

## Nonspecific Mitogen Responses of Peripheral Lymphocytes in Levamisole-Treated Patients with Herpetic Stromal Keratitis\*

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### ABSTRACT

For the purpose of determining the effect of levamisole on cell-mediated immunity of patients with herpetic stromal keratitis, the response of peripheral lymphocytes to three mitogens (PHA, Con A and PWM) was examined in a total of 19 cases of herpetic stromal keratitis (16 effective cases) including 12 patients undergoing levamisole administration.

As controls, a similar study was made on 35 healthy individuals without any ocular disease and on 19 patients with herpetic stromal keratitis who underwent steroid therapy.

The response of lymphocytes to the three types of mitogens in cases undergoing levamisole therapy did not show any significant difference from that of healthy individuals.

The response of peripheral lymphocytes to PHA and Con A in cases more than 7 months after cessation of levamisole administration and in cases undergoing steroid therapy was significantly elevated when compared with that of healthy cases.

These results suggest that levamisole possesses the action of modulating the function of chiefly T-cells among the lymphocytes, through which it is effective against herpetic stromal keratitis.

### INTRODUCTION

Corticosteroids in combination with antiviral drug have been recommended in treating herpetic stromal keratitis, but complete control is not achieved in all cases. Levamisole, an immunomodulating drug, has recently been reported to be effective for this condition<sup>5-7)</sup>, but the mechanism by which levamisole exerts its therapeutic effect has not been completely elucidated.

The present investigation was carried out to evaluate the effect of levamisole on the cellular immune response of peripheral lymphocytes of patients with herpetic stromal keratitis.

### SUBJECTS

A total of 19 patients with herpetic stromal keratitis composed of 12 patients undergoing levamisole therapy and 7 cases more than 7 months after cessation of levamisole treatment were examined. Of these patients, 13 were diagnosed as a type of disciform keratitis, 2 as localized parenchymatous keratitis, 2 as diffuse parenchymatous keratitis, 1 as keratouveitis and 1 as corneal deep ulcer. The age of the patients ranged from 22 to 68 years with an average of 39.3 years. In 9 of 19 patients, mitogen responses were pairly measured at the pretreatment stage and during treatment. 16 (84%)

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\*<sup>1)</sup> 坂田広志, 日山 昇, 近藤英明, 平川裕二, 吉田秀人: 実質型角膜ヘルペス患者の mitogen response について  
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of 19 patients, so far with stromal keratitis, responded to levamisole therapy. Levamisole at dose of 100 mg was given orally three consecutive days every week until keratitis disappeared, and once every week for three months after corneal inflammation became inactive.

As controls, a similar study was made on 35 healthy individuals (mean age of 41.5 years and age range from 21 to 64 years), and on 19 patients with herpetic stromal keratitis who were receiving steroid therapy (mean age 44.4 years and age range from 21 to 64 years).

**METHODS**

1. Peripheral lymphocyte culture: After isolation of lymphocytes from the peripheral whole blood by Conray-Ficoll's method modified by Takaishi et al.<sup>10</sup>, lymphocyte suspensions were prepared. Each culture which contained  $2.0 \times 10^6$  cells in 1 ml of Eagle's minimum essential medium containing 5% fetal calf serum and  $5 \times 10^{-5}$  Mercaptoethanol was maintained at 37 °C in an atmosphere of CO<sub>2</sub> in air.

2. Mitogens used: Phytohemagglutinin (PHA, Wellcome, England), concanavalin A (Con A, Sigma, USA) and pokeweed mitogen (PWM, Gibco, USA) were used. The dose and culture duration of PHA were 12.5 µg/ml and 3 days, those of Con A 10.0 µg/ml and 3 days, and those of PWM 6.25 µg/ml and 5 days, respectively.

3. Measurement of isotope incorporation: For studies in which responses to each mitogen were evaluated by isotope uptake, the microtstplate method was applied. After 24 hr of lymphocyte culture with each mitogen, 0.4 µCi of <sup>3</sup>H-thymidine was added to each well of the microplates and 3 to 5 days later the suspension from each well was transferred onto a membrane (GF/C, Whatman) placed in a micro-cell-harvester. The dry membrane was placed in scintillation vials. The materials were solubilized and counted.

4. HSV neutralizing antibody determination: In some cases, HSV neutralizing antibody was measured by a method using VR3 strain of herpes simplex virus I and cell line of MA 104.

**RESULTS**

**1. Mitogen response of peripheral lymphocytes**

(1) PHA response: In cases undergoing levamisole therapy, PHA response was almost the same as that of healthy humans. In cases more than 7 months after cessation of levamisole administration, PHA response was comparable as that of cases undergoing steroid

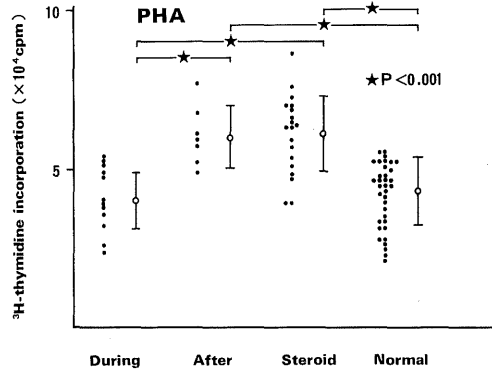


Fig. 1. PHA responses. All values are means ± S.D. The level of significance is indicated and was obtained by using Student's test. The responses in the cases undergoing levamisole therapy were similar to those in normal individuals. During: cases during levamisole treatment, After: cases 7 or more months after levamisole therapy; Steroid: cases undergoing steroid therapy; Normal: healthy humans without ocular disease.

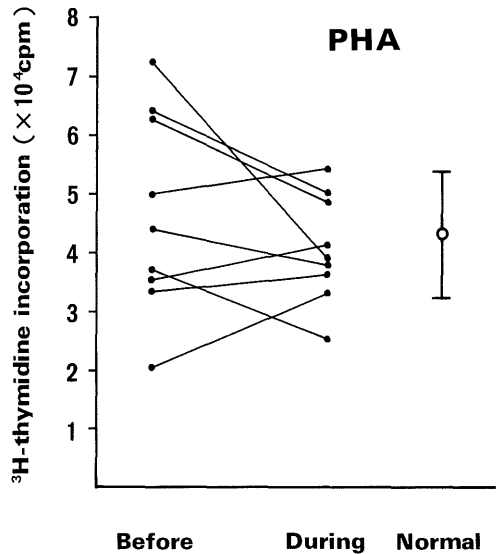
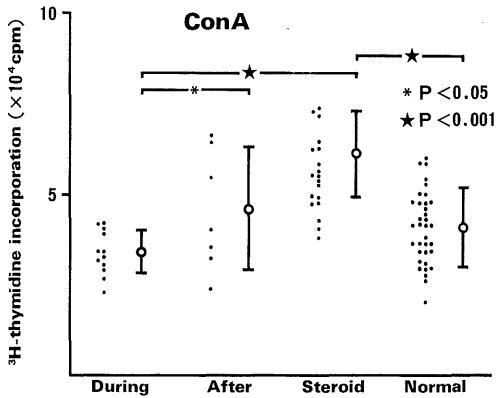
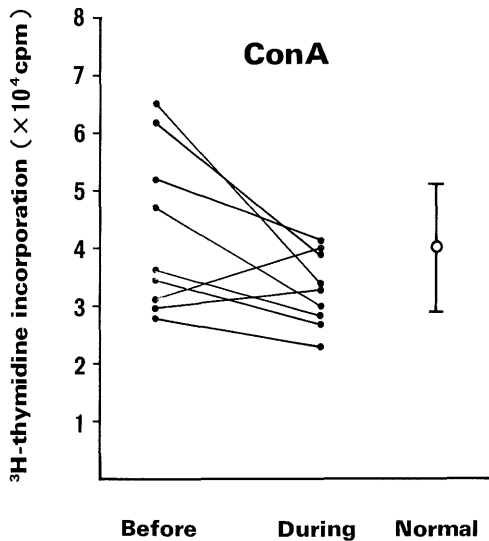


Fig. 2. PHA responses pairly measured at the pretreatment stage and during treatment. The responses showed a tendency to be restored to the normal range.

therapy, but was significantly higher than that of healthy humans ( $p < 0.001$ , Fig. 1). In 9 patients who were measured at the pretreatment stage and during treatment, impaired PHA responses tended to be restored to the normal



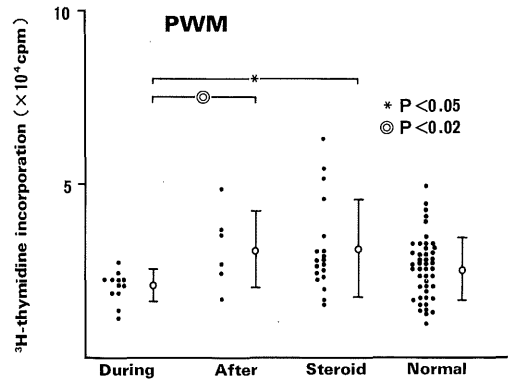
**Fig. 3.** Con A responses. All values are means  $\pm$ S.D. The level of significance is also indicated and was obtained using Student's test. The responses in undergoing levamisole therapy were similar to those in normal individuals as in the case of PHA responses. During: cases during levamisole treatment; After: cases 7 or more months after levamisole therapy; Steroid: cases undergoing steroid therapy; Normal: healthy humans without ocular disease.



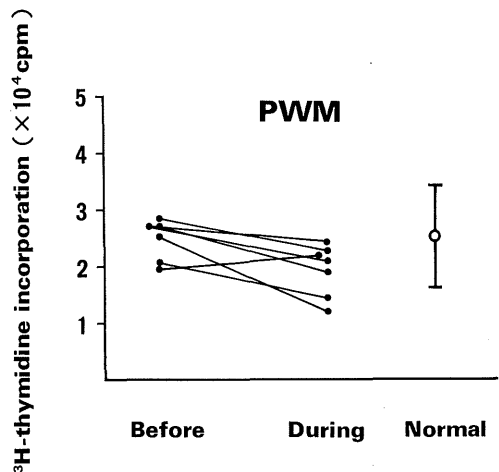
**Fig. 4.** Con A response paired measured at the pretreatment stage and during treatment. The responses had a tendency to be restored to the normal range as in the cases of PHA response.

range (Fig. 2).

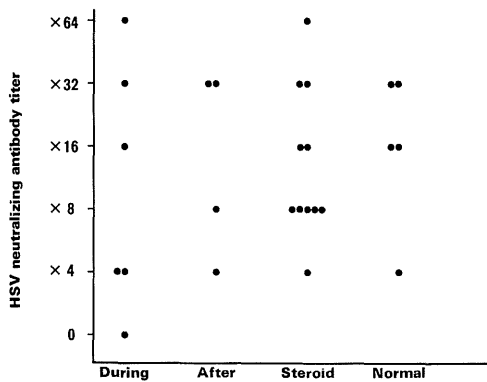
(2) Con A response: In cases undergoing levamisole therapy, Con A response was almost the same as that of healthy humans. In cases more than 7 months after cessation of levamisole treatment, Con A response was comparable as that of cases undergoing steroid therapy, but was significantly higher than that of healthy humans ( $p < 0.05$ , Fig. 3). In 9 patients who were measured at the pretreatment stage and during treatment, impaired Con A responses



**Fig. 5.** PWM responses. All values are means  $\pm$ S.D. The level of significance is also indicated and was obtained using Student's test. The level of significance was higher than that in PHA or Con A responses. During: cases during levamisole treatment; After: cases 7 or more months after levamisole therapy; Normal: healthy humans without ocular disease.



**Fig. 6.** PWM responses paired measured at the pretreatment stage and during treatment. The values at two time points were in the normal range.



**Fig. 7.** HSV neutralizing antibody. There was no special tendency in four groups, although antibody titer was not measured in all cases.

During: cases during levamisole treatment; After: cases 7 or more months after levamisole therapy; Normal: healthy humans without ocular disease.

tended to be restored to the normal range (Fig. 4).

(3) PWM response: In cases undergoing levamisole therapy, PWM response was similar to that of healthy humans, but was lower than that of cases more than 7 months after cessation of levamisole therapy or of cases undergoing steroid therapy although the probability was  $p < 0.05$  and  $p < 0.02$ , respectively (Fig. 5). The magnitude of PWM response which was measured at the pretreatment stage and during treatment in 9 patients remained unchanged (Fig. 6).

## 2. HSV neutralizing antibody

Although HSV neutralizing antibody was not measured in all cases, no significant differences were observed between the four groups (Fig. 7).

## DISCUSSION

In 1979, Kato et al.<sup>5)</sup> obtained favourable results in some patients treated with levamisole based on the experimental studies in rabbits by Smolin et al.<sup>9)</sup> Since then, the agent has been reported to be effective against herpetic stromal keratitis by several authors<sup>5-7)</sup>. It is thought that levamisole acts by restoring the impaired cellular immune state to the normal level<sup>3)</sup>. In the present study, too, paired measurement of PHA or Con A (T-cell mitogens) responses at the pretreatment stage and during treatment in 9 patients showed a tendency for it to be

restored to the normal range after administration of the drug. In addition, the mean value of PHA or Con A response in patients who were undergoing levamisole therapy were comparable as those of healthy humans, while those in cases undergoing steroid therapy were higher.

Levamisole acts on T-cell function<sup>4,8)</sup>, but has little effect on existing serum immunoglobulin levels or function of B-cells<sup>1,4)</sup>. Our results of measurement of HSV neutralizing antibody also did not demonstrate any difference between the two groups undergoing levamisole or steroid therapy. Furthermore, levamisole did not change the magnitude of PWM (B- and T-cell mitogen) response in 9 patients who were measured at the pretreatment stage and during treatment.

The response of peripheral lymphocytes to PHA and Con A in cases more than 7 months after cessation of levamisole administration was significantly higher than that in cases undergoing levamisole therapy. This indicates that effect of the drug on T-cell function disappears 7 months of more after treatment. The reason why recurrence rate is lower in levamisole-treated patients with herpetic stromal keratitis<sup>6,7)</sup>, despite the lack of effect of levamisole on T-cell function after the cessation of the drug, is unknown.

The use of purified HSV antigen as mitogen permits a specific response of peripheral lymphocytes to HSV. However, in the analysis of the data obtained from the method using HSV antigen, considerable care must be taken because it is not determined whether HSV antigen stimulates T-cells or B-cells, and the magnitude of the response is much lower than that induced by the nonspecific mitogens and shows little difference when compared with that in the normal host. Under these circumstances, it is more practical and rational that the status of T-cell function in patients with herpetic stromal keratitis is evaluated by the method using nonspecific mitogens which provides precise results and high reproducibility. The results of the previous study<sup>2)</sup> on the association between the nonspecific mitogen (PHA) response of peripheral blood and stromal herpetic keratitis are almost identical to our results, although they differed in that differences of PHA response between patients with herpetic stromal keratitis

and healthy humans were greater than those in the previous study<sup>2)</sup>. The reason for this difference between the present results and the previous results is unknown, but this may be explained by the fact that the whole blood technique was used in the previous study. In measuring mitogen responses of peripheral lymphocytes, isolated lymphocytes is a necessary condition because false response can be induced by red cells.

The question whether the improved systemic lymphocyte response is related to the improvement of herpetic stromal keratitis has been raised, since the results may be an epiphenomenon. In order to resolve the question, further testing should be made to determine the role of lymphocyte subpopulation in patients with herpetic stromal keratitis.

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