Interleukin-2 Receptor α and β Chain Expression by Circulating αβ and γδ T Cells in Inflammatory Bowel Disease

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The pathogenetic role of activated αβ and γδ T cells in inflammatory bowel disease (IBD) is not well defined. To elucidate this, interleukin-2 receptor (IL-2R) α and IL-2Rβ single chain expression and coexpression by peripheral blood TCRαβ+ cells and TCRγδ+ cells was studied in 21 patients with ulcerative colitis (UC), 25 with Crohn’s disease (CD), and 15 controls. The percentages of IL-2Rα+β−, IL-2Rα−β+, and IL-2Rα+β+ TCRαβ+ cells were increased in IBD patients with moderate and severe disease activity, as compared to controls (P < 0.01). In contrast, the percentages of IL-2Rα−β+ and IL-2Rα+β+ TCRγδ+ cells were increased in patients with inactive UC (P < 0.01), but not in CD. The results suggest that activated αβ T cells are involved in the development of IBD. The differences in γδ T cell IL-2R expression between inactive UC and CD may correspond to a yet undefined etiopathogenetic difference between these two diseases.

KEY WORDS: ulcerative colitis; Crohn’s disease; interleukin-2 receptors; receptors; antigen; gamma-delta T cells; alpha-beta T cell.
The α chain can be detected in both a cell membrane-associated and a soluble form (sIL-2R). Increased IL-2Ra cell expression, as well as increased levels of sIL-2R in the blood stream and mucosal biopsy specimens have been demonstrated in active IBD (6, 16-20). In contrast, there is only little current information available on the expression of the other IL-2R chains in IBD.

The aim of this study was to elucidate the role of activated αβ and γδ T cells in the development of IBD. To accomplish this, we have assessed the expression and coexpression of IL-2Rα and IL-2Rβ chains by circulating TCRαβ+ and TCRγδ+ cells from patients with different activity stages of UC and CD.

MATERIALS AND METHODS

Patients and Controls. Twenty-five patients with CD (21), 15 males and 10 females with a median age of 38 (range 14–61) years, and 21 patients with UC (22), 12 males and 9 females with a median age of 39 (range 15–70) years were included. None of the patients received corticosteroids; 27 patients received sulfasalazine or 5-ASA, 0.8–4 g/day; 19 did not receive any medical treatment at the time of study. Fifteen UC patients had clinically active disease according to a semiquantitative scale (23): seven slight and eight moderate or severe disease. The remaining six patients were in total remission.

CD activity was assessed according to the Simple Index (24). The CD patients were stratified into three groups: inactive (score 0–4): nine patients; slightly active (score 5–9): nine patients; moderate or severe CD (score 10 or more): seven patients.

Fifteen matched healthy volunteers, six males and nine females with a median age of 36 years (range 22–63), served as controls.

Cell Separation. Mononuclear cells were isolated from heparinized peripheral venous blood by gradient centrifugation on Lymphoprep (Nycomed, Oslo, Norway), washed in Hanks’ balanced salt solution (HBSS, pH 7.4), and resuspended in phosphate buffered saline (PBS, pH 7.2–7.4) supplemented with 10% (v/v) fetal calf serum (Gibco, Grand Island, New York).

Phenotypic Analysis. Lymphocyte triple labeling was performed using: (1) biotinylated anti-αβ TCR (Becton Dickinson Immunocytometry Systems, San Jose, California) or anti-γδ TCR (Becton Dickinson) with streptavidin-RED 613 as a second reagent (Gibco), (2) phycoerytherin (PE) conjugated anti-IL-2Ra (CD25) (Becton Dickinson), and (3) fluorescein isothiocyanate (FITC) conjugated anti-IL-2Rβ (Endogen, Boston, Massachusetts). In the first step, test samples and antibodies were added simultaneously. After incubation and subsequent washing, the second step reagent, streptavidin-RED 613, was added. After a final incubation and washing, the cells were fixed in paraformaldehyde (1%). Immunofluorescence analysis was performed on a FACScan (Becton Dickinson).

Ethics. This study was performed in accordance with the Second Helsinki Declaration and was approved by the Scientific Ethical Committee of the Copenhagen County.

Statistical Analysis. Values are expressed as medians and ranges, and statistical significance of differences between groups were analyzed using Wilcoxon’s two sample rank sum test. P < 0.05 (2α) was considered as statistically significant.

RESULTS

TCRαβ+ and TCRγδ+ Cell Percentages. The median percentage of TCRαβ+ cells was significantly lower in inactive IBD patients (49%, range 30–73%) as compared with controls (70%, range 56–82%) (P < 0.05). This difference was also present when CD and UC patients were analyzed separately (P < 0.05) (Figure 1). No differences were found in the median percentages of TCRγδ+ cells between patients and controls (Figure 1).

IL-2R Expression by TCRαβ+ Cells. As shown in Figure 2 (panel A), the median percentage of IL-2Rα+β− TCRαβ+ cells was significantly higher in moderate or severe active IBD patients (23%, range 12–59%) as compared with controls (14%, range 7–20%) (P < 0.01). The median percentage of IL-2Rα−β+ TCRαβ+ cells (Figure 2, panel B) was slightly higher in moderate or severe active IBD patients (1%, range 1–14%) as compared with controls (1%, range 0–1%) (P < 0.05). The same applied to the median percentage of IL-2Rα+β+ TCRαβ+ cells (Figure 2, panel C) (1%, range 1–10% and 0%, range 0–0%, respectively) (P < 0.01). No differences were found between UC and CD in any of the three situations (P > 0.05) (panels A–C).

IL-2R Expression by TCRγδ+ Cells. As shown in