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CLINICAL REPORT

The Potential of Using Enzyme-linked Immunospot to Diagnose Cephalosporin-induced Maculopapular Exanthems

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There is no reliable test to diagnose cephalosporin-induced maculopapular exanthems (MPE). This study aimed to evaluate the role of enzyme-linked immunospot assay in the diagnosis of cephalosporin-induced MPE compared with skin testing. A total of 25 patients with a history of cephalosporin-induced MPE were skin tested and the frequencies of cephalosporin-specific interferon-γ-, interleukin-5-, and interleukin-10-releasing cells/10^6 peripheral blood mononuclear cells were measured after stimulating with the culprit drug, compared with 20 non-allergic controls. Values greater than means+2 standard deviations of the values in non-allergic controls were considered diagnostic. The study showed that the combination of interferon-γ and interleukin-5 enzyme-linked immunospot assays was more sensitive than skin testing to diagnose cephalosporin allergy (40% vs. 8%, $p=0.008$) and sensitivity increased to 57.1% when the test was performed within 2 years of the drug reaction. Enzyme-linked immunospot assay is a promising tool for confirming the diagnosis of cephalosporin-induced MPE. *Key words: drug hypersensitivity; adverse drug reaction; ELISPOT.*

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Cephalosporins are one of the most commonly prescribed types of antibiotics causing hypersensitivity reactions, particularly maculopapular exanthems (MPE) (1, 2). Re-administration of β-lactam antibiotics is generally avoided in these patients, even though rashes associated with cephalosporins are usually non-life threatening and it is unclear how frequently cross-reactivity reactions actually occur (3, 4). In fact, rashes may be caused by concurrent infections, underlying diseases, or concomitant drug use, not only by cephalosporins.

The diagnosis of drug-induced exanthema remains a problem in clinical practice. Skin testing in patients with non-immediate reactions to β-lactams has demonstrated low sensitivity even in cases confirmed with positive drug challenges (5–7). In contrast, *in vitro* tests have been reported to be more sensitive than skin testing in detecting drug-induced non-immediate reactions including MPE (8, 9). Although drug provocation testing is the gold standard to confirm non-immediate drug reactions, the advantages of *in vitro* techniques would be the ability to assess immune responses to multiple drugs simultaneously and without bearing the risks of inducing severe adverse reactions or re-sensitization in tested patients (10, 11).

Traditionally, the 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 culprit drugs (12). However, the LTT is best performed at the acute stage within one week after the onset of MPE (13). The enzyme-linked immunospot (ELISPOT) assay is one of the most sensitive *ex vivo* techniques to analyse low-frequency antigen-specific, cytokine-producing cells in peripheral blood. ELISPOT has been shown to have better sensitivity than LTT to detect drug-specific T cells in patients with drug-induced non-immediate reactions (14–16). Drug-specific cytokine-releasing cells can be observed by ELISPOT as long as 12 years after strict avoidance of the culprit drug (17). Heterogeneous cytokine involvement including Th1 (i.e. interferon-gamma; IFN-γ), Th2 (i.e. interleukin 5; IL-5), and anti-inflammatory cytokines (i.e. IL-10), has been demonstrated in different types and stages of cutaneous drug reactions (18–20). Therefore, the measurement of multiple cytokines may increase the sensitivity of the test (21).

This study aimed to investigate the differences in cephalosporin-specific cytokine responses between patients with a history of cephalosporin-induced MPE and non-allergic individuals, and to compare the sensitivity of ELISPOT with the results of skin testing.

MATERIALS AND METHODS

Patients and skin tests

A total of 25 patients, with probable or definite history of ceftriaxone or ceftazidime-induced MPE according to the Naranjo probability scale (22), were recruited for skin testing and ELISPOT assays. Ceftriaxone and ceftazidime, at a concentration of 10 mg/ml, were initially tested using a prick method and then 0.05 ml of the solutions was intradermally injected on the volar forearm skin. An increased wheal diameter 3 mm ≥ the initial wheal, accompanied by an erythema at 20 min after the injection, was considered a positive immediate reaction (23, 24). An infiltrated erythema with a diameter of ≥5 mm observed later, after

48 and 72 h, was considered a positive delayed intradermal test (24). Patch-tests were performed with both drugs at a concentration of 10 mg/ml using Finn Chambers (Hyrylia, Finland). The results were interpreted according to the European Academy of Allergy and Clinical Immunology (EAACI) position paper for the diagnosis of drug hypersensitivity (24).

ELISPOT assay

The numbers of IFN-γ- (or IL-5- or IL-10-) releasing cells were determined using ELISPOT assay kits (Mabtech, Stockholm, Sweden). Briefly, 96-well nitrocellulose membrane plates (MAIP S45; Millipore, Bedford, USA) were coated for 16 h at 4ºC with 5 µg/ml anti-IFN-γ antibody, (or anti-IL-5 antibody or anti-IL-10 antibody) provided in the kit and blocked with R10 medium (RPMI1640 supplemented with 100 U/ml penicillin, 100 U/ml streptomycin and 10% heat-inactivated foetal bovine serum (FBS, Bio Whittaker, Maryland, USA)) for 1 h at room temperature. Peripheral blood mononuclear cells (PBMC) (2.5×10^5 in 100 µl) were incubated for 48 h at 37ºC in 5% CO_2 in the presence of ceftriaxone or ceftazidime at 1 mg/ml. Plates were washed 6 times with phosphate-buffered saline (PBS)/Tween 0.05% and incubated for 1.5 h at 37ºC with the corresponding biotinylated antibody and then washed extensively. Spot-forming cells (SFC) were developed using streptavidin-alkaline phosphatase, incubated for 1 h at 37ºC, and washed extensively before adding the substrate. Results are expressed as the numbers of IFN-γ (or IL-5 or IL-10) SFC/10^6 PBMC cultured with the drug, subtracted by the values obtained from PBMC cultured without the drug.

Statistical analysis

Student's *t*-test and χ^2 test were used to analyse the quantitative data and categorical data, respectively. McNemar's test was used to compare the sensitivity between skin test and ELISPOT assay.

RESULTS

Baseline characteristics: clinical presentations and skin test results in patients with a history of cephalosporin-induced maculopapular exanthems

A total of 25 patients with a history of cephalosporin-induced MPE were recruited into this study. Ceftriaxone and ceftazidime were the suspected culprit drugs in 20 and 5 patients, respectively. The adverse drug reaction (ADR) probability scale was classified as probable ADR in 22 patients and definite ADR in 3 patients. Skin-prick testing was negative in all patients, while the intradermal test showed a positive delayed reading in 1 case (4%). Patch-tests were positive in 2 patients (8%). No cross-reactivity between ceftriaxone and ceftazidime could be detected using skin testing. Patients' clinical characteristics are summarized in Table SI (available from: http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1386).

Measurement of drug-specific cytokines-secreting T cells in patients with cephalosporins-induced MPE compared with non-allergic individuals

Patients with ceftriaxone allergy were categorized into 2 groups: recent allergy (last reaction ≤2 years) and remote allergy (last reaction >2 years). Mean numbers of cefriaxone-specific IFN-γ-secreting cells measured in all cases of ceftriaxone allergy were 20.3 ± 6.4 SFC/10^6 PBMCs compared with 7.0 ± 2.7 SFC/10^6 PBMCs in non-allergic controls ($p = 0.067$). The frequency of ceftriaxone-induced IFN-γ-secreting cells in patients with a recent allergy was 25.8 ± 8.8 compared with 7.0 ± 2.7 SFC/10^6 PBMCs in non-allergic controls ($p = 0.027$). The frequency of IL-5-secreting cells in patients with recent ceftriaxone allergy was much higher than that in non-allergic controls (25.8 ± 17.9 vs. 0.6 ± 0.4, $p = 0.036$). A robust IFN-γ and IL-5 response to ceftriaxone could be observed only in patients with recent exposure, but it decreased noticeably after 2 years. In contrast, the frequency of ceftazidime-specific IL-10-secreting cells was much higher in non-allergic subjects (159.3 ± 63.4 SFC/10^6 PBMCs), while that in patients with ceftazidime-induced MPE was undetectable ($p = 0.02$). ELISPOT data are shown in Table SII and Fig. SI (available from http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1386).

Sensitivity of IFN-γ and IL-5 ELISPOT in the diagnosis of cephalosporin-induced MPE in patients with recent and remote allergic reactions

In order to identify the abnormal cytokine responses in cephalosporin-allergic patients, any values in tested patients beyond 2 SD of the mean values of the responses in non-allergic subjects were considered diagnostic. Numbers of cephalosporin-induced IFN-γ- and IL-5-secreting cells were higher in cephalosporin-allergic patients than in the control group. Therefore, the values greater than the means +2 SD of IFN-γ and IL-5 SFC/10^6 PBMCs were used as cut-off points to diagnose cephalosporin allergy. By using this criterion, the sensitivity of IFN-γ and IL-5 ELISPOT to diagnose cephalosporin-induced MPE would be 24% if each cytokine was separately analysed and up to 40% if both assays were combined. The positive ELISPOT rate was 57.1% if the test was performed within 2 years and 18.2% if the test was performed more than 2 years after the last reaction (Fig. 1). Among the 10 patients with positive ELISPOT assays, 4 patients were positive with IFN-γ alone, 4 patients were positive with IL-5 alone and 2 patients were positive with both IFN-γ and IL-5 ELISPOT assays. No patients with a remote history of cephalosporin-induced MPE could be diagnosed by skin test. The sensitivity of ELISPOT was significantly higher than skin testing in detecting cephalosporin-induced MPE (40% vs. 8%, $p = 0.008$).

Factors influencing the outcomes of ELISPOT assays in patients with a history of cephalosporin-induced MPE

Clinical characteristics between patients with positive and negative ELISPOT results were comparatively ana-

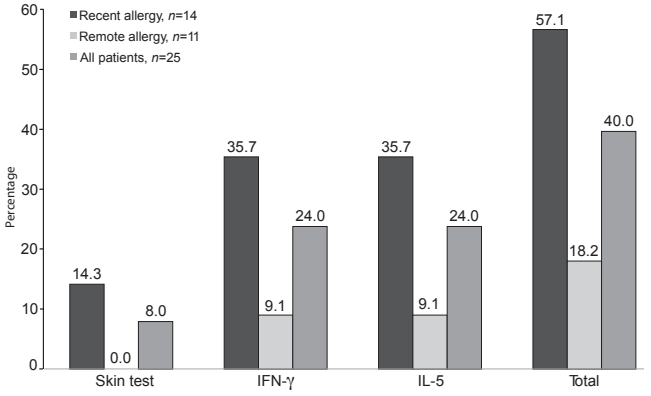

Fig. 1. Percentages of patients with a history of cephalosporin-induced maculopapular exanthems (MPE) can be diagnosed using skin testing or enzyme-linked immunospot (ELISPOT) assays. The combination between interferon gamma (IFN-γ) and interleukin-5 (IL-5) ELISPOT assays yielded higher sensitivity if the test was performed early (within 2 years after the last reaction). No patients with a remote history of cephalosporin-induced MPE (last reaction > 2 years) could be diagnosed by skin testing.

lysed (Table I). There were no significant differences in terms of predominant age and gender, underlying diseases, or ADR probability between these 2 patient groups. The duration from the last exposure was also not different, although there were more patients with recent allergic reactions in the group yielding positive ELISPOT assays ($p = 0.046$). Interestingly, the mean time after the initiation of cephalosporins until MPE developed in patients with positive ELISPOT was significantly longer than those whose ELISPOT yielded negative results (180.6 ± 49.5 h vs. 48.9 ± 8.4 h, or 7.5 ± 2.1 days vs. 2.0 ± 0.4 days, $p = 0.004$).

DISCUSSION

Clinical assessment is currently the main tool in the diagnosis of cephalosporin-induced MPE. Our study applied ELISPOT assays to detect drug-specific cytokine responses in these patients and demonstrated that the frequencies of IFN-γ- and IL-5-secreting cells in patients with cephalosporin-induced MPE were significantly higher than those in non-allergic subjects. Forty percent of patients could be diagnosed by ELISPOT and up to 57.1% of the cases yielded positive results if the test was performed within 2 years of the last reaction. In contrast, only 8% of patients were detectable by skin testing and none of them showed positive responses if the test was delayed longer than 2 years.

The frequencies of cephalosporin-specific circulating leukocytes in patients with positive ELISPOT in this 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, which were in the same range as drug-specific T cells in patients with amoxicillin-induced MPE (16). Timing is a crucial factor in determining the sensitivity of the test, since the degree of cephalosporin-specific cytokine response remarkably decreased after 2 years. The supplementary dendritic cell co-culture or depletion of regulatory T cells in conjunction with the ELISPOT technique may be able to enhance the cytokine responses in patients with a history of remote exposure to cephalosporins (25, 26).

Our data demonstrated the potential roles of ELISPOT in the diagnosis of cephalosporin-induced MPE. ELISPOT is a good candidate to detect cephalosporin-specific T cells since it is sensitive enough to detect the low frequencies of IFN-γ- and IL-5-secreting cells in patients with cephalosporin-induced MPE, while the numbers may be too low for other techniques. The measurement of cephalosporin-specific IL-10-secreting cells was not feasible for diagnosing cephalosporin allergy in this study, although the numbers in allergic patients were low. The ratio between allergenic T cells (IFN-γ- and IL-5-producing T cells) and regulatory T cells (IL-10-producing T cells) upon drug stimulation, nevertheless, may be another approach to predicting drug allergy status, rather than measuring the absolute numbers of IL-10-secreting cells.

Theoretically, ELISPOT assays could provide the assessment of potential cross-reactivity among related drugs. In this study, there were 3 patients with a history of ceftriaxone-induced MPE who reacted to both ceftriaxone and ceftazidime, and another 3 patients with a history of ceftriaxone allergy whose ELISPOT was considered positive at a low level to ceftazidime alone (patients 8, 14 and 19). As they had not previously received ceftazidime, the clinical relevance of ceftazidime sensitivity in these patients with positive ELISPOT remains unknown. Prospective studies focusing on the correlation between the results of ELISPOT and clinical allergic reactions are worth further exploration.

There are some limitations in the interpretation of the study results. The sensitivity of the test shown here may be underestimated, since some of these patients

Table I. *Clinical comparison between patients with a history of cephalosporin-induced maculopapular exanthems who yielded positive and negative enzyme-linked immunospot (ELISPOT) assays*

	ELISPOT		
Clinical characteristics	Positive ($n=10$)	Negative ($n=15$)	p
Gender, M/F, *n*	4/6	7/8	NS
Age, years, mean ± SD	59.5 ± 5.1	53.6 ± 4.5	NS
Underlying diseases, allergic/malignancy/others, *n*	1/2/7	3/6/6	NS
Naranjo ADR, probable/definite, *n*	8/2	14/1	NS
Time from last exposure, weeks, mean ± SD	70.9 ± 19.5	96.3 ± 17.6	NS
Recent/remote allergy, *n*	8/2	6/9	0.046
Time to notice rash after drugs used, h, mean ± SD	180.6 ± 49.5	48.9 ± 8.4	0.004

NS: not significant; ADR: adverse drug reaction; SD: standard deviation.

may not really have been allergic to the suspected drug. The arbitrary cut-off value needs to be verified using large-scale cohorts, and the clinical correlation of ELISPOT to diagnose cephalosporin-induced MPE should be confirmed either by a follow-up study of cephalosporin use in real life or, if possible, using drug provocation testing. The modification of the ELISPOT protocol to increase the accuracy of the test should also be encouraged.

In conclusion, the detection of drug-specific T-cell responses by ELISPOT is more sensitive than skin testing in diagnosing cephalosporin-induced MPE, particularly if performed within 2 years after the last reaction. This approach is a promising tool for clinicians to confirm the diagnosis and to study cross-reactivity among related drugs.

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The authors declare no conflicts of interest.

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