

Production of Interleukin-2 and Interleukin-2 Inhibitor in Patients with Palmoplantar Pustulosis

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We studied production of interleukin-2 (IL-2) and IL-2 inhibitor from peripheral blood of patients with palmoplantar pustulosis (PPP). Nineteen patients were divided into two groups: those with and those without arthro-osteitis. Although IL-2 production in both groups of patients was within normal limits, those with arthro-osteitis showed greater fluctuation in relation to the disease activity. The IL-2 production of five PPP patients with arthro-osteitis was greatly enhanced in the inactive stage compared with the active stage. Sera from two patients treated with a combination of etretinate and colchicine contained extremely low levels of IL-2 inhibitory activity. The increased IL-2 production in the inactive stage may be due in part to the depletion of IL-2 inhibitor-producing cells by the treatment. (Received February 19, 1987.)

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Palmoplantar pustulosis (PPP) is a chronic skin disease characterized by recurrent sterile pustules on the palms and soles. Although the classification and etiology of PPP are still controversial, it has been regarded as a psoriasis-related disorder. However, while much has been learned about immunologically mediated mechanisms in the pathogenesis of psoriasis (1), little comparable studies have been done on PPP. Previous studies have revealed that PPP, like psoriasis, is often associated with arthro-osteitis (2). Considering that immunologically mediated mechanisms are suggested to play an important role in the pathogenesis of psoriatic arthritis (3), immunological study may provide a new approach towards the understanding of the pathogenesis of PPP. Interleukin 2 (IL-2) is one of the most important immunoregulatory molecules and the defects in the production of and response to IL-2 have been reported in patients with various immunologically mediated diseases such as systemic lupus erythematosus (SLE) (4) and rheumatoid arthritis (5). Moreover, inhibitors of the IL-2 have been described previously in these diseases (6). We therefore decided to investigate whether there existed intrinsic lymphocyte abnormalities in PPP patients, especially those with arthro-osteitis, and, if so, whether successful treatment could improve the abnormalities. In this study, we focused our attention on the production of IL-2 and IL-2 inhibitor in patients with PPP.

MATERIALS AND METHODS

Patients

A total of 19 patients with PPP were selected for this study. Patients with evidence of psoriasis at other sites were excluded. The patients were divided into two groups: eleven patients with arthro-osteitis clinically and radiographically at the time of study; eight patients with neither any sign of arthro-osteitis at the time of study nor any past history of it. The former group consisted of seven men and four women, aged 25-71 years (mean 42 years); the latter group consisted of six men and three women, aged 28-69 years (mean 40 years). The duration of the disease ranged from 0.5 to 6 years.

Clinically active disease was defined as the formation of pustules on the palms and soles. All patients were treated with topical corticosteroids during this study. In addition, five patients were treated with non-steroidal anti-inflammatory drugs, three with etretinate (0.2–0.8 mg/kg) and two with a combination of etretinate and colchicine (0.5–1 mg) for a period of 3 to 8 months.

Assay for IL-2 production

Peripheral blood lymphocytes (PBL) were prepared from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation. PBL were cultured at 2×10^6 /ml with 10 µg/ml of purified phytohemagglutinin (PHA; Burroughs-Wellcome, Greenville, NC) in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS; Flow Laboratories, Stanmore, N.S.W., Australia), 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 10 mM HEPES, 100 U/ml penicillin and 100 µg/ml streptomycin for 48 h at 37°C. Supernatants were harvested, passed through 0.45 µm Millipore filter, and then stored at -70°C until assayed for IL-2 activity. IL-2 activity was measured in terms of its ability to support the proliferation of the murine IL-2-dependent helper T cell line, 1SS.1K, kindly provided by Dr H. Narimatsu, as described previously (5, 7). Briefly, test samples were serially diluted in 96-well flat-bottomed microtiter plates (Falcon No. 3042) containing RPMI 1640 medium supplemented with 10% FBS. 2×10^4 1SS.1K cells were then added to each well and the plates were incubated for 24 h. Individual wells were pulsed with 1 µCi/well of ^3H -thymidine (Radiochemical Center, Amersham, England, code TRK, 120) for 24 h. The cells were harvested on a glass fiber filter by a Titertek Multiple Cell Harvester and their radioactivity was measured in a liquid scintillation counter. The IL-2 activity that induced 50% of maximal thymidine incorporation was assigned a value of 1 U/ml.

Assay for IL-2 inhibitor

IL-2 inhibitory activity in sera was assayed by culturing 2×10^4 1SS.1K cells in 100 µl of complete medium together with 50 µl of a standard IL-2 obtained from Con A-activated rat spleen cells (9) and 50 µl of serum dilutions. The percent inhibition of ^3H -thymidine uptake by the addition of human sera was calculated as: % Inhibition = $100 \times (1 - \text{cpm with human sera} / \text{cpm without human sera})$. Preliminary experiments had demonstrated that the presence of normal human sera at a final concentration of 5% inhibits the growth of 1SS.1K by 99%.

RESULTS

IL-2 production in patients with PPP

PBL from PPP patients with and without arthro-osteitis were tested for their ability to produce IL-2 after stimulation with PHA in vitro. As shown in Fig. 1, no significant difference was observed between PPP patients with and those without arthro-osteitis, although the former showed great fluctuation. Because two PPP patients with arthro-osteitis showing extremely low levels of IL-2 production were in the active stage, it was likely that the IL-2 production might have fluctuated along with the disease activity. Therefore we next performed experiments to determine whether there was a correlation between the disease activity and individual IL-2 values. Five PPP patients with arthro-osteitis treated with etretinate or a combination of etretinate and colchicine were selected for this study and were tested in order to determine their IL-2 production in the active and inactive stage respectively, because their IL-2 production showed greater fluctuation along with the disease activity than that in patients without arthro-osteitis. As shown in Fig. 2, the IL-2 production in the inactive stage was significantly higher than that in the active stage ($p < 0.005$ by Student's *t*-test). All of the samples in inactive stage showed IL-2 levels over the normal irrespective of the treatment regimen, whereas none of those in active stage had IL-2 levels above the normal range.

IL-2 inhibitor in sera of PPP patients

A serum factor, designated as IL-2 inhibitor, which controls the activity of IL-2 has been shown to exist in normal animals (9, 10) and the defect might be related to the abnormal IL-2 production in various diseases reported (6). To determine whether IL-2 inhibitor in

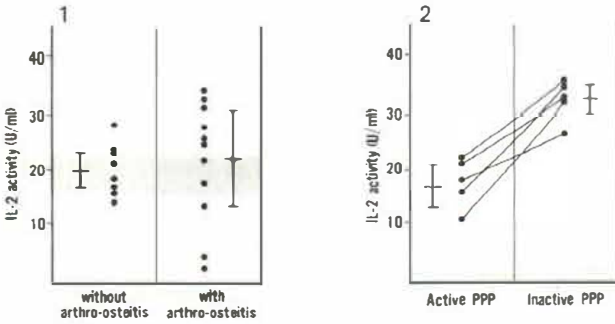


Fig. 1. IL-2 production (U/ml) by PBL from PPP patients with and without arthro-osteitis. Bars are mean \pm SD for each group. The mean \pm SD for 10 normal controls is indicated by the shaded area.

Fig. 2. Increased IL-2 production by PBL from PPP patients with an improvement of symptoms. Bars are mean \pm SD for each group.

the sera of PPP patients is related to the increased IL-2 production in the inactive stage, sera from same patients as those in Fig. 2 were tested to determine their inhibitory activity to preformed IL-2. Sera were obtained from patients in the inactive stage. As Fig. 3 shows, sera from two patients treated with a combination of etretinate and colchicine exhibited decreased IL-2 inhibitory activity, whereas those from three patients treated with etretinate alone contained IL-2 inhibitory activity comparable to that of normal human sera.

DISCUSSION

The defect of IL-2 production in response to mitogens in vitro has been demonstrated in PBL from patients with various immunologically mediated diseases such as SLE (4, 5). The defective IL-2 production has been suggested to contribute to the immunopathogenesis of those diseases (6). In this report we have found that IL-2 production by lymphocytes from PPP patients, unlike that from SLE patients, is comparable to normal controls, but greatly fluctuates along with the disease activity. Several possible mechanisms can be evoked to explain these observations. First, increased IL-2 production in the inactive stage could have contributed in part to the remission of the disease. This possibility is likely because IL-2 plays an important role not only in the clonal expansion of T cells but also in the production of interferon- γ by T cells (11) and the generation of natural killer cell

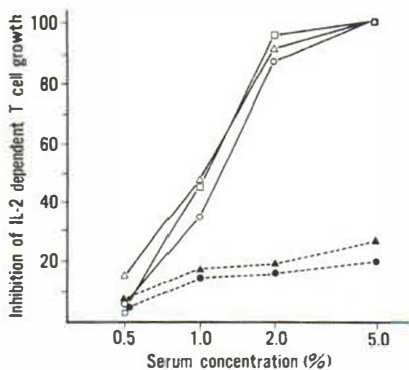


Fig. 3. Percent inhibition of growth of IL-2-dependent T cell lines in the presence of serum from PPP patients treated with etretinate (Δ , \circ , \square) and those treated with a combination of etretinate and colchicine (\blacktriangle , \bullet).

activity (12), most of which seem to have favourable effects upon the resolution of the disease. Alternatively, it is possible that increased IL-2 production in the inactive stage could be an epiphenomenon and the fluctuation along with disease activity could reflect a secondary manifestation of the disease. This view is supported by our finding that the defective IL-2 production was not observed even in active PPP with a few exceptions and that IL-2 production in inactive PPP was higher than that in normal controls. It is also possible that the dose of etretinate or colchicine could have affected the IL-2 production to some extent, because the dosage was decreased or stopped during the inactive stage.

Of interest is the finding that sera from two PPP patients treated with a combination of etretinate and colchicine had extremely low levels of the IL-2 inhibitor. The physiological role of IL-2 inhibitor is considered to prevent the activation of irrelevant T cells by restricting IL-2 action (10). Decreased IL-2 inhibitor levels in sera were reported in patients with SLE (6) or in mice treated with cyclophosphamide (9). Sera from newborn and nude mice also lack the factor. Thus, as suggested by Djeu et al. (6), decreased IL-2 inhibitor levels in these patients and animals might result from a reduced requirement of this factor secondary to decreased IL-2 production. However, decreased levels of serum IL-2 inhibitor in this study seem to be associated with increased IL-2 production, but not with decreased IL-2 production. It is therefore possible that increased IL-2 production observed in the inactive stage may be due in part to the depletion of IL-2 inhibitor-producing cells. Further studies in large numbers of patients are needed to investigate whether the depletion of IL-2 inhibitor-producing cells results in increased IL-2 production. The decrease in the IL-2 inhibitor levels in PPP patients could be attributable to colchicine, although we cannot exclude the possibility that the decrease was aggravated by the etretinate therapy. Since Takigawa et al. (13) reported that oral administration of colchicine is effective in preventing pustule formation in PPP patients, colchicine therapy has been widely used for the treatment of PPP. However, recent studies (14, 15) did not necessarily confirm the beneficial effect. Whenever colchicine is used, it should be kept in mind that prolonged administration of this drug, even at low dose, might lead to the depletion of IL-2-inhibitor producing cells which regulate IL-2 action.

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