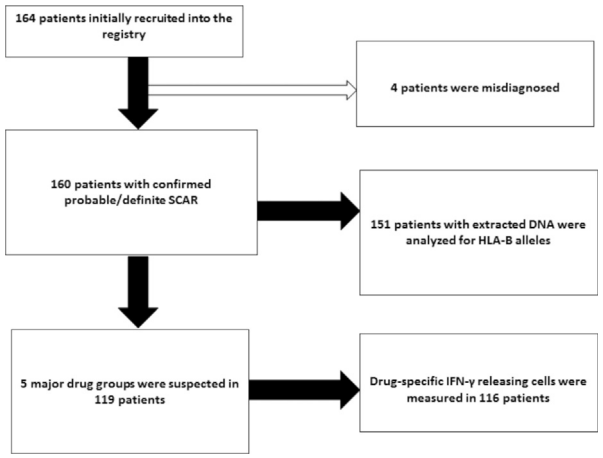

FIGURE 1. Schematic diagram of patient selection for this study. A total of 160 patients with confirmed probable or definite SCARs were recruited in the registry between 2013 and 2016. HLA-B genotyping was performed in 151 patients who had genomic DNA extracted. Drug-specific IFN-γ-releasing cells were 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 μg/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 μL) 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

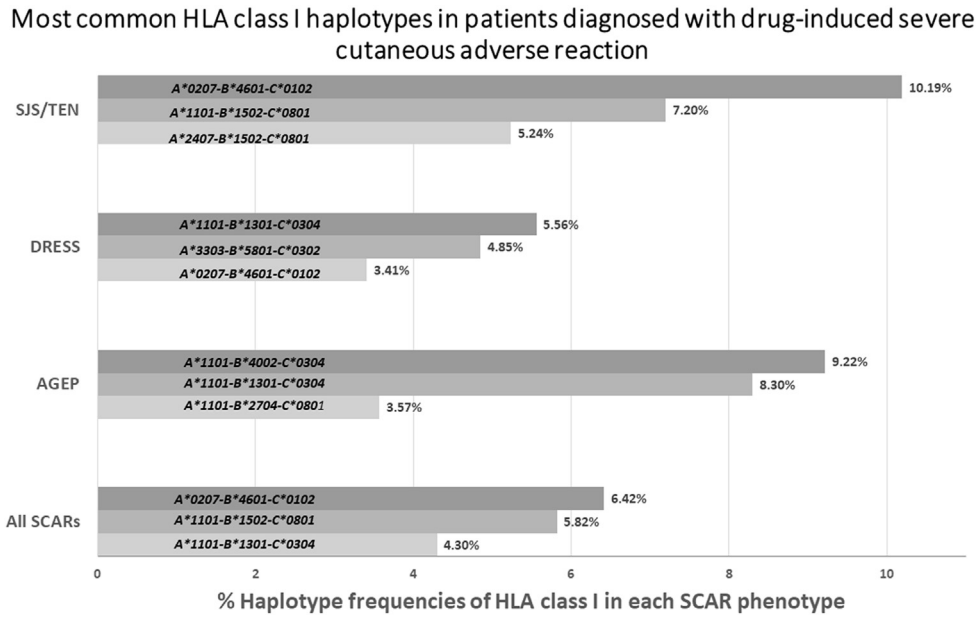

FIGURE 2. Allele frequency of the top 3 HLA class I haplotypes in patients diagnosed with each phenotype of drug-induced severe cutaneous adverse reaction (N = 151). *A*0207-B*4601-C*0102*, *A*1101-B*1502-C*0801*, and *A*1101-B*1301-C*0304* were the most common HLA class I haplotypes in 151 patients diagnosed with drug-induced SCARs in Thailand. The most frequent HLA class I haplotypes observed in the AGEP, 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. *AGEP*, 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)

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

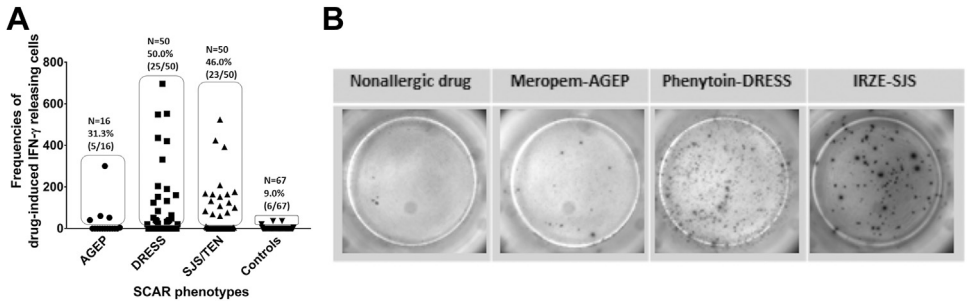

FIGURE 3. Quantification of drug-specific IFN-γ-releasing cells in different SCAR phenotypes during the acute allergic phase. **A,** A scatter dot plot of frequencies of drug-specific IFN-γ-releasing cells/10^6 PBMCs in 116 patients diagnosed with different phenotypes of severe cutaneous adverse reactions (16 AGEP, 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 AGEP] 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 AGEP, (3) phenytoin-induced DRESS, (4) antituberculosis agents (IRZE)-induced SJS. *AGEP*, 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*		
AGEP	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)

AGEP, 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 AGEP 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

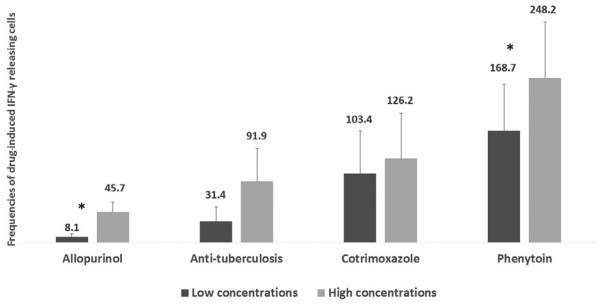

FIGURE 4. Dose-response of drug-specific IFN-γ-releasing cells for selected culprit drugs. Average frequencies of drug-specific IFN-γ-releasing cells/10^6 PBMCs at different concentrations of selected culprit drugs are shown. IFN-γ ELISpot responses were significantly higher on stimulation with high 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

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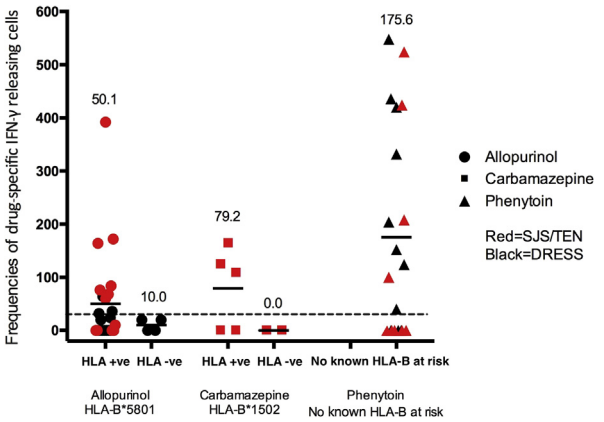

FIGURE E1. Frequencies of drug-specific IFN-γ-releasing cells/10^6 PBMCs of allopurinol, carbamazepine, and phenytoin are categorized by HLA-B risk alleles. Values above the dashed line indicate the positive IFN-γ ELISpot assay. There were trends that the absence of HLA-B risk alleles in patients with 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.

ORIGINAL ARTICLE

The measurement of drug-induced interferon γ-releasing cells and lymphocyte proliferation in severe cutaneous adverse reactions

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Abstract

Background The lymphocyte transformation test (LTT) is a standard laboratory method to identify culprit drugs in patients with a history of drug-induced non-immediate hypersensitivity and is mainly performed during the recovery phase. The measurement of drug-specific interferon γ (IFN-γ)-releasing cells has been introduced to confirm culprit drugs, even during the acute phase of drug allergy.

Objectives This study aimed to evaluate the capability of the enzyme-linked immunospot assay (ELISpot) to detect drug-specific IFN-γ-releasing cells during the acute phase and the capability of LTT to identify culprit drugs during the recovery phase in patients presenting with severe cutaneous adverse reactions (SCARs).

Methods Peripheral blood mononuclear cells (PBMCs) from 23 SCAR patients were collected during the acute and recovery phases and assayed for drug-specific IFN-γ-releasing cells and lymphocyte proliferation, respectively.

Results Drug-specific IFN-γ-releasing cells were detectable in 73.9% of SCAR subjects (55.6% and 85.7% in patients who were and were not taking systemic steroids, respectively), whereas LTT results were positive in 52.2% of SCAR subjects. The frequencies of drug-specific IFN-γ-releasing cells were significantly higher in patients with positive LTT than in those with negative LTT (260.1 ± 110.0 and 46.6 ± 20.7 cells/10^6 PBMCs, $P = 0.01$). A significant correlation between the results of the IFN-γ ELISpot assay and LTT was demonstrated ($r = 0.65$, P value <0.01).

Conclusion The IFN-γ ELISpot assay could be a useful tool to identify culprit drugs in SCAR patients when culprit drug identification is urgently needed during the acute phase of drug allergy.

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Conflicts of Interest

All authors declare that there are no conflicts of interest. All authors have read and approved the final manuscript.

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Introduction

Adverse drug reactions are a major health problem worldwide. Up to 6.7% of these reactions could be considered severe.[1] Severe cutaneous adverse reactions (SCARs) are comprised of acute generalized exanthematous pustulosis (AGEP), drug reaction with eosinophilia and systemic symptoms (DRESS), and Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN).[2] Although the immunopathogenesis of each phenotype is not completely understood, all are believed to be T-cell mediated.[3] Culprit drug identification in SCAR is difficult and mainly based on clinical history. Causality assessments of drug allergy based on various approaches often lead to different conclusions, and inter-rater reliability can be low.[4,5] A drug provocation test is rarely performed in SCARs, because re-administration of the suspected culprit drug can lead to serious consequences.[6]

Skin patch testing is a preferred *in vivo* method to identify the culprit drug. However, the recommended time interval for allergological evaluation is between 6 weeks and 6 months after the complete healing of cutaneous reactions, and the sensitivity is generally low.[7,8] Intradermal testing has a better sensitivity but is not encouraged due to the possibility of developing a severe skin reaction and aggravating the previous SCAR reaction.[9]

The lymphocyte transformation test (LTT) is the most commonly used *in vitro* technique to identify the culprit drug in drug-induced non-immediate hypersensitivity reactions.[10] The recommended time to perform LTT is during the remission stage after drug allergy symptoms subside, although the optimum test time may depend on the drug-allergic phenotypes as reported by some research groups.[11] Therefore, LTT may not be the ideal *in vitro* test for patients in whom culprit drug identification is urgently needed during the acute period.

The enzyme-linked immunospot (ELISpot) assay is a sensitive technique to detect antigen-specific T cells and has been introduced to identify drug-specific T cells in SCAR patients.[12] The turnaround time of this technique is shorter than that of LTT and does not involve radioisotope use. There is evidence that the frequencies of drug-specific IFN-γ-releasing cells are significantly correlated with the lymphocyte proliferation response measured by LTT in patients with a history of beta-lactam-induced maculopapular exanthema.[13] Measurement of drug-specific T cells is more sensitive than the skin patch test to identify the culprit drug in cephalosporin-allergic patients.[14] The detection of drug-induced IFN-γ-releasing cells in various manifestations of drug allergy suggests that the IFN-γ ELISpot assay is a potential *in vitro* tool for culprit drug confirmation in SCAR patients. In fact, a study in children reported that the ELISpot assay could be performed during the acute phase of a severe drug hypersensitivity reaction and yielded sensitivity equal to or better than that of LTT in identifying the culprit drug.[15]

It would be useful to compare the ability of the IFN-γ ELISpot assay and LTT in terms of culprit drug confirmation in the same SCAR subjects. The objective of this study was to evaluate the potential for measuring drug-specific IFN-γ-releasing cells by ELISpot assay during the acute phase compared with performing the lymphocyte transformation test after the recovery phase to identify the causative drugs in SCAR patients. The correlation between the results of the IFN-γ ELISpot assay and LTT in SCAR individuals was also analysed.

Materials and methods

Patient characteristics and blood acquisition

A total of 23 patients diagnosed with probable or definite SCAR according to the RegiSCAR criteria were enrolled in this study.[16–18] These patients are part of the Thailand Severe Cutaneous Adverse Reactions (ThaiSCAR) cohort registered at ClinicalTrials.gov (NCT02574988). This study was approved by the Ethics and Research Committee of the Faculty of Medicine, Chulalongkorn University, and informed consent was obtained from all participants. The suspected culprit drugs were assessed according to the Naranjo adverse drug reaction probability scale and later confirmed by using two different *in vitro* techniques.

The determination of drug-specific IFN-γ-releasing cells by ELISpot assay

Peripheral blood mononuclear cells were collected within 2 weeks of acute drug-allergic reaction and resuspended in freezing medium to create a cell suspension of 5×10^6 cells per mL. The cells were transferred to −80°C overnight before cryopreservation in liquid nitrogen storage until later use. The number of drug-specific IFN-γ-releasing cells was measured by using ELISpot assay kits (Mabtech, Stockholm, Sweden), as described in our previous study (14). In brief, 96-well plates (MSIP N4550; Millipore, Bedford, MA, USA) were coated with 5 μg/mL anti-human IFN-γ antibody at 4°C overnight, and then the plate was blocked with R10 medium (RPMI1640 with 100 U/mL penicillin, 100 U/mL streptomycin and 10% heat-inactivated foetal bovine serum; Bio Whittaker, Walkersville, MD, USA) for at

Biological specimens analysed in this study were acquired from patients enrolled in the Severe Cutaneous Adverse Reactions in Thailand registry. The registry was approved by the Ethics and Research Committee of the Faculty of Medicine, Chulalongkorn University, and listed on ClinicalTrials.gov as NCT02574988.

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least 1 h at room temperature. PBMCs (2.5×10^5 cells/well) were cultured at 37°C with 5% CO_2 for 40 h with the suspected culprit drugs. After incubation, the cells were removed and washed six times with phosphate-buffered saline, then incubated for 1.5 h at 37°C with 1 μg/mL biotinylated anti-IFN-γ antibody and washed extensively. Spot-forming cells were developed using streptavidin–alkaline phosphatase, incubated for 1 h at 37°C and washed extensively before the substrate was added. The results are expressed as the numbers of IFN-γ spot-forming cells (SFC) per 10^6 PBMCs cultured with the drug, after subtracting the values obtained from PBMCs cultured without the drug.

Peripheral blood mononuclear cells from 20 non-allergic individuals were also tested with beta-lactams, phenytoin, oxypurinol and NSAIDs (five drugs each) to evaluate non-specific IFN-γ responses. The average background frequency of IFN-γ-releasing cells after subtraction of the values obtained from PBMCs cultured without drug in non-allergic individuals was 3.4 ± 7.3 SFC/10^6 PBMCs (data not shown). Therefore, the frequency of drug-specific IFN-γ SFC was considered positive in this study if it was >18.0 IFN-γ SFC/10^6 PBMCs (mean ± 2 SD).

Lymphocyte transformation assay (LTT)

Patients' PBMCs were freshly isolated on the follow-up visit between 3 and 6 months after drug-allergic symptoms subsided. All patients were free of systemic steroids for at least 4 weeks before blood collection. The cells were washed twice and resuspended in RPMI 1640 supplemented with 100 U/mL penicillin, 100 U/mL streptomycin, 10% pooled AB plasma and 0.025% human holo-transferrin. The lymphocyte transformation assay was performed as described previously.[19] In brief, PBMCs (200 000 cells/well) were cultured at 37°C in 5% CO_2 with three different concentrations of the suspected culprit drug. After 6 days, 0.4 μCi ^{3}H-thymidine (PerkinElmer, Boston, MA, USA) was added and incubated overnight before measurement of thymidine incorporation in counts per minute by using a β-counter (PerkinElmer). Proliferative responses were expressed as a stimulation index (SI): SI = counts per minute in the cell cultures with the suspected culprit drug divided by counts per minute in the culture with medium alone.

Statistical analysis

The concentrations of drugs used for the IFN-γ ELISpot assay and LTT are listed in Table S1. The results of the IFN-γ ELISpot assay are shown as the highest frequencies of IFN-γ-releasing cells (SFC/10^6 PBMCs) upon stimulation with two concentrations of the suspected culprit drugs, after subtracting the value obtained from PBMCs cultured without drugs. In the case of LTT, the results were considered positive when the stimulation index was ≥2. Spearman's correlation analysis was used to evaluate the correlation between the frequencies of drug-specific IFN-γ-releasing cells and the stimulation indexes of LTT. McNemar's test was used to compare the positive rates between the IFN-γ ELISpot assay and LTT. Statistical analysis was performed by using Prism version 5 Software (GraphPad Prism, San Diego, CA, USA). Results were considered statistically significant at $P < 0.05$.

Results

Clinical characteristics of SCAR patients recruited in this study

A total of 23 patients who developed drug-induced SCAR (4 AGEP, 9 DRESS and 10 SJS/TEN) were included in this study. The suspected culprit drugs were anticonvulsants (39.1%), allopurinol (26.1%), beta-lactam antibiotics and analgesic drugs (17.4% each). Seventeen patients (73.9%) were female, with an average age of 49.2 ± 15.3 years. Nine of them (3 DRESS, 6 SJS/TEN) had received systemic corticosteroids prior to PBMC collection for the IFN-γ ELISpot assay, as shown in Table 1.

Measurement of drug-specific IFN-γ-releasing cells in the acute allergic phase and results of the lymphocyte transformation test (LTT) in the recovery phase of SCARs

The frequencies of drug-specific IFN-γ-releasing cells measured by using the ELISpot assay (Fig. 1), and the stimulation indexes measured by LTT in different phenotypes of SCARs are shown in Fig. 2a. The average frequency of drug-specific IFN-γ-releasing cells and stimulation index after stimulation with the suspected culprit drugs were 157.9 ± 61.3 SFC/10^6 PBMCs and 20.5 ± 11.1, respectively. The maximal cellular responses were observed in DRESS phenotypes as evaluated by either IFN-γ or LTT assays. A significant correlation between the frequencies of drug-specific IFN-γ-releasing cells measured during the acute phase and the stimulation indexes measured during the recovery phase of severe cutaneous adverse reactions are demonstrated in Fig. 2b ($r = 0.65$, $P < 0.01$).

Comparative sensitivities of the IFN-γ ELISpot assay and LTT for culprit drug identification in SCARs

Positive LTT assays, as shown by SIs ≥2, were demonstrated in 52.2% (12/23) of SCAR subjects, whereas drug-specific IFN-γ-releasing cells were detectable in 73.9% (17/23) of SCAR subjects in this cohort. Considering values greater than the means +2 SD of IFN-γ-releasing cells stimulated by irrelevant drugs in non-allergic controls (18.0 SFC/10^6 PBMCs) as positive for ELISpot assay (Fig. 3), the sensitivity of the IFN-γ ELISpot for culprit drug confirmation is 69.6% (77.8% for DRESS, 50.0% for SJS/TEN and 100.0% for AGEP). The sensitivity of a positive LTT assay (SI ≥ 2) was 66.7% for DRESS, 40.0% for SJS/TEN and 50.0% for AGEP. According to the McNemar's test, however, the positivity rate of IFN-γ ELISPOT was not significantly higher than that of LTT in identifying the culprit drugs ($P = 0.22$).

Table 1 Clinical characteristics of 23 SCAR patients and results of IFN-γ-releasing cell measurement by enzyme-linked immunospot (ELISpot) assay in the acute phase and results of the lymphocyte transformation test (LTT) in the recovery phase

No.	Sex	Age	Clinical manifestations	Culprit drugs	Concurrent steroid	ELISpot (SFC/10^6 PBMCs)	LTT (SI)
1	F	37	DRESS	Phenytoin	None	332	58.4
2	F	54	DRESS	Phenytoin	None	204	246.9
3	F	46	DRESS	Phenytoin	Dexa, 15 mg/day	124	43.7
4	F	44	DRESS	Allopurinol	Dexa, 16 mg/day	56	1.5
5	M	51	DRESS	Allopurinol	Pred, 40 mg/day	12	1.0
6	F	68	DRESS	Phenytoin	None	1392	76.2
7	F	72	TEN	Allopurinol	None	28	2.7
8	F	32	SJS/TEN	Carbamazepine	Dexa, 20 mg/day	124	2.5
9	M	36	SJS/TEN	Carbamazepine	None	44	2.1
10	F	40	SJS	Tramadol	Dexa, 8 mg/day	0	2.7
11	F	40	SJS	Carbamazepine	Dexa, 20 mg/day	108	1.2
12	F	32	SJS	Phenytoin	Dexa, 20 mg/day	0	1.3
13	M	65	AGEP	Meropenem	None	60	14.2
14	F	44	AGEP	Ceftriaxone	None	220	1.9
15	F	34	AGEP	Ibuprofen	None	40	1.3
16	F	75	SJS	Allopurinol	None	76	1.7
17	F	51	DRESS	Allopurinol	None	84	2.9
18	F	44	AGEP	Amoxicillin	None	300	2.3
19	M	58	DRESS	Allopurinol	None	428	2.1
20	F	47	SJS	Ceftriaxone	Dexa, 20 mg/day	0	1.4
21	M	21	SJS	Ibuprofen	Dexa, 12 mg/day	0	1.0
22	F	63	SJS	Mefenamic acid	None	0	1.2
23	M	78	DRESS	Phenytoin	None	0	1.1

M, male; F, female; AGEP, acute generalized exanthematous pustulosis; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis; DRESS, drug reaction with eosinophilia and systemic symptoms; LTT, lymphocyte transformation test; ELISpot, enzyme-linked immunospot, SI, stimulation index; SFC, spot-forming cells; Dexa, dexamethasone; Pred, prednisolone; PBMCs, peripheral blood mononuclear cells.

The frequencies of drug-specific IFN-γ-releasing cells in SCAR patients categorized by concurrent systemic steroid use and LTT results

The results of drug-specific IFN-γ-releasing cell measurement were analysed in relation to the concurrent systemic steroid usage and LTT results in Fig. 4. The frequencies of drug-specific IFN-γ-releasing cells in patients who received systemic steroids and those who did not were 47.1 ± 18.9 SFC/10^6 PBMCs and 229.2 ± 96.6 SFC/10^6 PBMCs, respectively (P = 0.07). SCAR patients with a positive LTT result had significantly more drug-specific IFN-γ-releasing cells compared to those with a negative LTT result (260.1 ± 110.0 SFC/10^6 PBMCs vs. 46.6 ± 20.7 SFC/10^6 PBMCs, P value = 0.01). IFN-γ ELISpot assays were positive in 44.4% (4/9) and 85.7% (12/14) of SCAR patients with and without systemic steroid administration, respectively. The rates of LTT positivity in the same patient groups during the recovery phase were 33.3% (3/9) and 64.3% (9/14), respectively.

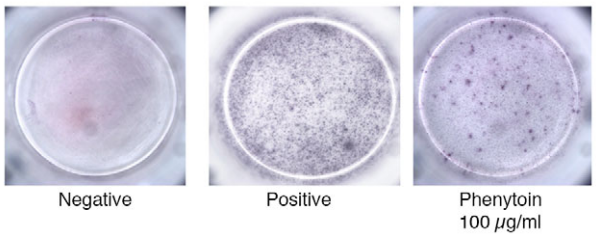

Figure 1 Representative figures of drug-specific IFN-γ response as demonstrated by the results of enzyme-linked immunospot assay after stimulation of PBMCs from a patient with a history of phenytoin-induced DRESS with different stimuli. PBMCs, peripheral blood mononuclear cells.

Discussion

The identification of culprit drugs in SCARs is a difficult task. Even the intradermal test can carry a risk of provoking SCAR symptoms. Because a drug provocation test is contraindicated in SCARs, laboratory diagnostic testing could be another option to identify culprit drugs without putting patients at risk. LTT is a standard proliferation-based *in vitro* test for drug allergy confirmation, commonly performed during the recovery phase. This study aimed to evaluate ELISpot in comparison with LTT as a tool to confirm the culprit drug during the acute symptomatic phase of SCARs.

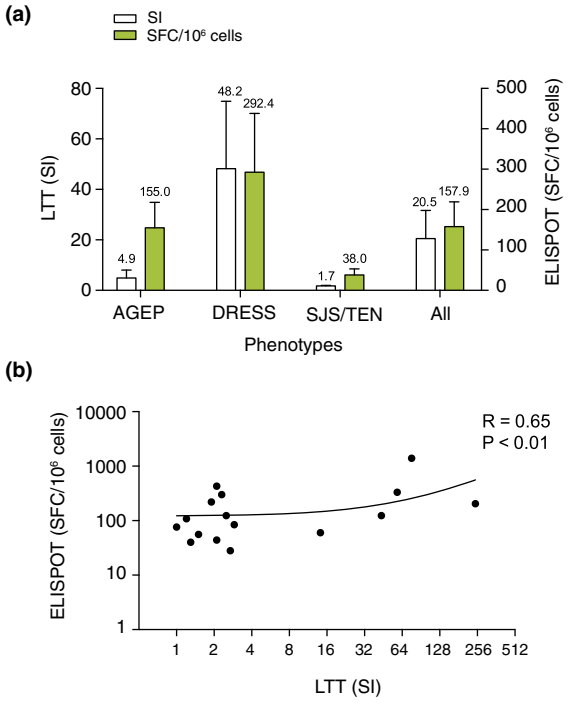

Figure 2 (a) The average frequencies of drug-specific IFN-γ-releasing cells and the stimulation indexes in different SCAR phenotypes. (b) Correlation between the frequencies of drug-specific IFN-γ-releasing cells measured during the acute phase and the stimulation indexes measured during the recovery phase of severe cutaneous adverse reactions. SCAR, severe cutaneous adverse reaction.

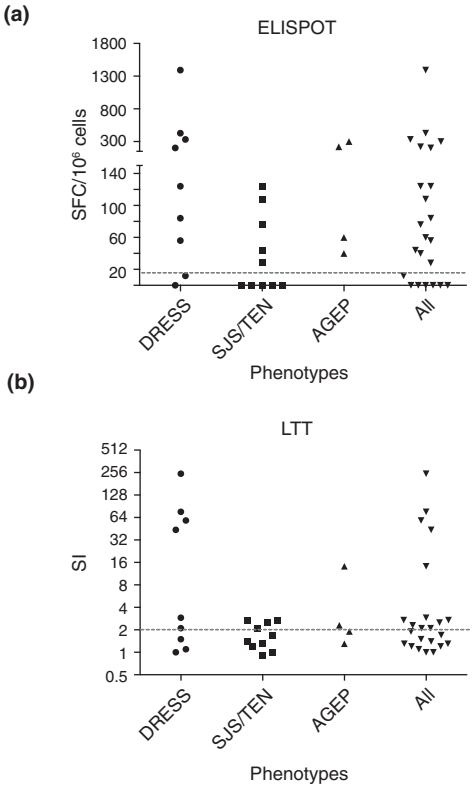

Figure 3 Results of the IFN-γ enzyme-linked immunospot assay and lymphocyte transformation test in different SCAR phenotypes (9 DRESS, 10 SJS/TEN and 4 AGEP). (a) Frequencies of IFN-γ-releasing cells measured by ELISpot (positive rate 69.6%) and (b) Stimulation indexes measured by LTT (positive rate 52.2%). AGEP, acute generalized exanthematous pustulosis; SCAR, severe cutaneous adverse reaction; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis; LTT, lymphocyte transformation test.

In addition to LTT, the results of our study indicate that the IFN-γ ELISpot assay could be utilized to confirm drug-inducing SCARs during the acute symptomatic phase. Both tests could serve different purposes. While LTT is still considered as a standard *in vitro* test for drug allergy diagnosis, IFN-γ ELISpot could be a useful tool to identify the culprit drug under certain conditions when culprit drug identification is urgently needed or in patients who cannot wait for LTT assay results after the test. The results of both tests, although not identical, were significantly correlated.

Both IFN-γ ELISpot and LTT assays demonstrated similar results in that drug-specific T cells in DRESS showed the highest response, whereas the confirmation of drug-induced SJS/TEN was more difficult. The frequencies of drug-specific IFN-γ-releasing cells were significantly higher in SCAR subjects with a positive LTT than in those with a negative LTT. The sensitivity of ELISpot depends on the diagnostic criteria, which are not yet well established. According to our study, the sensitivity of ELISpot was somewhat higher than that of LTT, but statistical significance was not reached. At present, different approaches have been applied to calculate the cut-off values of drug-specific IFN-γ-releasing cells to identify the culprit drugs.[12] The consensus criteria for culprit drug identification based on the IFN-γ ELISpot technique need to be established.

We observed lower frequencies of drug-specific IFN-γ-releasing cells in groups of patients receiving systemic steroids, although statistical analysis did not indicate significance. Nevertheless, it was difficult to conclude whether it was solely due to the effect of concurrent steroid usage, because the results of LTT performed after steroid discontinuation also showed the same trend. Further studies analyse the association between the results of IFN-γ ELISpot and the skin patch test or drug provocation test, if possible, would be helpful to evaluate the clinical diagnostic values of this technique in SCARs. Several possibilities could explain why the drug-specific IFN-γ-releasing cells were not detectable in about one-fourth of patients. The culprit drug identification, which is based on the Naranjo algorithm scale,

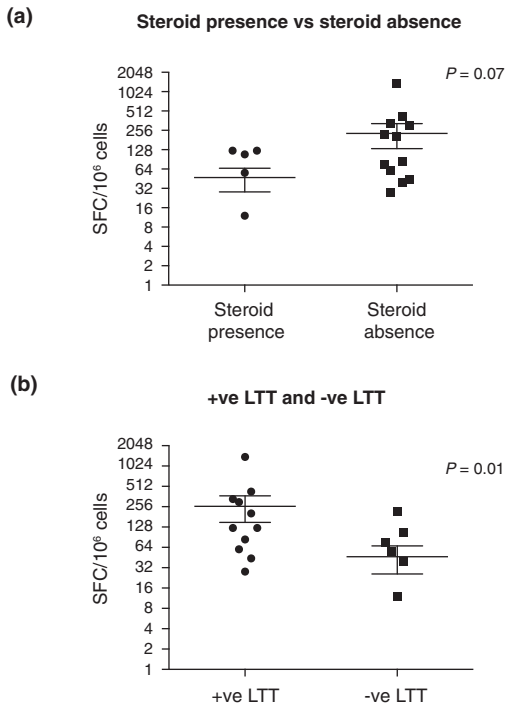

Figure 4 The frequencies of drug-specific IFN-γ-releasing cells in SCAR patients categorized by concurrent systemic steroid use and LTT results. (a) Patients with and without concurrent steroid use and (b) Patients with positive and negative LTT results. SCAR, severe cutaneous adverse reaction; LTT, lymphocyte transformation test.

may not always be accurate. Most of the patients with negative IFN-γ ELISpot results belonged to SJS phenotype, which granulysin, not IFN-γ, is known as a key molecule responsible for cell death in this SCAR group.[20] Some patients may be allergic to the active metabolites rather than to the native drug; as a result, the incubation of PBMCs with immunogenic metabolites may increase the yield of positive ELISpot responses in certain subjects. All of these could contribute to the negative IFN-γ ELISpot results in our cohort.

In conclusion, the IFN-γ ELISpot assay could be a useful tool for determining culprit drugs in patients suffering from SCAR during the acute allergic phase. Further studies are required to confirm diagnostic values of this test in different SCAR phenotypes. Additional factors affecting IFN-γ ELISpot results should be explored.

Acknowledgements

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Drug concentrations for interferon-gamma enzyme-linked immunospot assay (IFN-γ ELISpot) and lymphocyte transformation test (LTT)

BJD
British Journal of Dermatology

In vitro test to confirm diagnosis of allopurinol-induced severe cutaneous adverse reactions

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Conflicts of interest
None declared.

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Summary

Background Allopurinol is a frequent cause of severe cutaneous adverse reactions (SCARs), such as drug reaction with eosinophilia and systemic symptoms (DRESS), Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). The reactions can potentially be fatal. As drug rechallenge in patients with a history of drug-induced SCARs is contraindicated, *in vitro* testing may have a diagnostic role as a confirmation test.

Objectives To study the diagnostic value of interferon (IFN)-γ enzyme-linked immunospot (ELISpot) assay as a confirmatory test in patients with a history of allopurinol-induced SCARs.

Methods Peripheral blood mononuclear cells (PBMCs) from 24 patients with a history of allopurinol-induced SCAR (13 DRESS, 11 SJS/TEN) and 21 control subjects were incubated with allopurinol or oxypurinol in the presence or absence of antiprogrammed death ligand 1 antibody (anti-PD-L1). The numbers of IFN-γ-releasing cells after stimulation in each group were subsequently measured with ELISpot.

Results The numbers of IFN-γ-releasing cells in allopurinol-allergic subjects were significantly higher than in control subjects when stimulating PBMCs with oxypurinol 100 μg mL^{-1}, especially when adding anti-PD-L1 supplementation. According to the receiver operating characteristic curve results, the optimal discriminatory power of IFN-γ ELISpot in confirming diagnosis of allopurinol-induced SCARs can be obtained using 16 spot-forming cells per 10^6 PBMCs as a cut-off value upon oxypurinol/anti-PD-L1 stimulation (79·2% sensitivity and 95·2% specificity).

Conclusions The measurement of oxypurinol/anti-PD-L1-inducing IFN-γ-releasing cells yields a high diagnostic value in distinguishing between allopurinol-allergic and control subjects. This technique is beneficial in confirming diagnosis of allopurinol-induced SCARs in patients whose reaction develops while taking multiple drugs.

What's already known about this topic?

- Allopurinol is among the most common drugs causing severe cutaneous adverse reactions worldwide.
- The skin tests and *in vitro* assays currently available exhibit low sensitivity in verifying the diagnosis of allopurinol hypersensitivity in suspected cases.

What does this study add?

- This is the first study to introduce an *in vitro* test, demonstrating high diagnostic value in confirming the diagnosis of allopurinol-induced severe cutaneous adverse reactions.

Allopurinol is one of the drugs most commonly associated with Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), and is reported to have a high mortality rate.[1,2] Screening for human leucocyte antigen (HLA)-B*58:01 before the use of allopurinol has been shown to be beneficial in reducing the risk of allopurinol-induced SJS/TEN in certain Asian ethnic groups.[3,4] However, skin patch tests with allopurinol and its metabolite have been shown to have very low diagnostic value.[5] The allopurinol provocation test is also contraindicated in patients with a previous history of severe cutaneous adverse reactions (SCARs), as readministration of the drug can potentially be fatal.[3]

The sensitivity of the lymphocyte transformation test (LTT), an *in vitro* technique currently used to detect drug-specific T-cell proliferation, is approximately 60–70%. However, LTT is rarely positive in TEN (sensitivity < 10%).[6] Enzyme-linked immunospot (ELISpot) assay has recently been introduced for drug allergy diagnosis. The measurement of interferon (IFN)-γ-releasing cells with ELISpot has been demonstrated to have higher sensitivity than LTT and patch testing in patients with a history of allergic reaction to beta-lactams.[7,8]

High plasma levels of oxypurinol are correlated with poor prognosis in allopurinol-induced SJS/TEN,[9] and an oxypurinol-specific lymphocyte response has been detected in allopurinol-allergic patients who carry the HLA-B*58:01 allele.[10] Considering these points in conjunction, it is possible that the detection of a T-cell response to oxypurinol may be more sensitive in the diagnosis of allopurinol hypersensitivity than using allopurinol itself. There is evidence that the detection of drug-specific T-cell response could be diminished by the enhanced activity of regulatory T cells (Tregs), particularly in the acute phase of drug reaction with eosinophilia and systemic symptoms (DRESS) in drug-allergic subjects.[11] As the programmed death (PD)1/programmed death ligand (PD-L)1 pathway plays a pivotal role in the maintenance of Treg function,[12] the addition of an antibody to PD-L1 (anti-PD-L1) to block this pathway may be helpful to identify the culprit drug. In particular, the study of Gibson *et al.*[13] reported that PD-L1/PD1 signalling negatively regulates the priming of drug antigen-specific T cells and PD-L1 blockade, resulting in an increase in IFN-γ secretion when drug-specific cell clones are stimulated.

Ideally, an *in vitro* test to diagnose allopurinol-induced SCARs should have the ability to differentiate between patients with allopurinol-induced SCAR and patients who develop an allergic reaction from the other drugs. The purpose of this study was to evaluate the diagnostic value of IFN-γ ELISpot as a confirmatory test in patients with a suspected history of allopurinol-induced SCARs. A comparative analysis was undertaken of the frequencies of IFN-γ-releasing cells from peripheral blood mononuclear cells (PBMCs) incubated with allopurinol and oxypurinol in the presence or absence of anti-PD-L1 between allopurinol-allergic patients and control subjects.

Materials and methods

Specimens from allopurinol-allergic patients and control subjects

Cryopreserved PBMCs from 24 patients diagnosed with allopurinol-induced SCAR [13 drug reaction with eosinophilia and systemic symptoms (DRESS), 11 SJS/TEN] were used for this study. The diagnosis of DRESS was made according to the RegiSCAR criteria.[14] Drug causality assessment in SJS/TEN was carried out using the ALDEN algorithm.[15] Cryopreserved PBMCs from 21 patients who presented with allergic reactions to other drugs or were allopurinol tolerant according to an oral provocation test were used as control subjects.

The measurement of interferon-γ-releasing cells after stimulation of peripheral blood mononuclear cells with allopurinol and oxypurinol

The numbers of IFN-γ-releasing cells were measured using ELISpot assay kits (Mabtech, Stockholm, Sweden), as described in our previous study.[8] Briefly, 96-well nitrocellulose membrane plates (MAIP S45; Millipore, Bedford, MA, U.S.A.) were coated for 16 h at 4 °C with the 5-μg mL^{-1} anti-IFN-γ antibody provided in the kit, and blocked with R10 medium (RPMI1640

supplemented with 100 U mL^{-1} penicillin, 100 U mL^{-1} streptomycin and 10% heat-inactivated fetal bovine serum; Bio Whittaker, Walkersville, MD, U.S.A.) for 1 h at room temperature. PBMCs ($2{\cdot}5 \times 10^5$ in 100 μL) were incubated for 48 h at 37 °C in 5% CO_2 with allopurinol or oxypurinol at 10 and 100 μg mL^{-1} (Sigma-Aldrich, St Louis, MO, U.S.A.) in the presence or absence of anti-PD-L1 (10 mg mL^{-1}; BioLegend, San Diego, CA, U.S.A.). The plates were washed six times with phosphate-buffered saline/Tween 0·05%, incubated for 1·5 h at 37 °C with biotinylated anti-IFN-γ antibody and then washed extensively. Spot-forming cells (SFCs) were developed using streptavidin–alkaline phosphatase, incubated for 1 h at 37 °C and washed extensively before adding the substrate. The results are expressed as the numbers of IFN-γ SFCs per 10^6 PBMCs cultured with the drug, after subtracting the values obtained from PBMCs cultured without the drug. An example of the IFN-γ ELISpot results from a patient with allopurinol hypersensitivity is shown in Figure 1.

Statistical analysis

Student's t-test was used to compare the quantitative data, and Levene's test was used to test for the homogeneity of variance between groups. The results are expressed as medians and ranges (nonparametric data) or means with SE (parametric data). Receiver operating characteristic (ROC) curve analyses were performed to determine the optimal cut-off value of IFN-γ-releasing cells, incubating with different stimulatory panels to confirm the diagnosis of allopurinol hypersensitivity. All statistical calculations were analysed using SPSS 21 (IBM, Armonk, NY, U.S.A.). $P < 0{\cdot}05$ were considered statistically significant.

Ethics

The PBMCs employed in this experiment were cryopreserved specimens from patients enrolled in the Thailand Severe Cutaneous Adverse Reactions (ThaiSCAR) registry. 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 is registered at ClinicalTrials.gov (NCT02574988).

Results

The baseline characteristics of the patients will allopurinol-induced SCARs and control subjects, and the IFN-γ ELISpot results are shown in Tables 1 and 2. PBMCs from 24 patients with allopurinol-induced SCARs were used for this study. Nine of the patients were male and 15 were female, with an average age of $62{\cdot}5 \pm 2{\cdot}4$ years. The median duration after symptom onset to collection of PBMCs was 7·5 days (range 1–365). Clinical presentations were DRESS and SJS/TEN in 13 and 11 patients, respectively. The HLA*B58:01 allele presented in 83% of patients. At the time of PBMC isolation, 75% of patients were in the acute phase (symptomatic drug-allergic reaction) and 50% of patients were receiving systemic steroid treatment.

PBMCs from 21 individuals were used as controls. Of these, 11 patients had a history of DRESS or SJS/TEN from other drugs besides allopurinol, four patients had a history of cephalosporin-induced maculopapular exanthems without a history of allopurinol hypersensitivity, and six patients had a suspected history of allopurinol-induced allergy for which a later provocation test was negative.

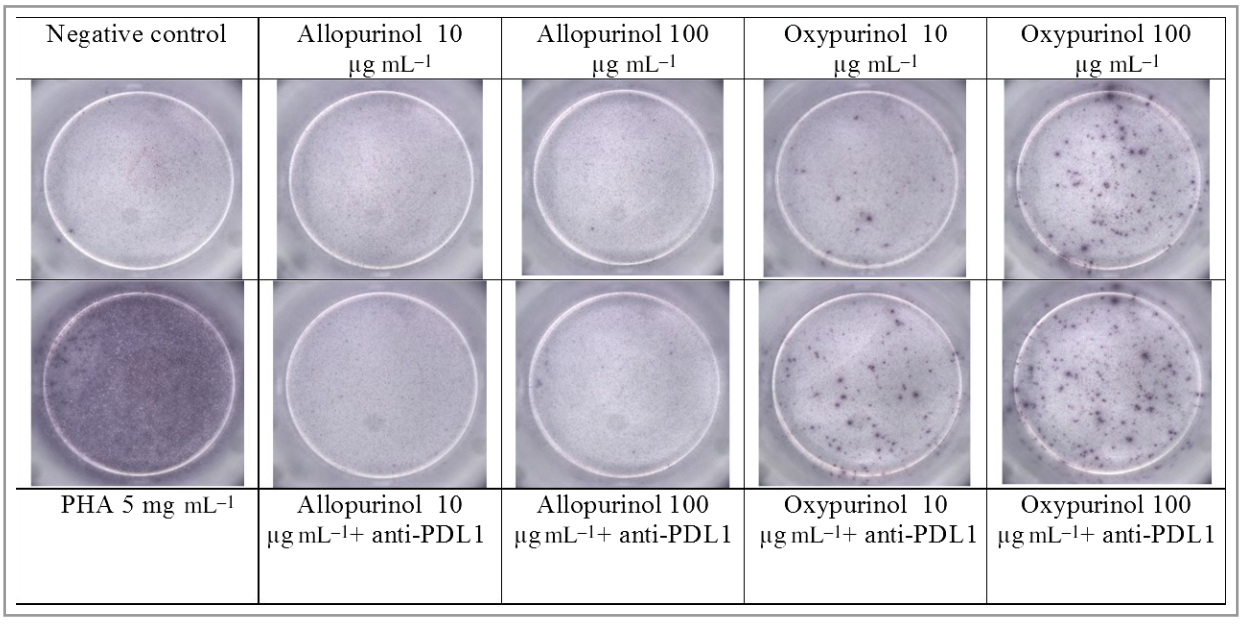

Fig 1. Interferon-γ (IFN-γ) enzyme-linked immunospot assay. Representative figures of IFN-γ-releasing cells after stimulating peripheral blood mononuclear cells with different reagents in allopurinol-allergic patients. PHA, phytohaemagglutinin; anti-PDL1, antiprogrammed death ligand 1 antibody.

Table 1 The results of interferon-γ (IFN-γ) enzyme-linked immunospot assay in patients with allopurinol-induced severe cutaneous adverse reactions (SCARs)

						Frequencies of IFN-γ -releasing cells per 10^6 PBMCs								
							Allopurinol (μg mL^{-1})		Oxypurinol (μg mL^{-1})		Allopurinol (μg mL^{-1}) + anti-PD-L1		Oxypurinol (μg mL^{-1}) + anti-PD-L1	
Patient no.	Sex, age (years)	SCAR type	HLA-B*58:01	Time after reaction	Concurrent steroid (mg per day)	PHA	10	100	10	100	10	100	10	100
1	M, 51	DRESS	Negative	3 days	Pred 40	2616	0	12	0	20	8	60	148	280
2	M, 68	SJS/TEN	Negative	7 days	Dexa 20	908	0	0	0	392	0	0	0	756
3	F, 72	SJS/TEN	Positive	1 year	None	1180	4	0	4	8	4	0	4	60
4	M, 64	DRESS	Positive	7 days	Pred 5	4236	4	0	0	52	4	4	0	104
5	M, 86	DRESS	Negative	80 days	None	3008	0	12	0	20	0	36	4	28
6	F, 53	DRESS	Positive	2 days	Dexa 10	2972	0	4	12	32	80	72	84	112
7	F, 73	DRESS	Positive	10 days	None	3584	0	0	352	236	64	148	200	420
8	F, 54	SJS/TEN	Positive	66 days	None	3056	0	0	10	0	0	0	32	48
9	M, 68	SJS/TEN	Positive	10 days	Dexa 10	2368	0	0	4	32	0	0	0	156
10	F, 84	SJS/TEN	Positive	7 days	Methylpred 60	2910	10	0	5	10	0	5	0	0
11	F, 47	SJS/TEN	Positive	1 day	None	2692	0	0	0	68	0	0	0	100
12	F, 70	SJS/TEN	Positive	1 year	None	3752	0	0	0	24	0	0	20	28
13	F, 75	SJS/TEN	Positive	2 days	None	3932	0	0	0	76	0	0	0	120
14	M, 46	DRESS	Positive	1 year	None	2628	0	0	0	0	0	0	0	0
15	F, 51	DRESS	Negative	6 days	None	2468	0	0	0	84	0	0	12	168
16	M, 55	SJS/TEN	Positive	84 days	None	2276	0	0	0	0	0	0	0	0
17	M, 58	DRESS	Positive	1 day	Pred 30	2220	0	0	72	428	0	0	248	572
18	F, 43	DRESS	Positive	5 day	Pred 30	4616	0	0	0	20	56	48	48	92
19	F, 65	DRESS	Positive	7 day	None	3612	0	0	0	36	0	0	16	32
20	F, 55	DRESS	Positive	9 day	Dexa 10	2048	0	0	0	0	0	4	0	36
21	F, 58	SJS/TEN	Positive	1 day	None	4664	0	0	16	60	0	0	0	0
22	F, 68	SJS/TEN	Positive	12 day	Methylpred 20	1032	0	0	0	0	0	0	0	0
23	M, 78	DRESS	Positive	27 day	Dexa 20	1792	0	0	0	0	0	0	0	64
24	F, 57	DRESS	Positive	8 day	Dexa 12	4120	0	0	0	0	0	0	0	130

HLA, human leucocyte antigen; PHA, phytohaemagglutinin; M, male; F, female; DRESS, drug reaction with eosinophilia and systemic symptoms; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis; Pred, prednisolone; Dexa, dexamethasone; Methylpred, methylprednisolone. Data from 24 patients with allopurinol-induced SCARs (13 DRESS, 11 SJS/TEN). Baseline characteristics and frequencies of interferon-γ-releasing cells [spot-forming cells per 10^6 peripheral blood mononuclear cells (PBMCs)] after PBMC stimulation with different concentrations of allopurinol and oxypurinol in the presence or absence of antiprogrammed death ligand 1 (anti-PD-L1) antibody (10 mg mL^{-1}). PBMCs were collected during the active stage of allopurinol hypersensitivity, except in patients 3, 5, 8, 12, 14 and 16.

Table 2 Results of interferon-γ (IFN-γ) enzyme-linked immunospot assay in control patients

						Frequencies of IFN-γ releasing cells per 10^6 PBMCs							
							Allopurinol (μg mL^{-1})		Oxypurinol (μg mL^{-1})		Allopurinol (μg mL^{-1}) + anti-PD-L1		Oxypurinol (μg mL^{-1}) + anti-PD-L1	
Patient no.	Sex, age (years)	Clinical presentation	Allergic drugs	Time after reaction	Concurrent steroid (mg per day)	PHA	10	100	10	100	10	100	10	100
1	F, 65	SJS	Celecoxib	8 days	Pred 40	2696	0	0	0	0	0	0	0	0
2	F, 31	DRESS	Bosutinib	4 days	Dexa 12	2408	0	0	0	0	0	0	0	0
3	M, 41	DRESS	Antituberculosis drugs	14 days	Dexa 5	1716	0	0	0	0	0	0	0	0
4	F, 54	DRESS	Antituberculosis drugs	21 days	Dexa 20	2368	0	4	0	0	0	4	0	0
5	M, 63	DRESS	Phenytoin	14 days	Dexa 15	2700	0	0	0	0	0	0	0	0
6	M, 64	DRESS	Dapsone	6 days	Pred 60	5495	0	0	0	0	0	0	0	0
7	F, 83	FDE	Allo (neg OPT)	2 years	None	2644	4	0	4	4	0	8	4	0
8	M, 78	MPE, eosinophilia	Allo (neg OPT)	14 days	None	3416	8	4	0	4	0	0	0	0
9	M, 73	DRESS	Allo (neg OPT)	7 days	Dexa 5	2244	0	0	0	0	0	0	0	0
10	M, 53	MPE	Allo (neg OPT)	180 days	None	2292	4	0	4	0	0	0	0	0
11	F, 60	FDE	Allo (neg OPT)	3 years	None	2840	0	4	0	0	0	0	0	4
12	M, 60	MPE	Allo (neg OPT)	2 years	None	4524	8	0	4	0	0	0	0	0
13	F, 43	MPE	Cefuroxime	240 days	None	872	0	0	0	0	0	4	0	0
14	M,	MPE	Ceftriaxone, amoxicillin	56 days	None	2712	0	0	0	4	8	4	4	0
15	F, 67	MPE	Ceftriaxone, Ceftazidime	2 years	None	3192	0	0	4	4	0	0	0	4
16	F, 49	MPE	Cefuroxime	168 days	None	2168	0	4	0	4	0	0	0	0
17[a]	F, 47	TEN	Ultracet	4 days	Dexa 12	1444	0	0	0	0	0	0	0	0
18[a]	F, 48	SJS	Phenytoin	6 days	None	1220	0	0	0	0	0	0	0	0
19[a]	F,37	DRESS	Phenytoin	5 days	Pred 30	3072	0	0	0	20	0	0	0	120
20[a]	F, 35	DRESS	Vancomycin	21 days	Pred 40	1656	0	0	0	0	0	0	0	0
21[a]	F, 30	SJS	Co-trimoxazole	7 days	None	2532	0	0	0	0	0	0	0	0

PHA, phytohaemagglutinin; M, male; F, female; DRESS, drug reaction with eosinophilia and systemic symptoms; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis; FDE, fixed drug eruption; MPE, maculopapular exanthems; Allo, allopurinol; neg OPT, negative oral provocation test; Pred, prednisolone; Dexa, dexamethasone. Data from 21 control subjects (11 patients with severe cutaneous adverse reaction (SCARs) from other drugs, four patients with cephalosporin-induced MPE and six allopurinol-tolerant subjects) were employed for comparative analysis. [a]Patients with SCARs from other drugs.

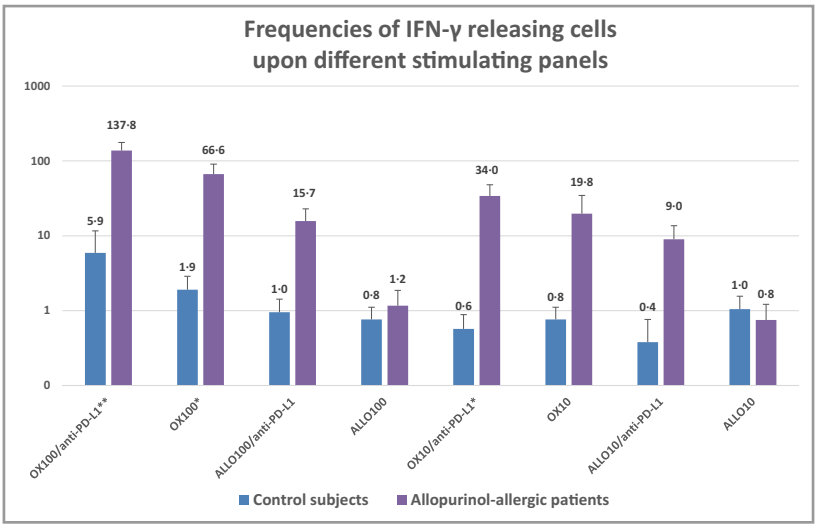

Fig 2. Differences in frequencies of interferon-γ-releasing cells between patients with allopurinol-induced severe cutaneous adverse reactions and control subjects. The differences are significant after stimulating peripheral blood mononuclear cells with oxypurinol 100 μg mL^{-1} (OX100), (OX100)/antiprogrammed death ligand 1 antibody (anti-PD-L1) and OX10/anti-PD-L1 (*$P < 0·05$, **$P < 0·01$). ALLO100, allopurinol 100 μg mL^{-1}.

Oxypurinol-specific IFN-γ-releasing cells were detected in subjects with allopurinol-induced SCARs and were significantly increased with anti-PD-L1 supplementation. Figure 2 illustrates the frequencies of IFN-γ-releasing cells in patients with a history of allopurinol-induced SCARs compared with the control subjects. The frequencies of IFN-γ-releasing cells in allopurinol-allergic patients were significantly higher than those in control subjects when stimulating PBMCs with oxypurinol 100 μg mL^{-1} (OXY100)/anti-PD-L1 ($137·8 \pm 39·0$ vs. $5·9 \pm 5·7$, $P < 0·01$), OXY100 alone ($66·6 \pm 23·9$ vs. $1·9 \pm 1·0$, $P < 0·05$) and OXY10/anti-PD-L1 ($34·0 \pm 13·9$ vs. $0·6 \pm 0·3$, $P < 0·05$). The magnitudes of IFN-γ responses after stimulation with other reagents in allopurinol-allergic patients were also higher than in the control group, but statistical significance was not reached.

Moreover, the measurement of IFN-γ-releasing cells upon stimulating PBMCs with OXY100/PD-L1 and OXY100 can predict allopurinol hypersensitivity. ROC curve analysis of IFN-γ-releasing cells was performed to evaluate the ability of IFN-γ measurement to confirm the diagnosis of allopurinol hypersensitivity. Our study indicates that the data acquired from OXY100/anti-PD-L1-stimulated PBMCs result in good discriminatory power in distinguishing between allopurinol-allergic and control subjects (area under the curve = 0·863, $P < 0·001$), and also with OXY100 alone (area under the curve = 0·806, $P < 0·001$) (Table 3).

The best discriminatory power in confirming the diagnosis of allopurinol-induced SCARs was obtained by using 16 SFCs per 10^6 PBMCs as a cut-off value after stimulating PBMCs with OXY100/anti-PD-L1 (79·2% sensitivity and 95·2% specificity), followed by using the cut-off value of 6 SPFs per 10^6 PBMCs using stimulation with OXY100 alone (70·8% sensitivity and 95·2% specificity). If the conclusion of allergic status is based on either OXY100 or OXY100/anti-PD-L1 positive criteria, 88% sensitivity (21 of 24) will be achieved. Other stimulation panels yield poor predictive values in distinguishing between allopurinol-allergic and control subjects. The sensitivities and specificities of these selected cut-off points are shown in Figure 3 and Table 3.

Table 3 Areas under the curve based on different stimulating panels

Test result variable(s)	Area	SE	Asymptotic significance	Asymptotic 95% confidence interval
OXY100/ anti-PD-L1	0·863	0·059	< 0·001	0·748–0·978
OXY100	0·806	0·068	< 0·001	0·672–0·939
ALLO100/ anti-PD-L1	0·591	0·085	0·30	0·425–0·758
ALLO100	0·475	0·087	0·78	0·304–0·646
OXY10/ anti-PD-L1	0·685	0·080	0·034	0·528–0·841
OXY10	0·595	0·085	0·28	0·429–0·762
ALLO10/ anti-PD-L1	0·602	0·084	0·24	0·437–0·768
ALLO10	0·467	0·087	0·71	0·296–0·638

OXY100, oxypurinol 100 μg mL^{-1}; ALLO100, allopurinol 100 μg mL^{-1}; anti-PD-L1, antiprogrammed death ligand 1 antibody.

We also considered the factors influencing the IFN-γ-enhancing effect of anti-PD-L1 upon PBMC stimulation with OXY100 in allopurinol-allergic patients. The enhancing effect of supplementary anti-PD-L1 on IFN-γ responses compared with stimulating with OXY100 alone in allopurinol-allergic patients is shown in Figure 4. Considering the stages of drug-allergic reactions, SCAR phenotypes and concurrent systemic steroid use, the enhancing effect of anti-PD-L1 is significantly demonstrated in patients with DRESS, those in the active stage of drug reaction and those with concurrent systemic steroid use. Interestingly, this effect is not significant in patients with SJS/TEN, those in the recovery stage or those not using systemic steroids at the time of PBMC collection.

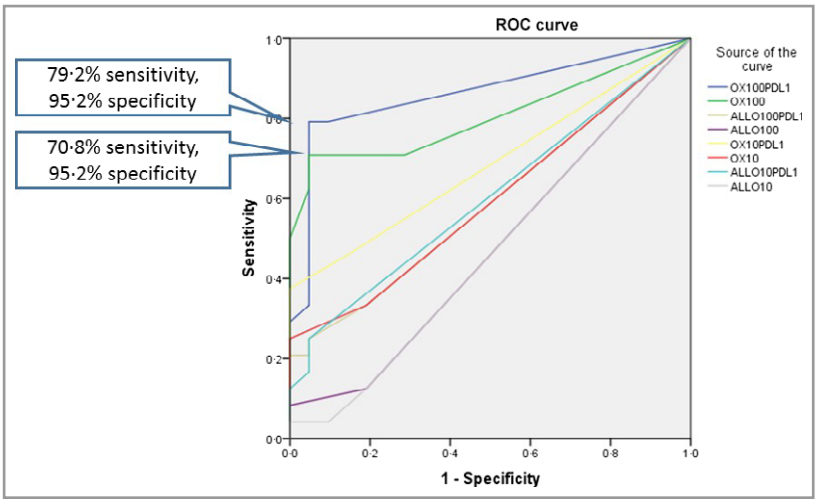

Fig 3. Receiver operating characteristic (ROC) curve analyses showing the sensitivity and specificity of interferon-γ enzyme-linked immunospot assay using different stimulating panels for the diagnosis of allopurinol-induced severe cutaneous adverse reactions. OX100, oxypurinol 100 μg mL^{-1}; ALLO100, allopurinol 100 μg mL^{-1}; PDL1, antiprogrammed death ligand 1 antibody.

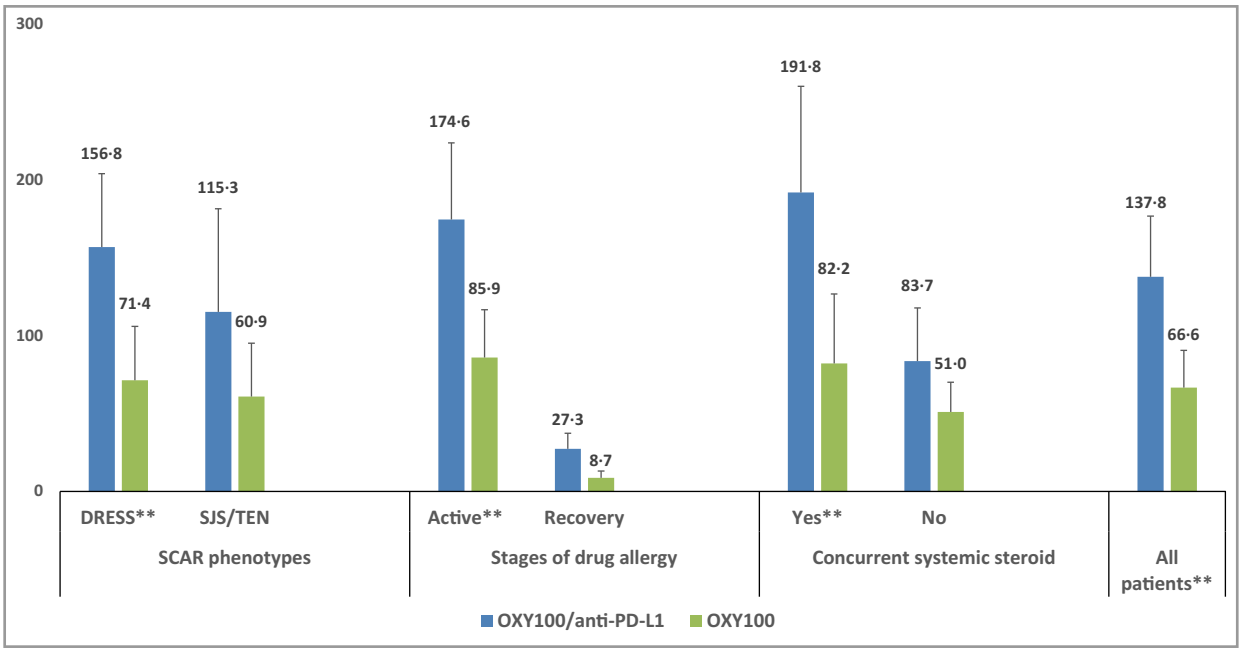

Fig 4. The interferon-γ-enhancing effect of antiprogrammed death ligand 1 antibody is significant in peripheral blood mononuclear cells collected from patients with drug reaction with eosinophilia and systemic symptoms (DRESS), patients during the active stage of drug allergy and patients on systemic steroids (**$P < 0·01$). SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis; SCAR, severe cutaneous adverse reaction; OXY100, oxypurinol 100 μg mL^{-1}; anti-PD-L1, antiprogrammed death ligand 1 antibody.

Discussion

Our study reveals that the measurement of IFN-γ-releasing cells upon stimulation with oxypurinol and anti-PD-L1 antibody is a useful tool for confirming the diagnosis of allopurinol hypersensitivity. According to our study, the frequencies of oxypurinol-induced IFN-γ-releasing cells are not only low in allopurinol-tolerant or cephalosporin-allergic subjects, but are also low or undetectable during the acute stage in patients who have developed SCARs from other drugs. Therefore, the argument that IFN-γ response to oxypurinol might be due to nonspecific immune activation is unlikely to hold. The fact that even allopurinol itself cannot induce IFN-γ response in patients with allopurinol-induced SCAR confirms that the response is immunogen specific. These findings indicate that this test can also be helpful in identifying the culprit drug in patients presenting with drug allergy symptoms after taking multiple drugs.

In general, the recommended timing for LTT performance is 4–8 weeks after the reaction, to avoid the interference of spontaneously activated T cells during the acute period.[16] It has been suggested that *in vitro* testing might be performed during acute SJS/TEN, but that it should be delayed in DRESS due to enhanced Treg activity during the active phase.[17] Polak

et al.[18] demonstrated a good sensitivity of IFN-γ ELISpot in the acute phase of delayed-type drug hypersensitivity reactions. In contrast, Valeyrie-Allanore *et al.*[19] found that reactive T cells are rarely detected in the acute stage of SCARs, and the overexpression of PD1 and PD-L1 in acute DRESS could result in low levels of detectable reactive T cells. However, in patients for whom an allergic reaction develops while taking multiple drugs, it is preferable to identify the culprit drug as early as possible so that patients can resume the drugs they need. Our study confirms that supplementation with anti-PD-L1 antibody could increase the sensitivity of IFN-γ ELISpot, particularly during the acute stage of drug allergy.

According to our study, the augmentation of the IFN-γ response from anti-PD-L1 supplementation was observed predominantly in the acute stage of SCARs, in patients with DRESS, and in patients treated with systemic steroids. The heightened function of Tregs during the acute drug allergic phase, particularly in DRESS, could provide an explanation for the significant enhancing effect of anti-PD-L1 in these patients. Corticosteroids have been reported to induce Treg populations and hamper the immune response,[20,21] which could also be counteracted by anti-PD-L1 supplementation.

In this study, while most patients with allopurinol-induced SJS/TEN were positive for HLA-B*58:01, approximately two-thirds had positive IFN-γ ELISpot values according to the OXY100/anti-PD-L1 stimulation results. Additional granulysin measurement may help to increase the test sensitivity in this patient group.[22,23] On the other hand, almost all patients with DRESS had positive IFN-γ ELISpot values, while only 77% were positive for the HLA-B*58:01 allele. Our data suggest that the measurement of IFN-γ-releasing cells using ELISpot could be an important tool for confirming the diagnosis of allopurinol-induced SCARs, especially in DRESS, for which prescreening for the negative HLA-B*58:01 allele is not fully protective. The diagnostic criteria could also be selected based on either OXY100 or OXY100/anti-PD-L1 positive cut-off values to maximize test sensitivity.

Factors influencing the sensitivity of the test should be explored in greater depth. The measurement of drug-specific IFN-γ-releasing cells at several time points throughout the acute and recovery phases in patients with DRESS and SJS/TEN should be analysed. It is worth mentioning that frequencies of IFN-γ-releasing cells may not necessarily correlate with the severity of cutaneous adverse reactions.

In conclusion, our study demonstrates that the application of ELISpot to measure IFN-γ-releasing cells upon PBMC stimulation with oxypurinol and anti-PD-L1 antibody has a beneficial role in confirming diagnosis in patients with a suspected history of allopurinol-induced SCAR, regardless of a patient's HLA*B58:01 status, concurrent systemic steroid use and SCAR phenotypes.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Video S1. Author video.