SHORT COMMUNICATION

Using CD40 Ligand Expression to Detect Antigen-specific T Cells in Patients with Drug Eruptions

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To determine the causes of drug eruptions, detailed medical interviews, patch tests, and the lymphocyte transformation test (LTT) or provocation tests have been used. Since the pathogenesis of drug eruptions mainly involves T cells, which maintain long-lasting reactivity to the causative drug (1, 2), an in vitro test, such as the LTT should be useful for diagnosis. We utilized CD40 ligand (CD40L = CD154) as an early activated T-cell marker. Alas, CD40L is difficult to detect because of immediate degradation via interaction with CD40 on antigen-presenting cells (3). However, antigen-specific CD4+ cells have been shown to be detectable, employing stabilizing intracellular CD40L with the secretion inhibitor Brefeldin A by flow cytometry (FCM), and this stimulatory assay can be performed in as little as 24 h (4, 5). We examined this method in patients with various drug eruptions.

PATIENTS AND METHODS

A total of 14 patients were clinically diagnosed with drug eruptions due to a single, definite drug, at first consultation at Toho University Sakura Medical Center or Tokyo Medical and Dental University. Three patients with prurigo who were taking medications for other diseases were included as disease control. The study was approved by the Institutional Review Boards of both universities.

Blood samples were collected in heparin-containing tubes. Peripheral blood mononuclear cells (PBMCs) were prepared by density gradient with Ficoll-Paque (GE Healthcare, Uppsala, Sweden). One tablet of causative drug was sonicated and filtrated in 5.0 ml distilled water. 0.5–1.0×10^6 cells were added with diluted suspensions of causative drug suspension (×50, ×250, ×1,250 and ×6,250) and anti-CD28 antibody (1 µg/ml; eBiosciences). The assay stimulation index (SI) was calculated as follows: SI = percentage of CD40L+ cells among CD4+ cells with drug suspension/percentage of CD40L+ cells among CD4+ cells without drug. SI >200% was defined as positive for CD40L detection, and results were compared with those of conventional drug tests, such as LTT (performed by SRL Inc., Tokyo, Japan), patch and provocation tests (Table SI).

RESULTS

Eight of the 14 patients were positive for CD40L detection. Case 1 had had trigeminal neuralgia for 3 years, and had experienced had experienced a maculo-papular (MP) type drug eruption to carbamazepine (CMZ). Due to an incorrect prescription, she had taken 1 CMZ tablet and maculo-papular erythema had generalized the next day. After treatment with systemic prednisolone, a patch test, LTT and the CD40L detection test were performed (Fig. 1). It is notable that the LTT was positive for another drug, ethyl lofzepate, which was thought to be a false positive. The patch test and CD40L detection were both positive for CMZ. Case 8 was a 69-year-old man who had taken phenobarbital for 12 days as a part of a treatment regimen for metastatic brain tumour, and erythema then appeared. On cessation of the drug, the...
eruptions disappeared promptly without treatment. LTT was negative in this case. We diagnosed this patient as having an EM-type drug eruption due to phenobarbital, based on his clinical course, and the positive result on the CD40L detection test (Fig. 1).

The positive CD40L results were performed relatively soon after the appearance of eruptions compared with 6 patients with negative results (mean 7.1 days and 20 days, respectively) (see Table SI). These results are consistent with those of a previous report, in which positive LTT reactions were obtained when the test was performed within one week after the onset (6).

DISCUSSION

There is a discrepancy between LTT and CD40L results in many cases. In EM patients with negative results (cases 6, 7, 13), LTT was positive when performed early in the course, but CD40L was not detected (negative) when performed after LTT. Thus, the timing of performing the stimulation assay is thought to be significant. In 6 cases performed after LTT. Thus, the timing of performing the assay 6–24 h after stimulation and maintained a plateau level up to 24 h (4, 5). Therefore we performed the assay 6–24 h after stimulation, and results were obtained the next day after blood sampling. The advantages of the detection of activated markers of T cells by FCM are thought to be shortening of the assay time and no requirement for radioactive materials. Thus, in addition to conventional drug tests, detection of T-cell activation markers is suggested to be a useful tool for determining the causative drug in cases with drug eruptions.

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REFERENCES