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Effects of Dexamethasone on Interleukin-6 and Immunoglobulins Production by Lamina Propria Mononuclear Cells Isolated from Biopsy Specimens in Patients with Ulcerative Colitis

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Lamina propria mononuclear cells (LPMNC) were isolated from 20 untreated patients with active ulcerative colitis (UC) and 11 patients with inactive UC. All patients with active UC were first attacks, and were not being treated with any drugs. The effects of dexamethasone on production of interleukin-6 (IL-6) and immunoglobulins by LPMNC were assessed. IL-6 production by LPMNC, stimulated by pokeweed mitogen (PWM), was higher in patients with active UC (663.3±213.1 pg/ml) compared with normal controls (129.0 ±39.0 pg/ml) and patients with inactive UC (219.6±63.4 pg/ml). IgG was produced in greater amount by the LPMNC from patients with active UC (1395.5±876.6 ng/ml) than by those from controls (413.0±471.2 ng/ml) and patients with inactive UC (488.3±552.0 ng/ml) ($p < 0.001$). The amount of IgA and IgM did not vary among three groups.

Dexamethasone suppressed the production of IL-6 and IgA, IgG and IgM by PWM-stimulated LPMNC in a dose-dependent manner in the dose range between 10^{-5} and 10^{-2} mg/dl. We speculate that the suppression of IL-6 production by dexamethasone will contribute to the suppression of UC-associated inflammation

Key words : dexamethasone, interleukin-6, immunoglobulins, lamina propria mononuclear cells, ulcerative colitis

Introduction

Although the etiology of ulcerative colitis (UC) is unknown, it is probable that abnormal immune responses of the colorectal mucosa are involved in the onset of this disease[1)-3)]. To date, the inflamed intestinal mucosa of patients with UC has been reported to show the presence of activated T and B cells and excessive expression of MHC class II antigen in epithelial cells. Elevated blood immunoglobulin levels and the presence of anti-colon antibodies have also been reported[4)-7)]. On the other hand, TCR $\gamma\delta$ type T cells ($\gamma\delta$ T cells) and interleukin-2 (IL-2) - producing CD4 cells have been reported to decrease in patients with UC[8)9)]. Regarding the ability of lamina propria mononuclear cells (LPMNC) to produce cytokines, it has been reported that the LPMNC of UC patients produce less IL-2 but more IL-6, compared with the LPMNC of normal controls[4),10),11)]. Our previous studies also demonstrated an increased IL-1 and IL-6 production in UC patients.

IL-6 is produced by various cells such as macrophages/monocytes, T cells, B cells, endothelial cells, and fibroblasts. The induction of IL-6 is stimulated by endotoxins, IL-1, IL-2, tumor necrosis factor (TNFα) and some other factors. IL-6 induces the differentiation of B cells, thus enhancing the antibody production by B cells. IL-6 also works as a proinflammatory cytokine, which stimulates the proliferation of T cells and the IL-2 production by T cells and is thus involved in acute-phase responese and inflammation. It also serves as an anti-inflammatory cytokine, which suppresses endotoxin-induced IL-1 and TNF production[12),13)].

We isolated LPMNC from untreated patients with active or inactive UC. The effects of steroid hormones on the ability of LPMNC to produce IL-6 and on immunoglobulin production were examined.

MATERIALS AND METHODS

PATIENT EXAMINED

Twenty patients with active UC (eight women, twelve men ; mean age 44.1 years and range 18-70 year old), 11 with inactive UC (three women, seven men ; mean age 38.3 and range 15-57 year old), and 13 patients with normal controls (five women, eight men ; mean age 58.3 and range 23-88 year old) were studied. Mucosal biopsy specimens were obtained, with informed consent under the clinical endoscopic procedure. In the case of active disease, the biopsy specimens obtained from the site where the most intense inflammation was macroscopically observed. In the inactive case, the specimens were obtained from the site where inflammation had been evident during the active phase. The control group confirmed with normal colonic

mucosa histologically normal control were patients who were undergoing polypectomy for colonic polyps.

The diagnosis of UC was based on the clinical picture and laboratory findings, including colonoscopy and histological features of the biopsy. Active and inactive UC were defined by clinical features, colonoscopic findings, and histological features of the biopsy when available. All patients with active UC were first attacks, and were not being treated with any drugs. Eight patients with inactive UC were receiving sulfasalazine alone (median 3 g/day, range 1.5-4) and other patients were untreated.

ISOLATION OF LAMINA PROPRIA MONONUCLEAR CELLS

LPMNC were isolated from inflamed mucosa and normal mucosa by a modification of the enzymatic technique of Bull and Bookman[14], as previously described[15]. Briefly, tissue was obtained from fresh endoscopic biopsy specimens. After washing, the tissue was incubated in 1 mM dithiothreitol solution (DTT ; Sigma, St. Louis, Mo.) for 15 mins at room temperature. To remove the epithelial cells, it was shaken with 5 mM ethylene-diaminetetraacetic acid (EDTA; Sigma, St. Louis, Mo.) for 30 mins at 37 ℃. After washing, the tissue was digested with collagenase from *Clostridium* histolyticm (Boehringer, Germany) at a concentration of 1 mg/ml in PRMI 1640 medium (Gibco, Grand island N. Y.) with 10 % fetal calf serum (FCS) for three hours at 37 ℃. LPMNC were obtained by Ficoll-Paque (Pharmacia, Sweden) gradient centrifugation (400 g for 30 mins at 4 ℃).

CELL CULTURE

LPMNC were suspended in culture medium PRMI 1640 containing 10 % FCS, at a concentration of 1×10^5 cells/ml, and 1 ml of the cell suspension were put into each well of a 24-well flat-bottom plate (Nunc, Denmark). In the case of IL-6 production, LPMNC were cultured in the presence of pokeweed mitogen (PWM ; Sigma, St. Loius Mo.) for five days. In the case of immunoglobulin production, LPMNC were cultured in the presence of PWM for seven days. Incubation of the culture was performed in 5 % CO_2, 95 % air at 37 ℃, and then the supernatant was collected by centrifugation at 400 g for 5 mins and stored at -70 ℃ until analysis.

Efficacy of PWM used in these studies was confirmed in experiments on peripheral blood mononuclear cells from healthy individuals.

MEASUREMENT OF IL-6 AND IMMUNOGLOBULINS

The amount of IL-6 in the culture supernatants were assayed using radioimmunoassay (Amersham, U. K.). The amount of immunoglobulins (IgA, IgG and IgM) present in the supernatants were assayed using ELISA-kit (Ohtsuka Assay Inc. , Japan). Both assays were performed in duplicate according to the manufacture's instructions.

DRUGS USED IN THE STUDIES

Dexamethasone (Banyu, Tokyo, Japan) was dissolved in distilled water at 1 mg/ml, and serially diluted with RPMI 1640 (10 % FCS) to final assay concentration of 1×10^{-2} to 1×10^{-7} mg/dl.

STATISTICAL ANALYSIS

Statistical analysis was made using Bonferroni/Dunn (Dunn's procedure as a multiple comparison produce) after Bartlett's test. All data was expressed as mean ± standard deviation (mean±SD), and probability less than 5 % was considered to be significant.

RESULTS

MUCOSAL SPECIMENS STUDIES

The biopsy specimens were obtained with ordinary biopsy forceps. Criteria were applied to included only technically adequate and representatively biopsy specimens. The average mononuclear cell yield in active UC was $51.2\pm9.4\times10^5$/g. In contrast, the average mononuclear cell was $37.1\pm5.3\times10^5$/g in inactive UC and $36.9\pm8.1\times10^5$/g in normal controls, respectively. Viability was 90.5±2.6% in active UC, 89.7±9.3 % in inactive UC and 92.5±4.7 % in normal controls, as determined by trypan blue exclusion. Daxamethasone (10^{-2}～10^{-7} mg/dl) did not influence cell viability.

IL-6 PRODUCTION

There was significantly more IL-6 produced by LPMNC isolated from active UC mucosa compared with both LPMNC from inactive UC mucosa and from normal control mucosa ($p<0.001$) (Figure 1).

IMMUNOGLOBULIN PRODUCTIONS

As shown in Figure 2, the amount of IgG produced by LPMNC was significantly higher in patients with active UC than in controls, and also than inactive UC ($p<0.001$). Nevertheless, the amount of IgA and IgM did not vary significantly among these groups studied.

EFFECTS OF DEXAMETHASONE ON IL-6 PRODUCTION

Dexamethasone had a marked inhibitory effect on

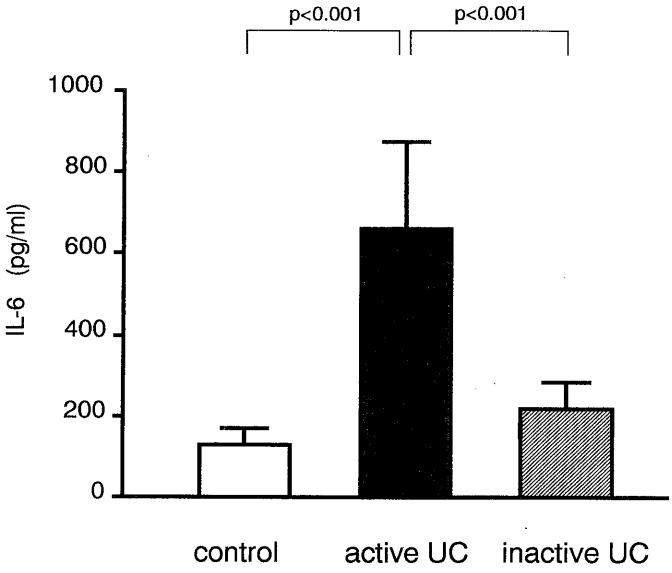

Figure 1. IL-6 production in a 5 days culture with PWM stimulation by isolated LPMNC.

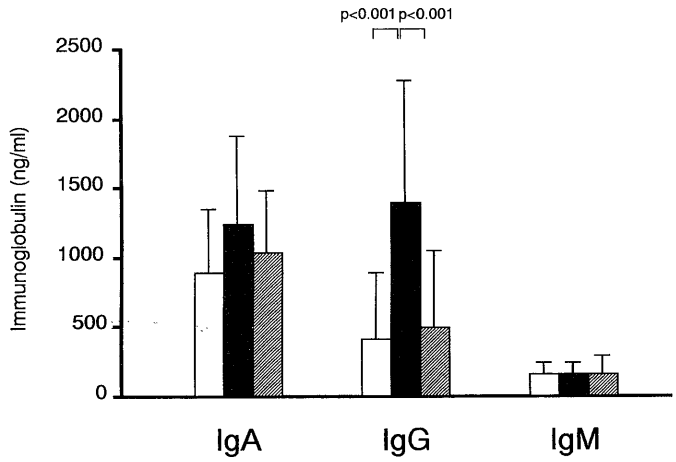

Figure 2. IgA, IgG, IgM production in a 7 days culture with PWM stimulation by isolated LPMNC in untreated patients with active UC (■), patients with inactive UC (▨) and normal controls (□).

PWM-stimulated IL-6 production. The mean percentage of inhibition of IL-6 production induced by dexamethasone was dose-dependent with 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} mg/ml (Figure 3).

EFFECTS OF DEXAMETHASONE ON IMMUNOGLOBULIN PRODUCTION

Dexamethasone showed a dose-dependent inhibition of IgA, IgG and IgM production. The mean percentage of inhibition of IgA production induced by dexamethasone was dose-dependent with 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} mg/ml (Figure 4). Similar results were observed for IgG and IgM

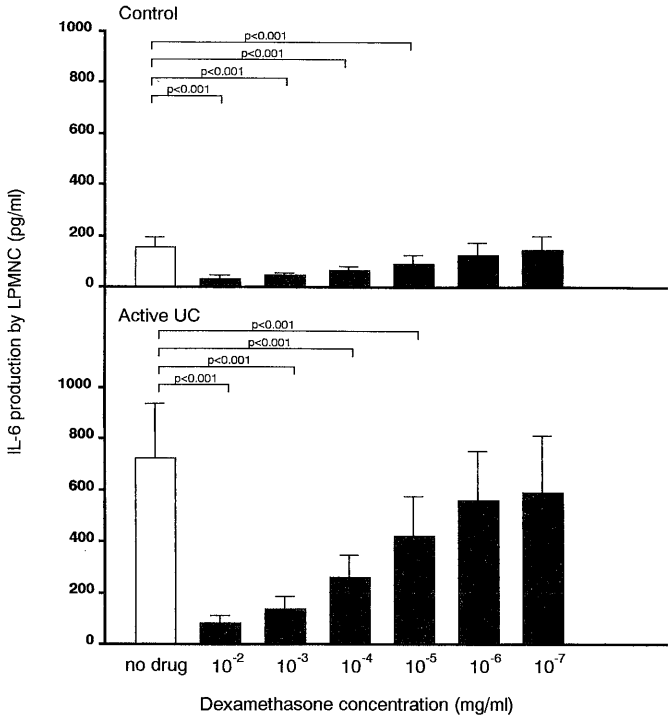

Figure 3. Effect of dexamethasone on IL-6 production by LPMNC. LPMNC were stimulated with PWM, and incubated with dexamethasone at concentrations of 10^{-2}~10^{-7} mg/ml for 5 days.

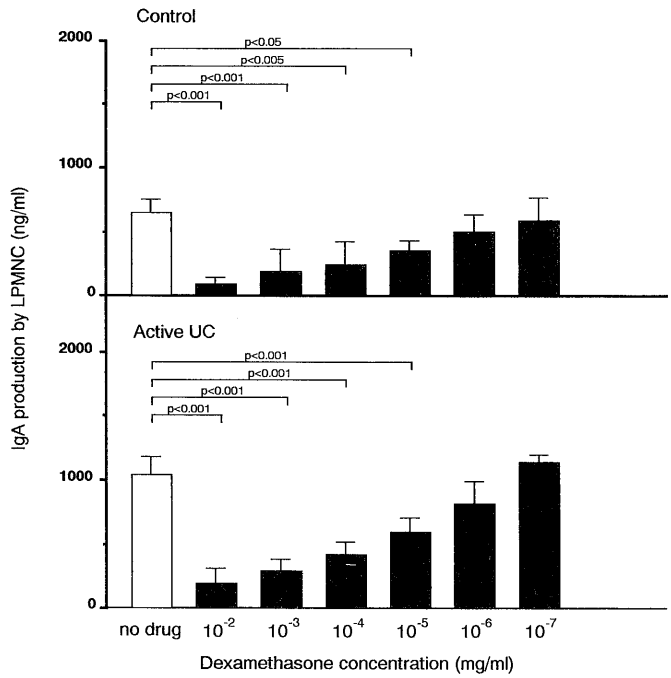

Figure 4. Effect of dexamethasone on IgA production by LPMNC. LPMNC were stimulated with PWM, and incubated with dexamethasone at concentrations of 10^{-2}~10^{-7} mg/ml for 7 days.

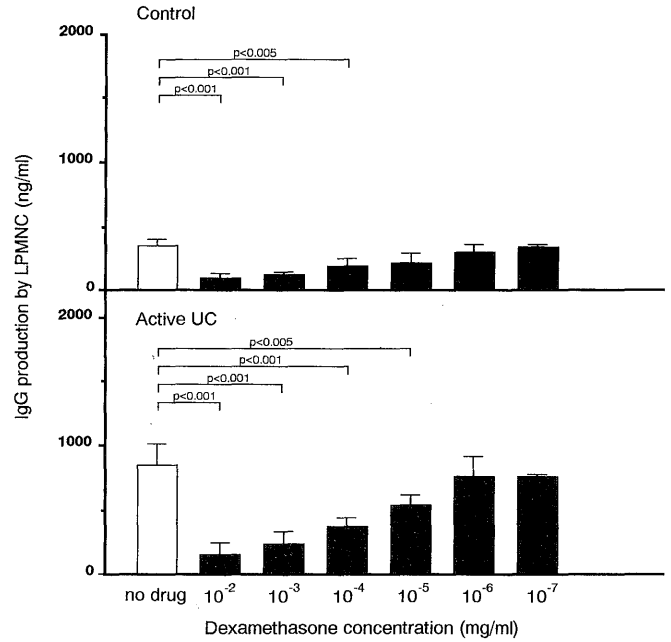

Figure 5. Effect of dexamethasone on IgG production by LPMNC. LPMNC were stimulated with PWM, and incubated with dexamethasone at concentrations of 10^{-2}~10^{-7} mg/ml for 7 days.

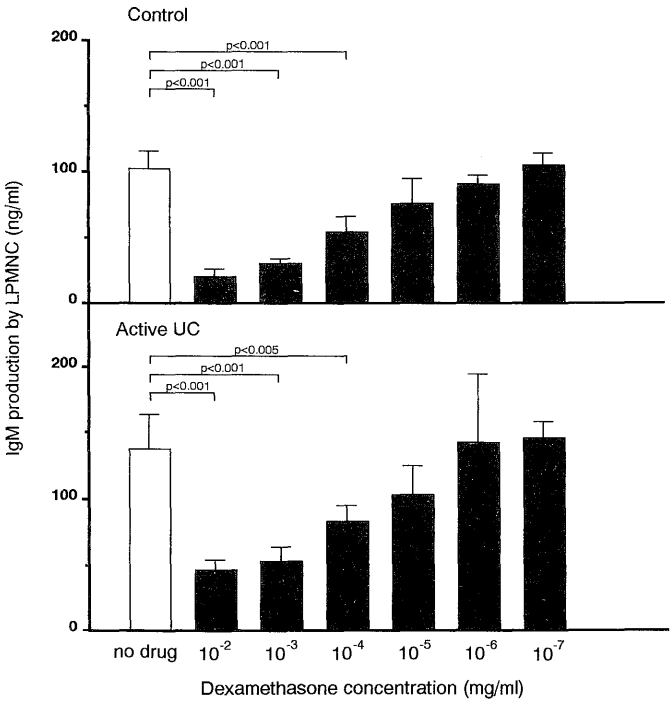

Figure 6. Effect of dexamethasone on IgM production by LPMNC. LPMNC were stimulated with PWM, and incubated with dexamethasone at concentrations of 10^{-2}~10^{-7} mg/ml for 7 days.

production by LPMNC (Figure 5 and 6).

Discussion

Our previous report indicated that LPMNC of UC patients produce excessive amounts of IL-6, and that this is more marked in patients with active UC than in patients with inactive UC. Similar results were also obtained in the present study. Kusugami and coworkers suggested that activated macrophages in the LPMNC are responsible for the production of IL-6, on the grounds that IL-6 production continued to be seen even after elimination of lymphocytes from the LPMNC[11]. Although no cytokines other than IL-6 were examined in the present study, it is likely that abnormal production of cytokines in local areas of the intestine is closely related to the pathogenesis of UC, considering previous reports that excessive IL-1 and TNFα production was also noted in UC patients[16), 17)].

In the present study, dexamethasone suppressed the production of immunoglobulins by the LPMNC of UC patients in a dose-dependent manner. Since anti-colon antibodies and antibodies with antibody-dependent cell-mediated cytotoxicity (ADCC) can damage colorectal epithelial cells and cause exacerbation of UC, the effect of dexamethasone in suppressing excessive production of Igs will contribute to the suppression of UC-associated inflammation.

Steroids exert potent anti-inflammatory effects by suppressing the biosynthesis of prostaglandins, thromboxane, leukotrienes, inducing vasodilation, elevate vascular permeability or cause leukocyte infiltration. Steroids also suppress the production of cytokines, resulting in anti-inflammatory effects. For example, low levels of steroids suppress the production of IL-1 and TNFα by monocytes and the IL-2 production by lymphocytes. Steroids are thought to suppress the production of IL-6 by inhibiting the expression of IL-6 mRNA[18), 19)].

The present study demonstrated that dexamethasone suppresses the production of IL-6 by the LPMNC of UC patients in a dose-dependent manner. IL-6 acts not only as an anti-inflammatory cytokine but also as a proinflammatory cytokine that induces the proliferation of T cells and aggravates inflammation. In addition, when IL-6 induces the differentiation of B cells, autoantibody production is stimulated, resulting in deterioration of UC. Therefore, we speculate that the suppression of IL-6 production by steroid hormone will contribute to the suppression of UC-associated inflammation in two ways.

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