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Novel interferon-γ enzyme-linked immunoSpot assay using activated cells for identifying hypersensitivity-inducing drug culprits

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ABSTRACT

Background: The drug-induced lymphocyte stimulation test (DLST), also referred to as lymphocyte transformation test (LTT), is used to identify the culprit drug in cases of cutaneous adverse drug reactions (cADR). Although DLST is a widely used in vitro test, its sensitivity and specificity are unsatisfactory. Recent reports suggest that the detection of drug-induced interferon (IFN)-γ production using enzyme-linked immunoSpot (ELISpot) assay (conventional IFN-γ ELISpot) is useful for identifying culprit drugs in cADR cases.

Objective: The aim of this study was to establish a novel method for identifying culprit drugs in patients with cADR by efficiently detecting drug-specific IFN-γ production using activated cells.

Methods: Sixteen patients with cADR, including drug-induced hypersensitivity syndrome, erythema multiforme-like eruption, maculopapular exanthema, Stevens-Johnson syndrome, and toxic epidermal necrolysis, caused by clinically convincing culprit drugs were enrolled in this study. In some cases, the blood samples were obtained at two or three different time points. Peripheral blood mononuclear cells (PBMCs) from total 20 samples were analyzed using both the DLST and drug-induced conventional IFN-γ ELISpot. In addition, drug-induced IFN-γ ELISpot was performed using PBMCs, which were stimulated with anti-cluster of differentiation (CD)-3/CD28 antibody-coated microbeads and interleukin (IL)-2 for 7 days before exposure to the culprit drugs (modified IFN-γ ELISpot).

Results: Among the culprit drugs tested in each patient, the modified IFN-γ ELISpot was positive in 17 samples (13 patients) while DLST and conventional IFN-γ ELISpot were positive in eight and four samples (six and three patients), respectively.

Conclusion: The modified IFN-γ ELISpot using activated PBMCs was more sensitive than the conventional IFN-γ ELISpot was for detecting drug-induced IFN-γ production, which could be a useful in vitro tool for identifying culprit drugs in cADR cases.

Abbreviations: DLST, drug-induced lymphocyte stimulation test; LTT, lymphocyte transformation test; cADR, cutaneous adverse drug reactions; PBMCs, peripheral blood mononuclear cells; ELISpot, enzyme-linked immunospot; OVA, ovalbumin; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis; DIHS, drug-induced hypersensitivity syndrome; DRESS, drug rash with eosinophilia and systemic symptoms; EM, erythema multiforme; MPE, maculopapular exanthema; PHA, phytohemagglutinin; SMX/TMP, sulfamethoxazole/trimethoprim.

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1. Introduction

T cell-mediated delayed hypersensitivity is responsible for the pathogenesis of severe cutaneous adverse drug reactions (cADRs), including Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug-induced hypersensitivity syndrome (DIHS), also called drug rash with eosinophilia and systemic symptoms (DRESS) syndrome [1–4]. In severe cADR, the drug-induced lymphocyte stimulation test (DLST), also referred to as the lymphocyte transformation test (LTT), is used to identify culprit drugs because oral provocation test using culprit drugs cannot be recommended for ethical safety reasons. Although patch testing is safer than the oral provocation test and is useful when contrast media or anti-epileptics are suspected to be the causative drugs, its sensitivity is not always satisfactory [5]. In the DLST, peripheral blood mononuclear cells (PBMCs) are incubated with the culprit drug for approximately 7 days and then the cell proliferation is measured using ^{3}H-thymidine incorporation for the last 24 h. The DLST is commonly performed in Japan because this test is covered by health insurance as a diagnostic method for cADR. While DLST is a widely used in vitro test, its sensitivity and specificity are unsatisfactory. Previous reports indicate that the sensitivities of DLST are 47% in highly probable patients and 74% in definite patients of cADR [6].

The determination of antigen-specific IFN-γ production using ELISpot assay is a well-established in vitro diagnostic method for tuberculosis infections. T-SPOT.TB (Oxford Immunotec, Oxford, UK) is a commercially available IFN-γ ELISpot for detecting tuberculosis infection, which is highly sensitivity in high-risk populations (84.1–94.1%) and shows a high specificity in low-risk populations (98.7–99.1%) [7–12]. Hashizume et al. [13] reported the detection of drug-induced IFN-γ production using conventional IFN-γ ELISpot in PBMCs that were freshly isolated from phenobarbital-induced cADRs. Recent reports suggest that drug-induced conventional IFN-γ ELISpot is more sensitive than DLST is, and this method is suitable for identifying the culprit drug in cADR cases [14–18].

Previously, we reported a mouse model of TEN established using T cells from OT-I transgenic mice [19]. Most of the $CD8^{+}$ T cells in OT-I mice express a class I-restricted ovalbumin (OVA)-specific T cell receptor. These antigen-specific T cells activated by OVA peptide show cell division and IFN-γ production, leading to cytotoxicity. A previous study using T cells from OT-I mice demonstrated that the determination of IFN-γ production requires the activation of T cells by a 7-day stimulation with anti-cluster of differentiation 3 (CD)-3/CD28 antibody-coated microbeads and interleukin (IL)-2 before exposure to the OVA peptide [20]. Therefore, we speculated that IFN-γ production by drug reactive T cells might be efficiently detected following activation of patients' PBMCs with anti-CD3/CD28 microbeads and IL-2. We performed a drug-induced ELISpot assay using similarly activated human PBMCs. In this study, we demonstrated that drug-induced IFN-γ ELISpot using activated PBMCs was more sensitive than DLST and conventional IFN-γ ELISpot were.

2. Materials and methods

2.1. Patients and drugs

Sixteen patients with cADR caused by the clinically convincing culprit drugs sulfamethoxazole/trimethoprim (SMX/TMP), celecoxib, allopurinol, lamotrigine, and phenytoin and three healthy volunteers were enrolled in this study. The culprit drugs that caused the cADR in each case were determined based on the clinical course and drug history. In the assessment of all cADR cases using the Naranjo algorithm, which is one of the most widely used causality assessment tools, enrolled cases were categorized as 14 probable cases and two definite cases, but none of them were unlikely cases or possible cases.

In cases 2, 5, and 10, the blood samples were obtained at two or three different time points. PBMCs freshly prepared using Ficoll gradient separation were used for the three different assays: DLST, as well as conventional and modified IFN-γ ELISpot. The samples used in the three methods were collected during the same blood draw and were assayed within 24 h after their collection. Irrelevant drugs were selected as those administered safely in each patient without the recurrence of rashes after the onset of cADR. The culprit drugs were dissolved in phosphate-buffered saline (PBS) or PBS with 0.025% dimethyl sulfoxide (Wako) if the drug was PBS-insoluble. This study was approved by our Institutional Review Board (Osaka University, No. 08088 and Nara Medical University, No. 1257) and conducted according to the Helsinki declaration. Informed consent for all diagnostic procedures and research was obtained from all the patients and healthy volunteers.

3. DLST/LTT

PBMCs were distributed in duplicate in 96-well flat-bottom microwell plates (2×10^{5} cells/well) in 1640 Roswell Park Memorial Institute (RPMI) medium supplemented with 10% AB serum, in the absence or presence of the culprit drugs (100, 10, and 1 μg/mL) or irrelevant drugs (100 μg/mL). The positive controls were phytohemagglutinin (PHA)-stimulated cultures (10 μg/mL). The cultures were incubated for 7 days at 37 °C in 5% CO_2, and ^{3}H thymidine was added to each well for the last 24 h. The drug-specific proliferation was determined based on ^{3}H thymidine incorporation. The results are expressed as the stimulation index (SI), which is the ratio of the highest count per minute of the samples cultured with diluted drug to that of the control cultured without a drug. An SI value > 2.0 was interpreted as a positive result.

3.1. Cell activation and IFN-γ ELISpot

For the modified IFN-γ ELISpot assay, the PBMCs were stimulated with Dynabeads Human T-activator CD3/CD28 according to manufacturer's protocol except for the timing of IL-2 addition. In addition, PBMCs were used without T cell purification. Briefly, the PBMCs were cultured in 10% AB serum in RPMI-1640 (1×10^{6} cells/mL) containing 25 μL/mL anti-CD3/CD28 antibody-coated microbeads (Gibco, Life Technologies) and 30 IU/mL human recombinant IL-2 (Miltenyi Biotec) for 7 days at 37 °C exposed to an atmosphere of 5% CO_2. IL-2 was added only at the beginning of cell activation. The activated cells were harvested, and the bound microbeads were detached using a magnetic device. Both the conventional and modified IFN-γ ELISpot were performed using a human IFN-γ ELISpot kit (Mabtech). Cells were distributed in duplicate in 96-well ELISpot plates (2×10^{5} cells/well) in 1640 RPMI medium supplemented with 10% AB serum, and incubated for 24 h in the absence or presence of the culprit drugs (100 and 10 μg/mL) or irrelevant drugs (100 μg/mL). The positive controls were PHA-stimulated cultures (10 μg/mL). The number of IFN-γ spots in each well was counted using an ELISpot plate reader (CTL), and the results are shown as the largest number of IFN-γ spots/2×10^{5} PBMCs. All the experiments were read using the ELISpot reader, and were also checked by visual reading. The results were interpreted by subtracting the spot count in the negative control (no drug) from the spot count in the drug-treated wells. Based on the criteria for T-SPOT.TB, a difference in the value of the IFN-γ spot > 6 was defined as a positive result in this study because differences in values of 5, 6, and 7 are interpreted as borderline in the T-SPOT.TB.

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