Materials and Methods

Patients

Seven patients with DRESS (Table 1) were enrolled in the present study after providing informed consent. All 7 patients had definitive diagnoses of DRESS, which fulfilled >5 of the scoring system for DRESS [13]. In all patients, HHV-6 reactivation was confirmed by significant increases in both HHV-6 IgG titers and HHV-6 DNA levels in whole blood samples. Causative drugs were carbamazepine (4 patients), salazosulfapyridine (2 patients), and zonisamide (1 patient). Lymphocyte transformation tests were positive for these drugs in all patients. No patients had a history of allergic diseases, such as atopic dermatitis and asthma. Six of the 7 patients were treated with systemic corticosteroids.

Blood samples were obtained from patients with DRESS during the active stage (before treatment and within 1 week after the onset) and the recovery stage of the disease (after treatment and 5–8 weeks after the onset). Seven healthy subjects were used as controls. The present study was approved by the Ethics Committee of Saitama Medical Center, Saitama Medical University.

Monoclonal Antibodies

Monoclonal antibodies (MAbs) against CLA [fluorescein isothiocyanate (FITC)-conjugated], CD4 [allophycocyanin (APC)-Cy7-conjugated], CD8 [peridinin chlorophyll protein-Cy5.5-conjugated], IFN-γ (FITC-conjugated), IL-4 [phycoerythrin (PE)-conjugated], IL-13 (PE-conjugated), and isotype controls were obtained from BD Biosciences. MAbs against IL-17 (PE-conjugated), IL-22 (APC-conjugated), and isotype controls were obtained from eBioscience.

Cell Preparation

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood obtained from DRESS patients and healthy subjects by density gradient sedimentation. Cells were stored at –80°C until use.

Flow Cytometry

For intracellular cytokine staining, PBMCs were stimulated for 4 h in RPMI medium containing 25 ng/ml phorbol 12-myristate 13-acetate (Sigma-Aldrich, Japan), 1 mM ionomycin (Sigma-Aldrich) and 10 μg/ml brefeldin A (Sigma-Aldrich). After harvesting, cells were directly labeled with MAbs against the cell surface markers CLA, CD4, and CD8 for 25 min at room temperature. Cells were washed and then incubated in 0.5 ml lysing solution and 0.5 ml permeabilizing solution (BD Biosciences) at room temperature. Finally, cells were incubated for 30 min at 4°C with MAbs specific to IL-4, IL-13, IL-17, IL-22, and IFN-γ. Samples were analyzed with 6-color staining using FACSCanto™ II (BD Biosciences).

Statistical Analysis

All results are expressed as the mean value ± SEM. Statistical analyses were performed using the unpaired or paired Student *t* test, and the Pearson correlation coefficient. Probability (*p*) values <0.05 were considered statistically significant.