

Patients and Methods

Patients

The study group included 20 patients attending the outpatient clinic of the Department of Dermatology at Rabin Medical Center. Inclusion criteria were an eruption clinically consistent with allergic contact dermatitis as well as a positive and relevant patch test result for chromate. The control group included 19 outpatients presenting with allergic contact dermatitis and a relevant positive patch test result for either nickel or fragrance. None of the participants had an atopic background. The European standard patch test series, including potassium dichromate in petrolatum 0.5%, was used in all cases. Subjects had given their informed consent. The study was approved by the local ethics committee.

Cell Cultures

Venous blood was collected from each patient and heparinized, and the lymphocytes were counted and divided into test tubes. Each study culture contained $0.5 \times 1,000,000$ cells, which were incubated with potassium dichromate 0.5 or 1 μg (the maximal concentration not toxic to lymphocytes). Three cultures containing only medium were used as controls. In order to have measurable cytokine levels, we had to stimulate all samples with phytohemagglutinin. The phytohemagglutinin (10 μg) was added to each culture, to a total volume of 1 ml. After incubation at 37°C for 24 h (based on previous data), the samples were centrifuged at 2,500 rpm, and the supernatants were collected and stored at -70°C until assayed [8].

Luminex Platform

The Human Cytokine Multi-Plex (BioSource International Inc., USA) is a solid-phase sandwich immunoassay. Beads of defined spectral properties conjugated to analyte-specific capture antibodies, buffers, and samples (including standards of known analyte concentration, control specimens, and unknowns) are pipetted into the wells of a filter bottom microplate and incubated for 2 h, until the analytes bind to the capture antibodies on the beads. Thereafter, the beads are washed, and biotinylated detector antibodies are added and incubated with the beads for 1 h. The biotinylated detector antibodies recognize epitopes and bind to the immobilized analytes. The excess biotinylated detector antibodies are then removed, and streptavidin conjugated to the fluorescent protein R-phycoerythrin (streptavidin-RPE) is added and incubated with the beads for 30 min. During this final incubation, the streptavidin-RPE binds to the biotinylated detector antibodies associated with the immune complexes on the beads, forming 4-member solid-phase sandwiches. The unbound streptavidin-RPE is removed by washing, and the beads are analyzed with the Luminex 100™ system. By monitoring the spectral properties of the beads and the amount of fluorescence associated with RPE, the instrument measures the concentration of 1 or more analytes present in the original specimen. For the present study, multiplex bead kits were purchased from BioSource International Inc. (Camarillo, CA, USA) [13, 14].

Procedure

The following cytokines in the supernatant were quantitated by the Luminex platform: granulocyte-macrophage colony-stimulating factor, IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN- γ , and tumor necrosis factor- α . Findings for the lymphocytes incubated with potassium dichromate were compared with those for lymphocytes from the same patients incubated with medium only and with the 19 patients with ACD with a positive patch test for nickel and fragrance, as a control.

Statistical Analysis

The data were analyzed using the nonparametric Wilcoxon test, and the significance of differences between groups was analyzed by the Student t test. We used Finner's procedure [15], based on the family-wise error rate. By taking into account the performance of both Wilcoxon test and Student t test, multiple comparison procedures adjusted p values, thus reducing risk flukes. Calculations were performed using the PEPI software [16]. As some researchers consider use of adjusted p values unnecessary, misleading, or inefficient [17], both unadjusted and adjusted p values were used. A p value of <0.05 was considered statistically significant.