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Appendix S1

SUPPLEMENTARY MATERIALS AND METHODS

Patients.

Serum samples were obtained from 20 Japanese patients with psoriasis vulgaris. Consecutive patients with moderate to severe psoriasis (psoriasis area and severity index (PASI) score ≧ 10) who had not received any prior systemic treatment within one year were included in this study. The diagnosis was made clinically, based on characteristic plaque-type psoriatic lesions. All patients mett the inclusion criteria outlined in the guidelines (1). The severity of psoriasis was assessed using the PASI score. Pruritus was assessed using the pruritus VAS score. Patients took apremilast 10 mg once in the morning. From day 2 to day 6, the medication dose was increased by 10 mg daily. After day 7, the dosage was adjusted to 30 mg twice daily (morning and evening). All studies were approved by the Ethics Committee of the University of Tokyo Graduate School of Medicine, and signed informed consent was obtained from all patients. It is important to note that patients or the general public were not involved in the design, conduct, reporting, or dissemination plans of this research.

Serum.

Venous blood samples (5–10 mL) were taken in vacutainer tubes under sterile conditions from patients. Blood samples were centrifuged at 3,000 rpm for 10 minutes at 4 °C. Sera were quickly frozen at −80°C and stored until processed.

Principles of microfluidic-ELISA system and its protocol.

The basic principle of microfluidic-ELISA was reported in previous papers (2,3). We successfully integrated an immunoassay into a microchip by applying basic ELISA methods to a microfluidic ELISA system. This system can measure the very low concentration of several proteins using bead-bed immunoassay, which is performed on a microchip composed of flow channels with depths ranging from 5 to 100 μm. We have also developed a laser-induced thermal lens microscope (TLM), which is especially useful for ultrasensitive determinations in microscopy (4). In this study, the microchip was filled with polystyrene beads coated with antibodies. After the introduction of beads, 1 μl of samples was loaded into the microchip. In this assay, a highly sensitive detection method with high spatial resolution is indispensable because there are very small amounts of analytes in a microchip. During the microfluidic ELISA, dye molecules produced by the enzymatic reaction are detected by our sensitive TLM. A comparison with conventional methods showed excellent performance in sensitivity (1–1000 fg/ml), analysis time (10–20 min), and sample volume (1 μl). For all the experiments, a single microchip was used repeatedly by changing the microbeads.

Antibodies used in microfluidic-ELISA system.

Serum levels of IL-1β, IL-6, IL-10, IL-12, IL-17A, IL-17F, IL-18, IL-21, IL-22, IL-23, IL-35, interferon (IFN)-α, transforming growth factor (TGF)-β1, and TNF-α levels were quantified using a microfluidic-ELISA system with commercially sourced capture and detection antibodies (eBioscience, Bender MedSystems, Vienna, Austria). Serum IL-36γ levels were measured using a microfluidic-ELISA system with commercial antibodies (AdipoGen, San Diego, CA, USA). Serum IL-17C (R&D Systems, Abingdon, UK) and IL-36γ (AdipoGen, San Diego, CA, USA) levels were also measured using microfluidic-ELISA system with commercial antibodies.

Statistical analysis.

The study investigated the rate of change and absolute difference in serum cytokine levels before and after treatment, along with their correlation with PASI score and pruritus VAS. Covariate effects were considered statistically significant if the p-value was less than 0.05. The Spearman's rank correlation test was used for the correlation analysis. All statistical analyses were conducted using JMP Pro 14. A p-value less than 0.05 was considered to indicate statistical significance. To account for multiple comparisons, Bonferroni correction was applied.

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