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Serum interleukin-8 level is a more sensitive marker than serum interleukin-6 level in monitoring the disease activity of recurrent aphthous ulcerations

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BACKGROUND: Recurrent aphthous ulcerations (RAU) are common oral inflammatory lesions. Interleukin (IL)-8 is a pro-inflammatory cytokine of host response to injury and inflammation. Our recent study has found that measurement of serum IL-6 level can detect only 24% RAU patients with an abnormal serum level. In this study, we examined both the serum IL-6 and IL-8 levels in a group of RAU patients. The abilities of IL-6 and IL-8 to detect patients with an abnormal serum level were compared in order to find out whether IL-8 was a more sensitive serum marker than IL-6 in monitoring the disease activity of RAU.

METHODS: In this study, we used a solid-phase, two-site sequential chemiluminescent immunometric assay to determine the baseline serum levels of IL-6 and IL-8 in 146 patients with RAU, 9 patients with traumatic ulcers (TU), and 54 normal control (NC) subjects. Eighty-two RAU patients, with the serum IL-6 or IL-8 levels higher than the upper limit of normal serum concentration, were treated with levamisole for 0.5–3.5 months, and their serum IL-6 and IL-8 levels were measured after treatment.

RESULTS: We found that 25% (37/146) RAU patients, as well as 33% (20/61) major-type, 19% (13/69) minor-type, and 25% (4/16) herpetiform-type RAU patients, had a serum level of IL-6 greater than the upper normal limit of 4.7 pg/ml. In contrast, 60% (87/146) RAU patients, as well as 59% (36/61) major-type, 59% (41/69) minor-type, and 63% (10/16) herpetiform-type RAU patients, had a serum level of IL-8 greater than the upper normal limit of 8.7 pg/ml. In 82 RAU patients with the serum IL-6 or IL-8 levels higher than the upper limit of normal serum concentration, treatment with levamisole for a period of 0.5–3.5 months could significantly reduce the serum IL-6 level from 12.0 ± 1.6 to 3.0 ± 0.5 pg/ml ($P < 0.001$), and could significantly lower the serum IL-8 level from 70.9 ± 11.2 to 13.8 ± 3.1 pg/ml ($P < 0.001$).

CONCLUSIONS: Because measurement of serum IL-8 level can detect 60% RAU patients with an abnormal serum level, while measurement of serum IL-6 level can detect only 25% RAU patients with an abnormal serum level, we conclude that serum IL-8 level is a more sensitive marker than serum IL-6 level in monitoring the disease activity of RAU. Levamisole can modulate both the serum IL-6 and IL-8 levels in RAU patients. IL-8, like IL-6, is also a useful serum marker in evaluating therapeutic effects of levamisole on RAU patients.

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Introduction

Recurrent aphthous ulcerations (RAU) are common inflammatory lesions characterized by recurrent and painful ulcerations of the oral non-keratinized mucosa. Previous investigations have demonstrated alterations of serum immunoglobulins (1), circulating immune complexes (2), T-lymphocyte subsets (3–7), and natural killer cell activity (8) in patients with RAU. Immunohistochemical studies have shown an increased number of CD4+ lymphocytes in the pre-ulcerative oral aphthous lesions and a predominance of CD8+ lymphocytes in the ulcerative oral aphthous lesions (4). Quantitative analysis has shown that the inflammatory infiltrate in active RAU lesions consists of 30–60% of CD4+ cells, 10–30% of CD8+ cells, 5–12% of B cells, 5–35% of macrophages, 2–5% of mast cells, and a low number of monocytes (9). Many activated T cells and very few activated B cells are found in both local active RAU lesions (10) and peripheral lymphocytes (11). The results of previous studies on peripheral blood mononuclear

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cells (PBMC) and tissue-infiltrated mononuclear cells (TIMC) favored the role of cell-mediated cytotoxicity in the immunopathogenesis of RAU (3–7, 9, 10, 12).

Interleukin (IL)-6 is a multifunctional cytokine that participates in inflammatory and immune responses. It is especially important for the acute phase response. Its immunological activities and production by many cell types through complicated autocrine and paracrine cytokine-stimulation mechanisms have been described previously (13). Yamamoto et al. (14) and our recent study (13) found a higher than normal serum IL-6 concentration in 18 and 45 RAU patients, respectively, and a decrease in serum IL-6 level in these RAU patients after treatment. These findings suggest that serum IL-6 level may be a useful marker in evaluating therapeutic effects of RAU.

IL-8 is an important mediator of host response to injury and inflammation (15). It possesses diverse functions as a neutrophil activator and a chemoattractant for neutrophils, T cells, and basophils (16). IL-8 is produced by a variety of cell types including monocytes/macrophages, T cells, neutrophils, endothelial cells, fibroblasts, and keratinocytes in certain pathological and inflammatory states (15, 17, 18). In healthy tissues, IL-8 is barely detectable, but it is rapidly induced by 10- to 100-fold in response to pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) or IL-1β, bacterial or viral products, and cellular stress (19). Higher serum IL-8 level has been found in active Behcet's disease (BD) patients than in inactive BD patients and/or normal control (NC) subjects (15, 16, 18, 20, 21). In addition, PBMC, isolated from active BD patients, secrete a higher level of IL-8 than those isolated from inactive BD patients or NC subjects (22). The spontaneous secretion and lipopolysaccharide-stimulated production of IL-8 by peripheral blood monocytes are significantly increased in BD patients compared to that in healthy controls (23). To the best of our knowledge, the serum IL-8 level in RAU patients has not been studied. However, elevated serum IL-8 level in BD patients has been found to be significantly associated with the presence of oral aphthous ulcers (18, 21). It has been reported that IL-8-activated neutrophils are a major source of enzymes involved in tissue destruction (24). Furthermore, because keratinocytes, endothelial cells, and inflammatory cells in pre-ulcerative oral aphthous lesion may secrete a significant amount of IL-8, which in turn activates neutrophils and attracts more T cells, including cytotoxic T cells, to the aphthous lesion, IL-8 may be involved in the pathogenesis of RAU.

Levamisole is an effective immunodulating agent that can restore the normal phagocytic activity of macrophages and neutrophils, modulate T-cell-mediated immunity, and potentiate the activity of human interferon and IL-2 (25–28). Our previous study has shown that levamisole has modulating effects on both cell-mediated and humoral immunity in RAU patients (29).

Our recent study has found that 24% RAU patients had a higher than normal serum level of IL-6. In 45 RAU patients, with the serum IL-6 level above 5 pg/ml, treatment with levamisole can reduce the high serum IL-6 concentration to a normal level (13). In this study, we measured both the serum IL-6 and IL-8 levels in a group of RAU patients in the active stage of disease at baseline, to clarify whether both IL-6 and IL-8 were elevated in the sera of RAU patients and to assess whether the serum IL-8 level was a more sensitive marker than the serum IL-6 level in monitoring the disease activity of RAU. In addition, we followed the serum IL-6 and IL-8 levels serially in RAU patients, both during and after therapy, to see whether levamisole could modulate both the serum IL-6 and IL-8 levels in RAU patients and to determine whether IL-8 was also a useful serum marker in evaluating therapeutic effects of levamisole on RAU patients.

Materials and methods

The study group consisted of 146 RAU patients (66 men and 80 women; mean age 39.4 years, range 10–79 years). All the RAU patients had at least one episode of oral ulcerations per month during the preceding years. The severity of RAU was subdivided into major, minor, and herpetiform types as described previously (13). The disease and NC groups consisted of 9 patients with traumatic ulcers (TU) and 54 healthy subjects without any oral mucosal disease, respectively. All the patients and control subjects were diagnosed and treated in the dental department of National Taiwan University Hospital. None of them had taken any prescription medication at least 3 months before entering the study. The age and sex distributions of the subjects in both the study and control groups are summarized in Table 1.

Eighty-two RAU patients with the serum IL-6 or IL-8 levels above the upper limit of normal serum concentration were treated by levamisole. Levamisole was administered at a dose of 50 mg two times per day for patients with 30–50 kg of body weight, or at a dose of 50 mg three times per day for patients with 50–70 kg of body weight for three consecutive

Table 1 Age and sex distributions of 146 patients with RAU, 9 patients with TU, and 54 NC subjects

	Age (years)														
	10–19		*20–29*		*30–39*		*40–49*		*50–59*		*60–69*		*70–79*		
	M	*F*	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*	*Total*
RAU	8	4	10	17	14	21	19	19	7	11	6	4	2	4	146
TU	0	0	0	1	2	3	0	2	0	1	0	0	0	0	9
NC	4	0	4	5	6	8	5	10	2	6	3	1	0	0	54

M, male; F, female.

days at the beginning of each 2-week interval. Compliance was monitored by asking the patients to record the time at which each drug was taken. Before the start of therapy, data on all cases were recorded according to a set protocol, and the patients were examined by the same dentist at each visit. Patients were monitored one or two times a month for the recording of changes of frequency, duration, and number of oral aphthous ulcerations.

Blood samples were withdrawn from the RAU patients in the active stage (from the onset to the complete healing of the oral mucosal ulceration) before treatment, and from the disease and NC subjects. To assess whether the serum level of IL-6 or IL-8 in patients with RAU could be modulated by treatment with levamisole, one or up to four serial blood samples were obtained after treatment until patients' serum IL-6 or IL-8 levels returned to normal.

The IL-6 concentration in serum samples was determined using Immulite IL-6 assay (Diagnostic Products Corporation, Los Angeles, CA, USA). The detailed procedure in determining the serum IL-6 level has been described previously (30). The IL-8 concentration in serum samples was determined using Immulite IL-8 assay (Diagnostic Products Corporation). Immulite IL-8 assay was nearly identical with Immulite IL-6 assay, except that the polystyrene bead was coated with monoclonal murine anti-IL-8 antibody instead of anti-IL-6 antibody, and that the alkaline phosphatase-labeled polyclonal sheep anti-IL-6 antibody was replaced by rabbit anti-IL-8 antibody. The detection limit was between 1 and 1000 pg/ml for IL-6 and between 5 and 7500 pg/ml for IL-8. The intra-assay coefficient of variation (CV) varied from 3.5 to 6.2% for IL-6 and from 3.6 to 3.8% for IL-8. The interassay CV varied from 5.1 to 7.5% for IL-6 and from 5.2 to 7.4% for IL-8.

Analysis of variance (ANOVA) and multiple comparison were used to test whether the mean baseline serum IL-6 or IL-8 levels were different among RAU patients, TU patients, and NC subjects. The serum levels of IL-6 or IL-8 at baseline and after treatment were compared to each other using a paired *t*-test. The result was considered to be significant if the *P*-value was less than 0.05.

Results

The baseline serum levels of IL-6 and IL-8 in 146 RAU patients, 9 TU patients, and 54 healthy control subjects were measured and compared between groups (Table 2). In 54 NC subjects, the mean serum IL-6 level was 2.1 ± 0.2 pg/ml (mean $\pm$ SEM). A value of 4.7 pg/ml (equal to approximate mean normal level plus 2 SD) was adopted as the upper limit of the normal range of IL-6. About 98.4% (62/63, this value is also called specificity) of the NC subjects and the TU patients had a serum level of IL-6 within the normal limit of 4.7 pg/ml. However, 25% (37/146, this value is also called sensitivity) RAU patients, as well as 33% (20/61) major-type, 19% (13/69) minor-type, and 25% (4/16) herpetiform-type RAU patients, had a serum level of IL-6 greater than 4.7 pg/ml (Table 2; Fig. 1). A significant difference in the mean baseline serum IL-6 level was found among three types of RAU patients and NC subjects ($P < 0.05$). The mean serum level of IL-6 in RAU patients (4.1 ± 0.5 pg/ml; $P < 0.05$) – in major-type RAU patients (4.8 ± 0.8 pg/ml; $P < 0.005$), in minor-type RAU patients (3.5 ± 0.6 pg/ml; $P < 0.05$), or in herpetiform-type RAU patients (3.9 ± 1.2 pg/ml; $P < 0.05$) – was greater than that in NC subjects (2.1 ± 0.2 pg/ml; Table 2).

As shown in Table 2, the mean serum IL-8 level in 54 NC subjects was 5.7 ± 0.2 pg/ml (mean $\pm$ SEM). A value of 8.7 pg/ml (equal to approximate mean normal level plus 2 SD) was adopted as the upper limit of the normal range of IL-8. About 93.7% (59/63, specificity) of the NC subjects and the TU patients had a serum level of IL-8 within the normal limit of 8.7 pg/ml. However, 60% (87/146, sensitivity) RAU patients, as well as 59% (36/61) major-type, 59% (41/69) minor-type, and 63% (10/16) herpetiform-type RAU patients, had a serum IL-8 level greater than the normal limit of 8.7 pg/ml (Table 2; Fig. 2). A significant difference in the mean baseline serum IL-8 level was found among three types of RAU patients and NC subjects ($P < 0.001$). The mean serum IL-8 level in RAU patients (46.7 ± 6.8 pg/ml; $P < 0.001$) – in major-type RAU patients (69.0 ± 14.1 pg/ml; $P < 0.001$), in minor-type RAU patients (29.5 ± 6.0 pg/ml; $P < 0.001$), or in herpetiform-type RAU patients (38.2 ± 14.0 pg/ml; $P < 0.001$) – was greater than that in NC subjects (5.7 ± 0.2 pg/ml; Table 2).

We monitored the serum IL-6 level in 25 RAU patients before and after treatment with levamisole, and found that levamisole could significantly reduce the serum IL-6 level from 12.0 ± 1.6 to 3.0 ± 0.5 pg/ml after treatment for a period of 0.5–3.0 months ($P < 0.001$; Table 3). A significant reduction in the serum IL-6 level after levamisole treatment

Table 2 Baseline serum levels of IL-6 and IL-8 in 146 patients with major-, minor-, or herpetiform-type RAU; 9 patients with TU, and 54 NC subjects

		Serum levels of IL-6 (pg/ml)			*Serum levels of IL-8 (pg/ml)*		
	Number of subjects	*Range*	*Mean ± SEM*	*Level > 4.7 pg/ml Case number (%)*	*Range*	*Mean ± SEM*	*Level > 8.7 pg/ml Case number (%)*
RAU[a]	146	1.0–30.8	$4.1 \pm 0.5^{***}$	37 (25)	5.0–449	$46.7 \pm 6.8^{*}$	87 (60)
Major type	61	1.0–30.8	$4.8 \pm 0.8^{**}$	20 (33)	5.0–449	$69.0 \pm 14.1^{*}$	36 (59)
Minor type	69	1.0–30.8	$3.5 \pm 0.6^{***}$	13 (19)	5.0–282	$29.5 \pm 6.0^{*}$	41 (59)
Herpetiform type	16	1.0–15.6	$3.9 \pm 1.2^{***}$	4 (25)	5.0–199	$38.2 \pm 14.0^{*}$	10 (63)
TU[a]	9	1.0–2.4	1.3 ± 0.2	0 (0)	5.0–5.5	5.1 ± 0.1	0 (0)
NC[a]	54	1.0–4.8	2.1 ± 0.2	1 (2)	5.0–11.3	5.7 ± 0.2	4 (7)

SEM, standard error of the mean.
[a]Comparison among RAU, TU, and NC groups: $P < 0.05$ for IL-6 level and $P < 0.001$ for IL-8 level.
Comparison between patients and NCs: $^{*}P < 0.001$, $^{**}P < 0.005$, and $^{***}P < 0.05$.

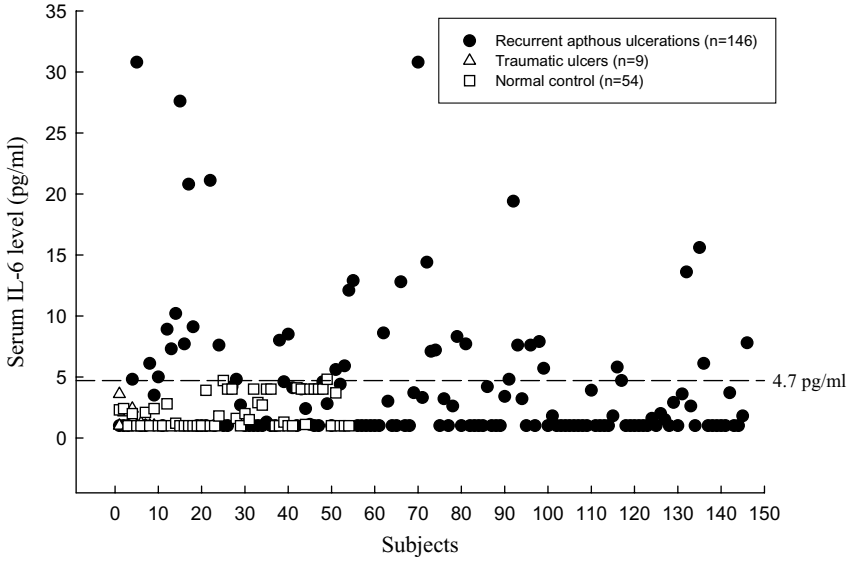

Figure 1 Scatter plots of baseline serum IL-6 levels in 146 patients with RAU, in 9 patients with TU, and in 54 NC subjects, showing that about 98.4% (62/63) of the NC subjects and the TU patients had a serum IL-6 level below the upper normal limit of 4.7 pg/ml, while 25% (37/146) RAU patients had a serum IL-6 level above the upper normal limit of 4.7 pg/ml.

was also found in 13 major-type ($P < 0.001$) and 12 minor-type RAU patients ($P < 0.001$; Table 3).

We also monitored the serum IL-8 level in 82 RAU patients before and after treatment with levamisole, and found that levamisole could significantly reduce the serum IL-8 level from 70.9 ± 11.2 to 13.8 ± 3.1 pg/ml after treatment for a period of 0.5–3.5 months ($P < 0.001$; Table 4). A significant reduction in the serum IL-8 level after levamisole treatment was also found in 34 major-type ($P < 0.001$), 38 minor-type ($P < 0.005$), and 10 herpetiform-type RAU patients ($P < 0.05$; Table 4).

Discussion

Our recent study has shown that the mean serum levels of IL-6 in major-, minor-, or herpetiform-type RAU patients in the active stage of the disease are significantly higher than that in NC subjects. In 31 major- or minor-type RAU patients with the serum IL-6 levels above the upper limit of normal serum concentration, treatment with levamisole for a period of 0.5–5 months could significantly reduce the serum IL-6 level to normal (13). Similar results were also found in the present study. The significant increase in the serum IL-6

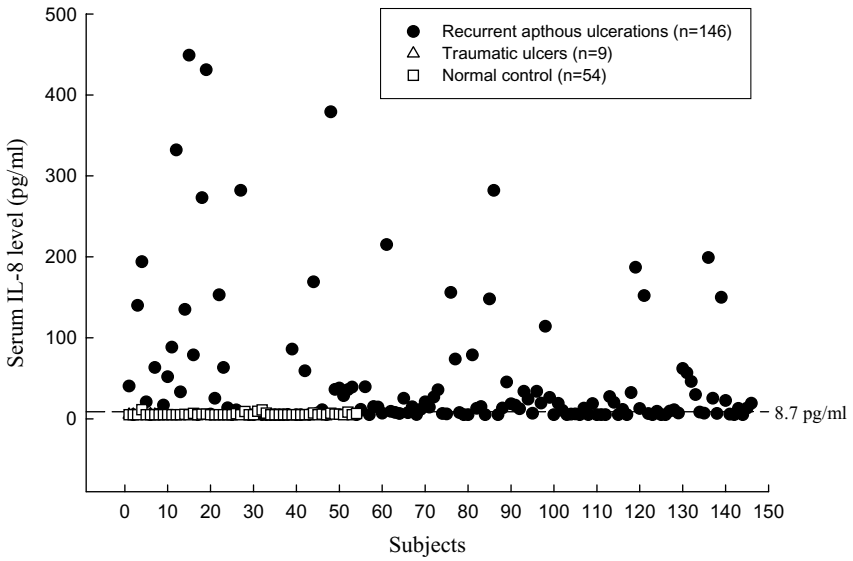

Figure 2 Scatter plots of baseline serum IL-8 levels in 146 patients with RAU, in 9 patients with TU, and in 54 NC subjects, showing that about 93.7% (59/63) of the NC subjects and the TU patients had a serum IL-8 level below the upper normal limit of 8.7 pg/ml, while 60% (87/146) RAU patients had a serum IL-8 level above the upper normal limit of 8.7 pg/ml.

Table 3 The serum levels of IL-6 before and after treatment with levamisole in major- or minor-type RAU patients with the serum IL-6 level above 4.7 pg/ml

		Duration of treatment (months)		*Serum levels of IL-6 (pg/ml)*		
Treatment	*RAU patients (number and type)*	*Range*	*Mean ± SD*	*At baseline (mean ± SEM)*	*After treatment (mean ± SEM)*	*Difference (mean ± SEM)*
Levamisole	13 major type	0.5–3.0	1.2 ± 0.8	12.6 ± 2.3	2.3 ± 0.7*	10.2 ± 2.4
Levamisole	12 minor type	0.5–2.5	0.9 ± 0.7	11.4 ± 2.1	3.7 ± 0.8**	7.8 ± 2.3
Total	25 patients	0.5–3.0	1.0 ± 0.8	12.0 ± 1.6	3.0 ± 0.5*	9.0 ± 1.7

SD, standard deviation; SEM, standard error of the mean.
Significant difference in the serum level of IL-6 between RAU patients at baseline and patients after treatment with $^{*}P < 0.001$ and $^{**}P < 0.005$ by paired *t*-test.

level in RAU patients in the active stage compared to the NCs could be because of local and systemic production of IL-6 by many cell types through complicated autocrine and paracrine cytokine-stimulation mechanisms. This has been clarified in detail in our previous study (13).

Here, we further explained why RAU patients had a significantly higher mean serum level of IL-8 than NC subjects. IL-8 can be produced by a variety of cell types, including monocytes/macrophages, T cells, neutrophils, endothelial cells, fibroblasts, and keratinocytes via the stimulation of pro-inflammatory cytokines, such as TNF-α or IL-1, and bacterial or viral products (15, 17–19). Previous studies have shown the presence of CD4+, CD8+ and B lymphocytes, mature tissue macrophages, recently recruited monocytes, and activated T cells in oral aphthous lesions (4, 9, 10). Activated T cells are also found in the peripheral blood of RAU patients (11). Elevated levels of TNF-α mRNA or proteins have been detected in oral aphthous lesion (31, 32). Increased production of TNF-α by peripheral blood leukocytes is found in active RAU patients (33). These locally and systemically produced TNF-α can induce many cell types to secrete IL-8 in the local tissues and peripheral blood.

IL-1 is also a potent inducer of IL-8 synthesis by PBMC, endothelial cells, fibroblasts, and oral keratinocytes (34–36). When PBMC are stimulated with bacterial endotoxin, 50% of the IL-8 is produced via an intermediate action of IL-1 (34). IL-8 can also be produced by endothelial cells when stimulated with activated platelets via an IL-1-dependent pathway (35). IL-1, in the concentration of 1 pg/ml, can induce IL-8 production in the fibroblast culture (34). Moreover, human oral keratinocyte cell lines, after treatment with IL-1, show an increase in the production of IL-8 mRNAs (36). A recent study showed a significant association between IL-1β gene polymorphism and an increased risk for the development of RAU (37). Therefore, we suggest that IL-1-induced IL-8 production by PMBC, endothelial cells, fibroblasts, and oral keratinocytes may also contribute to the elevated serum IL-8 level in RAU patients.

Taking these findings together, we suggest that keratinocytes, TIMC, endothelial cells, and fibroblasts in RAU lesional oral mucosa may be the local cellular sources of IL-8, and PBMC and endothelial cells may be the systemic cellular sources of IL-8. IL-8 can be produced by these cells through the stimulation of TNF-α and IL-1 in the local tissues and peripheral blood. The locally and systemically produced IL-8 finally results in an increased serum IL-8 level in RAU patients.

In this study, the abnormally high serum IL-6 and IL-8 levels in RAU patients could be significantly reduced to the normal level after treatment with levamisole for 0.5–3.5 months. This finding indicates that levamisole can modulate both the serum IL-6 and IL-8 levels in RAU patients. IL-8, like IL-6, is also a useful serum marker in evaluating therapeutic effects of levamisole on RAU patients.

The reasons why treatment with levamisole can result in a significant reduction of the serum IL-6 level in RAU patients have been described previously (13). Healing of RAU lesions after levamisole therapy may give rise to a reduction of the number of altered keratinocytes and TIMC that can secrete IL-8 in the local lesional tissues. Our previous study has shown that levamisole has modulating effects on

Table 4 The serum levels of IL-8 before and after treatment with levamisole in major-, minor-, or herpetiform-type RAU patients with the serum IL-8 level above 8.7 pg/ml

		Duration of treatment (months)		*Serum levels of IL-6 (pg/ml)*		
Treatment	*RAU patients (number and type)*	*Range*	*Mean ± SD*	*At baseline (mean ± SEM)*	*After treatment (mean ± SEM)*	*Difference (mean ± SEM)*
Levamisole	34 major type	0.5–3.0	1.2 ± 0.7	114.9 ± 22.3	19.1 ± 7.2*	95.8 ± 19.7
Levamisole	38 minor type	0.5–3.5	1.1 ± 0.8	39.7 ± 9.6	11.1 ± 1.9**	28.7 ± 8.4
Levamisole	10 herpetiform type	0.5–3.5	1.2 ± 1.0	41.4 ± 18.3	6.2 ± 0.6***	35.2 ± 18.0
Total	82 patients	0.5–3.5	1.1 ± 0.8	70.9 ± 11.2	13.8 ± 3.1*	57.2 ± 9.9

SD, standard deviation; SEM, standard error of the mean.
Significant difference in the serum level of IL-8 between patients at baseline and patients after treatment with $^{*}P < 0.001$, $^{**}P < 0.005$, and $^{***}P < 0.05$ by paired *t*-test.

cell-mediated immunity in RAU patients (29). Normalization of the cellular immunity after levamisole therapy may also reduce the number of PBMC or endothelial cells that are capable of producing IL-8 systemically, or may reduce the secretion of cytokines, such as TNF-α and IL-1, that are found to induce the production of IL-8 by keratinocytes, macrophages, and endothelial cells (19, 38). Therefore, healing of local RAU lesions and normalization of the local and systemic cellular immunity may explain why treatment with levamisole can decrease the serum IL-8 level in RAU patients.

Because measurement of serum IL-8 level can detect 60% RAU patients with an abnormal serum level, while measurement of serum IL-6 level can detect only 25% RAU patients with an abnormal serum level, we conclude that serum IL-8 level is a more sensitive marker than serum IL-6 level in monitoring the disease activity of RAU. Levamisole can modulate both the serum IL-6 and IL-8 levels in RAU patients. IL-8, like IL-6, is also a useful serum marker in evaluating therapeutic effects of levamisole on RAU patients.

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