

Serum cytokines, interleukin-2 receptor, and soluble intercellular adhesion molecule-1 in oral disorders

Tetsuya Yamamoto, DDS, Kazunori Yoneda, DDS, Eisaku Ueta, DDS,
and Tokio Osaki, DDS, DMD, Kochi, Japan

DEPARTMENT OF ORAL SURGERY, KOCHI MEDICAL SCHOOL, KOCHI, JAPAN

Serum levels of soluble intercellular adhesion molecule-1, soluble interleukin-2 receptor, and cytokines such as interleukin-3, interleukin-4, interleukin-6, tumor necrosis factor- α , and granulocyte-macrophage colony-stimulating factor were examined in patients with oral disorders with 20 healthy persons used as control subjects. Patients studied included 30 with squamous cell carcinoma, 26 with oral lichen planus, 20 with recurrent aphthous ulcer, 19 with acute odontogenic bacterial infection, 16 with pseudomembranous candidiasis, and 16 with herpetic gingivostomatitis. Compared with levels in control subjects, detectable serum levels of interleukin-3 (≥ 10 pg/ml) existed more frequently in pseudomembranous candidiasis (13/16), acute odontogenic bacterial infection (14/19), and squamous cell carcinoma (24/30) and of granulocyte-macrophage colony-stimulating factor (≥ 4 pg/ml) more frequently in recurrent aphthous ulcer (15/20) and squamous cell carcinoma (21/30). These cytokine levels were increased with T stage of squamous cell carcinoma. About 20 pg/ml of interleukin-4 was detected in serum from one third to one fourth of patients with oral lichen planus, recurrent aphthous ulcer, and squamous cell carcinoma. Tumor necrosis factor- α was hardly detected in most patients except those with oral lichen planus and squamous cell carcinoma in which about one third of the patients had more than 40 pg/ml of tumor necrosis factor- α in serum. More than 10 pg/ml of interleukin-6 was frequently detected in all disorders, especially recurrent aphthous ulcer (18/20), pseudomembranous candidiasis (12/16), and acute odontogenic bacterial infection (17/19). After treatment, the serum interleukin-6 level increased in pseudomembranous candidiasis, acute odontogenic bacterial infection, herpetic gingivostomatitis, and squamous cell carcinoma, but decreased in oral lichen planus and recurrent aphthous ulcer. As compared with levels in the control subjects, significantly higher levels of soluble intercellular adhesion molecule-1 were observed in recurrent aphthous ulcer, herpetic gingivostomatitis, and pseudomembranous candidiasis and levels of soluble interleukin-2 receptor were significantly increased in oral lichen planus, recurrent aphthous ulcer, and pseudomembranous candidiasis. These results seem to indicate the immunologic characteristics of the individual oral disorder. (ORAL SURG ORAL MED ORAL PATHOL 1994;78:727-35.)

Cytokines and their receptors and antagonists sometimes play the critical role in pathogenesis, and the pathologic condition is closely correlated with serum cytokine levels in many disorders.¹⁻⁴ Recently, the relationship between the clinical appearances of each disorder and levels of soluble adhesion molecules, cytokine receptors, and antagonists such as intercellular adhesion molecule-1 (ICAM-1), interleukin-1 receptor and its antagonist, interleukin-2 receptor (IL-2R), tumor necrosis factor- α receptor (TNF- α R) and TNF- α R antagonist has been investigated.⁵⁻¹⁰

Among interleukins, interleukin-6 (IL-6) is most frequently generated, and its serum level is increased in inflammatory disorders and in malignant neoplasms.^{4, 11-13} Other interleukins such as interleukin-3 (IL-3) and interleukin-4 (IL-4) are expected to be associated with many disorders because these interleukins occupy the important position in the interleukin network.¹⁴⁻¹⁷ TNF- α upregulates functions of lymphocytes and polymorphonuclear leukocytes¹⁸⁻²⁰ and works with other cytokines in activation of these

cells.^{21, 22} Therefore concomitant investigation of TNF- α production and release with the interleukins mentioned is recommended. In addition, the involvement of granulocyte-macrophage colony-stimulating factor (GM-CSF) should be investigated, because GM-CSF is one of the important cytokines that regulate monocytes and polymorphonuclear leukocyte proliferation and activation,²³⁻²⁵ and this cytokine plays a critical role in inflammation.^{26, 27}

Studies of soluble ICAM-1 (sICAM-1) have accumulated,^{5, 6, 28, 29} and the role of sICAM-1 in each specific disorder has gradually been clarified. When increased serum sICAM-1 binds lymphocyte function-associated antigen-1 molecules, which are expressed on lymphocytes, the lymphocytes cannot bind other cells through the adhesion molecules, which results in inhibition of the lymphocyte function.³⁰ In the same manner, abrogation of IL-2 action may occur when serum soluble IL-2R (sIL-2R) increases. To understand the pathophysiologic process of autoimmune disorders and oral mucous membrane diseases, it seems necessary to investigate both up-regulatory and down-regulatory cytokines, receptors, and also receptor antagonists described herein.

Table I. Clinical summary of healthy control subjects and patients studied

	Control subjects (n = 20)	OLP (n = 26)	RAU (n = 20)	HGS (n = 16)	PMC (n = 16)*	ABI (n = 19)	SCC (n = 30)
Sex							
Male	10	6	6	8	10	12	21
Female	10	20	14	8	6	7	9
Age (yr)							
Mean $\pm$ SD	34.9 $\pm$ 9.1	61.2 $\pm$ 12.5	37.2 $\pm$ 16.3	33.1 $\pm$ 15.0	59.4 $\pm$ 8.4	44.8 $\pm$ 13.8	61.0 $\pm$ 10.9
Range	25-48	25-82	7-66	6-65	43-73	19-69	40-89

RAU, HGS, PMC, and ABI were considered to be cured when the clinical findings disappeared. Recovery in OLP was determined as the time when subjective symptoms were gone and erosion, ulcer, and redness had disappeared. SD, Standard deviation.

*Complication of lung carcinoma in two patients with PMC; rheumatoid arthritis and diabetes mellitus in one patient with PMC each was observed.

In oral diseases, investigations on cytokines are few,³¹⁻³³ and serum cytokine levels and sICAM-1 and cytokine receptors have scarcely been examined.^{3,34} Cellular immunosuppression has been demonstrated in fungal and viral infections. Immunosuppression by herpes simplex virus-1 has been reported,³⁴⁻³⁶ but immunologic investigations in primary herpes infection are few.³⁴ Recently, fungal infection, especially candidiasis associated with viral infection, has frequently been reported.^{37,38} To clarify each characteristic of the immunologic pathophysiologic process, we investigated serum levels of cytokines, sICAM-1, and sIL-2R in carcinomas, chronic oral inflammatory disorders, and acute bacterial, fungal, and viral infections. Characteristic patterns of serum cytokine, sICAM-1, and sIL-2R levels were observed in each disorder.

MATERIAL AND METHODS

Patients and healthy control subjects

Thirty patients with oral squamous cell carcinomas (SCC), 26 with oral lichen planus (OLP), 20 with recurrent aphthous ulcer (RAU), 16 with oral pseudomembranous candidiasis (PMC), 19 with odontogenic acute bacterial infections such as space abscess and phlegmon (ABI), and 16 with herpetic gingivostomatitis (HGS) were examined. Twenty healthy persons were used as control subjects.

Patients with SCC (T2, 16; T3, 7; T4, 7 according to International Union Against Cancer classification³⁹) received inductive chemoradiotherapy for about 3 weeks and surgical removal of the remaining tumor 2 to 3 weeks after the inductive therapy. The first and second blood samplings were taken before the inductive therapy and before surgery, respectively. OLP was clinically and histopathologically confirmed. The histologic confirmation was based on the liquefaction of the basal cell layer and bandlike mononuclear cell infiltration in the dermis. All RAUs, which were clinically diagnosed, were of the small ul-

cer type. The primary infection of herpes simplex virus-1 was clinically and serologically determined by the antibody examination with paired sera (complement fixation test, neutralization test, herpes simplex virus-1-immunoglobulin G and herpes simplex virus-1-immunoglobulin M levels). *Candida* cell culture testing of the oral swabs was done in all patients with PMC, and PMC was finally diagnosed by the response to the antifungal agent amphotericin B. ABI was treated with antibiotics and antiinflammatory drugs.

The patients and healthy control subjects had not received any immunosuppressive or potentiating agents. No complicated systemic disorders were found except for in four cases of PMC. A summary of patients and control subjects studied is shown in Table I including gender, age, and complicated systemic diseases.

Sera

Blood samples were obtained at the first visit (symptomatic phase) and at the time of convalescence or the disappearance of each oral lesion (remission phase) or after chemoradiotherapy. Blood was centrifuged at 800 $\times$ g for 10 minutes, and serum was separated. All serum samples were stored at -80° C until use.

Titration of serum cytokines and soluble molecules

IL-3, IL-4, IL-6, sICAM-1, and TNF- α were measured by a solid-phase enzyme-linked immunosorbent assay with the use of commercially obtained kits according to the manufacturers' directions (kits for IL-3, IL-4, and IL-6: Quantikine, R & D Systems, Inc., Minneapolis, Minn.; kit for sICAM-1: Bender MedSystems, Vienna, Austria; kit for TNF- α : BIOK-INE, T Cell Science, Inc., Cambridge, Mass.). Briefly, serum and each diluted standard cytokine or sICAM-1 were incubated in microwells previously coated with murine monoclonal antibody to each cy-

tokine or sICAM-1. After removal of excessive reactants by washing with phosphate buffered saline, the wells were filled with horseradish peroxidase-conjugated murine monoclonal antibodies against each cytokine or sICAM-1, and unbound reagents were then removed. As the substrate for visualization, ϕ -phenylenediamine was used in TNF- α assay and tetramethylbenzidine was used in other cytokines. After the substrate was allowed to react with horseradish peroxidase complex in the presence of hydrogen peroxidase (for 15 minutes in ICAM-1, 30 minutes in TNF- α , and 20 minutes in IL-3, IL-4, and IL-6), the reaction was stopped by a detergent (4 N sulfuric acid for sICAM-1 and 2 N sulfuric acid for others) and the optical density of each well was determined with a spectrophotometer.

GM-CSF levels were also measured with an enzyme-linked immunosorbent assay kit (Factor-Test, Genzyme Co., Boston, Mass.). The only difference from the previously described procedure was the use of rabbit anti-human GM-CSF polyclonal antibody (second antibody) and biotin-labeled goat anti-rabbit polyclonal antibody (third antibody) instead of horseradish peroxidase-conjugated monoclonal antibodies. The cytokine-antibody complex was visualized after hydrogen peroxidase and ϕ -phenylenediamine treatment.

The level of sIL-2R was determined by a two-site, solid-phase enzyme immunometric assay with a commercial kit (Cellfree Interleukin-2 Receptor Beadassay Kit, T Cell Diagnostics, Cambridge, Mass.). Briefly, in test tubes containing horseradish peroxidase-conjugated murine monoclonal antibodies against human IL-2R, serum and diluted standard IL-2R were mixed with polystyrene beads coated with murine monoclonal antibodies to human IL-2R. After 90 minutes of incubation, the beads were washed three times with phosphate buffered saline, and the mixture of hydrogen peroxidase and ϕ -phenylenediamine was added to each test tube containing the beads. After 30 minutes of incubation, the reaction was terminated with 1 N sulfuric acid, and the optical density of each solution in the tube was then measured.

The concentration of each cytokine and soluble molecule in the serum samples was determined by the optical density curve drawn from serially diluted standards.

Statistical analysis

The statistical significance was evaluated with Student's *t* test, paired *t* test, or chi-square test. A *p* value of less than 0.05 was considered statistically significant.

Results

Serum cytokine levels were not largely elevated except for IL-6 (Table II). Compared with the findings for control subjects, detectable serum levels of IL-3 existed more frequently in PMC, SCC, and ABI. Among the 20 control subjects, levels greater than 10 pg/ml of IL-3 were detected in 9. Although out of 16 patients with PMC, 30 with SCC, and 19 with ABI serum IL-3 was detected in 13, 24, and 14 patients, respectively, the mean levels were nearly the same as those in the control subjects. IL-3 levels were increased in parallel with T stage (Fig. 1). Among 16 patients in T2 stage, serum IL-3 was detectable in 12 and the mean level was 21.9 ± 7.8 pg/ml. In the T3 and T4 cases, detectable serum IL-3 levels were 27.0 ± 8.0 pg/ml.

One third to one fourth of patients with OLP, RAU, and SCC showed an increase of serum IL-4 levels; however, the degree was very low (Table II). The slight increase was most frequently observed in patients with RAU (7 of 20 cases). In control subjects, in those with HGS, and in those with PMC serum IL-4 was scarcely detected (2 of 20, 0 of 8, and 1 of 16 cases, respectively).

Sera from six control subjects contained IL-6 levels of 18.3 ± 8.7 pg/ml, whereas IL-6 was detected in 15 patients with OLP, 18 with RAU, 5 with HGS, 12 with PMC, 17 with ABI, and 8 with SCC, and mean IL-6 levels were 50.1 ± 36.1 , 58.7 ± 39.4 , 27.8 ± 10.5 , 138.2 ± 93.8 , 47.6 ± 32.6 , and 22.6 ± 11.1 pg/ml, respectively. In PMC, five patients had very high IL-6 levels in serum. After treatment, serum IL-6 levels in patients with OLP and RAU decreased to near the control level, but increased in those with HGS, PMC, ABI, and SCC.

TNF- α was detected in sera from one case each of control subjects, HGS, and ABI. Four patients each with RAU and PMC and also nine each with OLP and SCC had TNF- α levels greater than 40 pg/ml in serum. In these cases, the cytokine level was constantly about 60 pg/ml or a little higher. In SCC the serum TNF- α level increased after inductive chemoradiotherapy with an increase of the ratio (7/16) of patients serum-positive for TNF- α .

Serum GM-CSF levels did not increase in most patients. However, serum GM-CSF was detected in 15 of 20 patients with RAU, 16 of 26 with OLP, and 21 of 30 with SCC. Patients in T3 and T4 stages exhibited higher GM-CSF levels (6.06 ± 0.96 pg/ml) than patients in T2 stage (5.44 ± 0.95 pg/ml) (Fig. 1).

Compared with the control serum level (275 ± 99 ng/ml), sICAM-1 levels were significantly higher in

Table II. Serum cytokine levels in oral diseases

Disease	IL-3 (≥ 10 pg/ml)	IL-4 (≥ 10 pg/ml)	IL-6 (≥ 10 pg/ml)	TNF- α (≥ 40 pg/ml)	GM-CSF (≥ 4 pg/ml)
Control	9/11* 20.4 $\pm$ 4.3†	2/18 20.0 $\pm$ 3.0	6/14 18.3 $\pm$ 8.7	1/19 95.0	13/7 5.00 $\pm$ 0.50
OLP					
Active phase	12/14 20.3 $\pm$ 6.5	7/19 25.9 $\pm$ 16.1	15/11 50.1 $\pm$ 36.1	9/17‡ 66.1 $\pm$ 14.3	16/10 5.67 $\pm$ 0.93
Remission phase	6/10 20.1 $\pm$ 4.8	6/10 23.6 $\pm$ 10.7	4/12 31.7 $\pm$ 10.0	7/9 65.0 $\pm$ 15.6	13/3 5.59 $\pm$ 1.36
RAU					
Active phase	12/8 14.9 $\pm$ 2.4	7/13 20.2 $\pm$ 7.3	18/2§ 58.7 $\pm$ 39.4	4/16 111.7 $\pm$ 52.7	15/5 5.46 $\pm$ 0.68
Remission phase	0/6 —	0/6 —	4/2 22.2 $\pm$ 8.6	2/4 95.0 $\pm$ 25.0	5/1 5.14 $\pm$ 0.93
HGS					
Before treatment	3/5 30.8 $\pm$ 14.2	0/8 —	5/3 27.8 $\pm$ 10.5	1/7 115.0	4/4 4.83 $\pm$ 0.48
After treatment	4/4 18.3 $\pm$ 6.9	0/8 —	5/3 50.9 $\pm$ 33.9	2/6 82.5 $\pm$ 42.5	7/1 5.20 $\pm$ 0.94
PMC					
Before treatment	13/3 18.6 $\pm$ 6.3	1/15 16.0	12/4 138.2 $\pm$ 93.8¶	4/12 76.7 $\pm$ 34.7	7/9 5.36 $\pm$ 0.95
After treatment	9/2 21.3 $\pm$ 15.3	0/11 —	8/3 182.9 $\pm$ 120.1¶	2/9 50.0 $\pm$ 5.0	4/7 5.37 $\pm$ 0.82
ABI					
Before treatment	14/5‡ 14.9 $\pm$ 5.8	4/15 38.1 $\pm$ 20.7	17/2§ 47.6 $\pm$ 32.6	1/18 60.0	8/11 4.72 $\pm$ 1.04
After treatment	4/1 16.0 $\pm$ 4.9	2/3 21.5 $\pm$ 7.5	4/1 107.6 $\pm$ 83.6	1/4 65.0	3/2 7.03 $\pm$ 1.72
SCC					
Before treatment	24/6‡ 24.2 $\pm$ 8.2	7/23 18.8 $\pm$ 7.1	8/22 22.6 $\pm$ 11.1	9/21 57.2 $\pm$ 14.8	21/9 5.77 $\pm$ 1.00
After treatment	9/7 20.3 $\pm$ 6.2	2/14 18.0 $\pm$ 3.0	10/6 35.6 $\pm$ 13.2#	7/9 66.3 $\pm$ 18.9	10/6 5.33 $\pm$ 0.84

*Ratio indicates number of patients with detectable cytokines/number without detectable cytokines.

†Mean $\pm$ standard deviation of cytokine levels above the detectable border.‡Significantly more frequent than in control subjects: $p < 0.05$ by chi-square test.§Significantly more frequent than in control subjects: $p < 0.001$ by chi-square test.||Significantly more frequent than in control subjects: $p < 0.02$ by chi-square test.¶Significantly higher than in control subjects: $p < 0.01$ by Student's t test.#Significantly higher than in control subjects: $p < 0.02$ by Student's t test.

patients with RAU (455 ± 147 ng/ml), HGS (398 ± 130 ng/ml), and PMC (451 ± 201 ng/ml) (Fig. 2). On the contrary, significantly lower levels were observed in patients with OLP (179 ± 76 ng/ml) and SCC (174 ± 60 ng/ml). In patients with OLP, RAU, PMC, and SCC the soluble molecule levels were almost constant before and after treatment. However, the sICAM-1 level in patients with ABI was significantly decreased after treatment and a slight decrease was observed in those with HGS.

Serum IL-2R levels were increased in all oral disorders examined; however, the increase was not significant in patients with HGS, ABI, and SCC before treatment (Fig. 3). A statistically significant increase of the cell free receptors was observed in patients with

OLP (821 ± 427 U/ml), RAU (1183 ± 476 U/ml), and PMC (1032 ± 236 U/ml) compared with the control level (439 ± 106 U/ml). In those patients with PMC whose cases were complicated with other systemic diseases such as diabetes mellitus, lung carcinoma, and rheumatoid arthritis, the sIL-2R level was greatly increased. The sIL-2R levels were almost constant in patients with OLP, RAU, and ABI. However, a large increase in serum sIL-2R levels in patients with PMC (from 1032 ± 236 to 1234 ± 372 U/ml), HGS (from 511 ± 248 to 673 ± 296 U/ml), and SCC (from 568 ± 206 to 653 ± 259 U/ml) was observed after treatment.

Serum cytokine levels of sICAM-1 and IL-2R were summarized with a comparison of levels before and

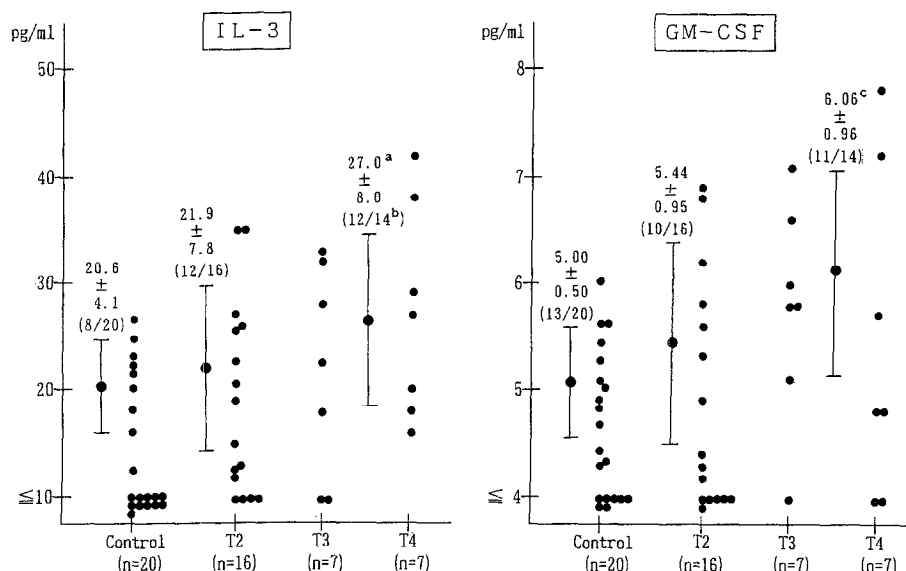


Fig. 1. Serum IL-3 and GM-CSF levels in different T stages. *a*, $p < 0.1$ by *t* test; *b*, $p < 0.05$ by chi-square test; *c*, $p < 0.01$ by *t* test.

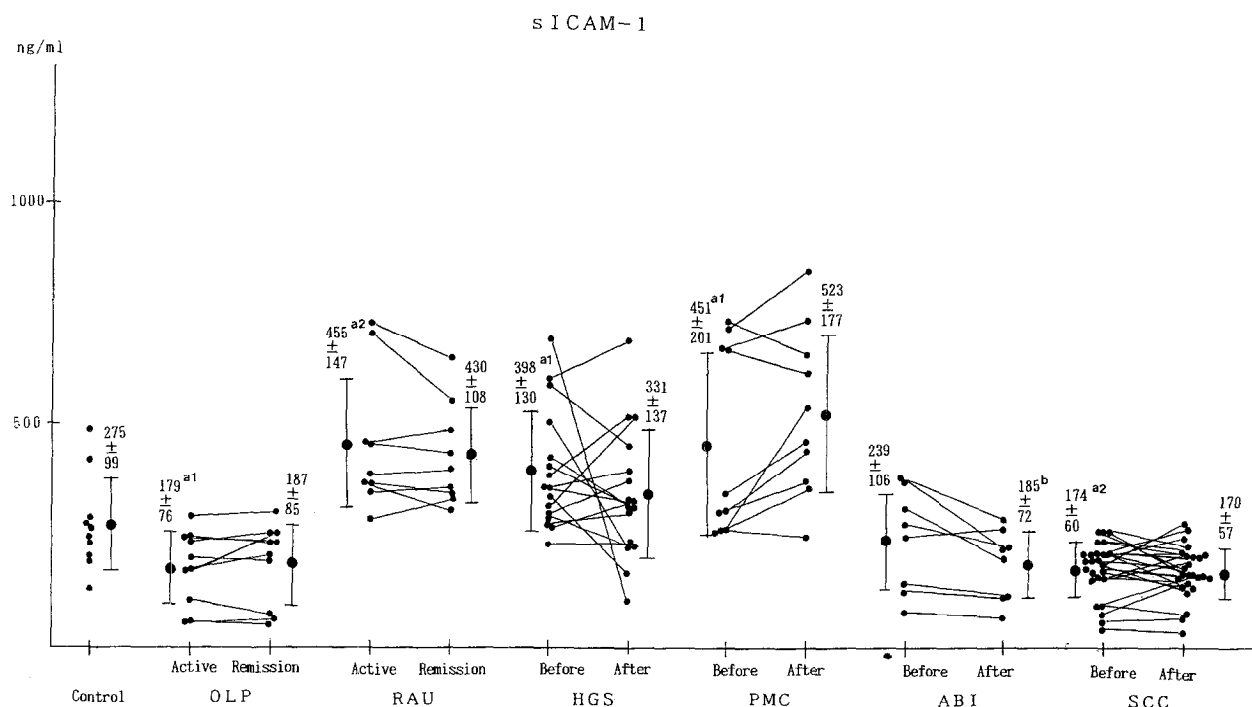


Fig. 2. Soluble ICAM-1 levels in oral diseases. *a*, Statistically significant in comparison with control values (*a*₁, $p < 0.05$; *a*₂, $p < 0.01$ by *t* test). *b*, Significantly lower than before treatment ($p < 0.05$ by paired *t* test).

after treatment (Table III). Large and wide increases of IL-6, sICAM-1, and IL-2R were clearly demonstrated.

DISCUSSION

Cytokine generation is regulated properly in healthy persons, and cytokine actions are usually balanced in

the normal condition. In pathologic conditions, cytokine imbalance occurs and large volumes of cytokines, which may be beneficial or harmful to the body, are generated.¹⁻⁴

The level of IL-3 was increased in the serum of patients with SCC, PMC, ABI, and HGS, but not clearly increased in those with OLP and RAU com-

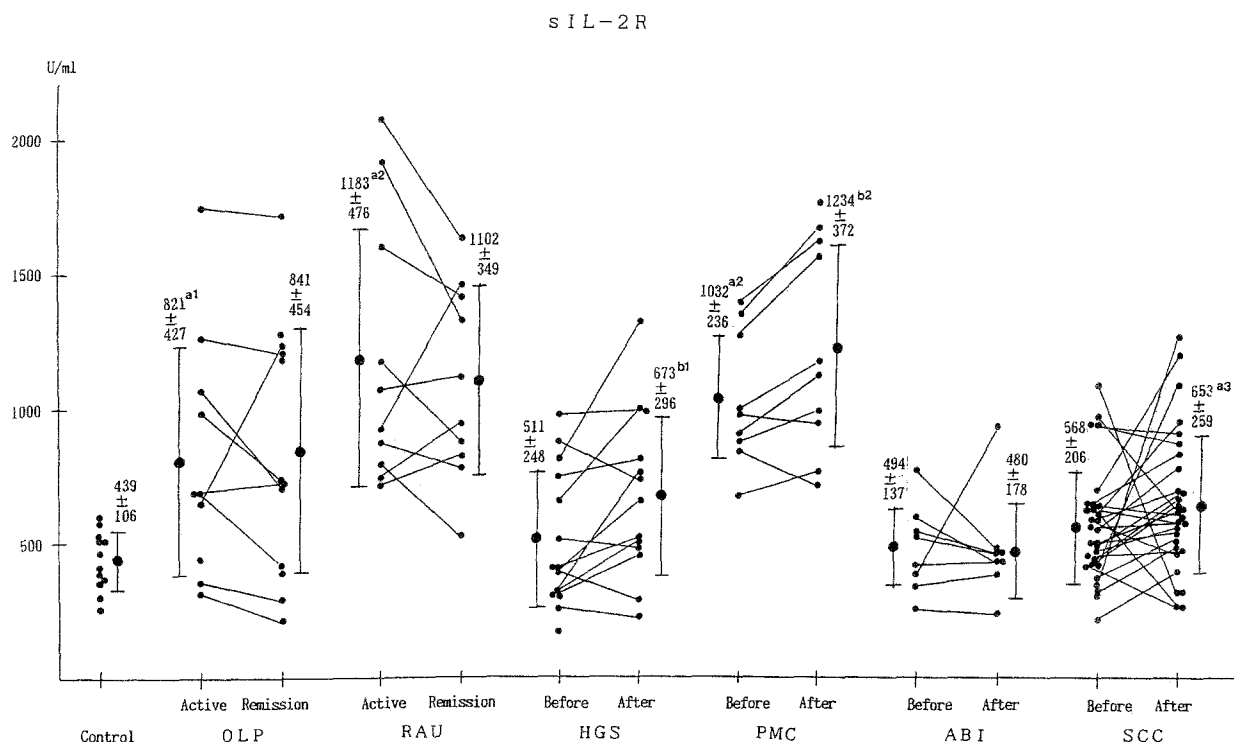


Fig. 3. IL-2R levels in serum from patients with oral disease. *a*, Significantly higher than control values (*a*₁, *p* < 0.01; *a*₂, *p* < 0.001; *a*₃, *p* < 0.02 by *t* test). *b*, Significantly higher than before treatment (*b*₁, *p* < 0.05; *b*₂, *p* < 0.02 by paired *t* test).

Table III. Summary of serum cytokine levels in oral disorders before and after treatment

Disorder	IL-3		IL-4		IL-6		TNF-α		GM-CSF		sICAM-1		IL-2R	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
OLP	→	→	↑	↑	↑↑	↑	↑	↑	↑	↑	↓	↓	↑↑	↑
RAU	↓	↓	↑	→	↑↑	↑	↑	↑	↑	→	↑↑	↑↑	↑↑	↑↑
HGS	↑	↑	→	→	↑	↑↑	→	→	→	→	↑↑	↑↑	→	↑
PMC	↑	↑	→	→	↑↑	↑↑↑	→	→	→	→	↑↑	↑↑	↑↑	↑↑↑
ABI	↑	↑	↑	↑	↑↑	↑↑↑	→	→	→	↑	→	↓	→	→
SCC	↑	↑	↑	→	↑	↑↑	↑	↑↑	↑	↑	↓	↓	→	↑

Pre, Before treatment; post, after treatment (remission phase in OLP, RAU; after improvement of the inflammatory manifestation in HGS, PMC, and ABI; after inductive chemoradiotherapy in SCC); Right arrow, near control value; up arrow, slightly higher than control value; double up arrow, moderately higher than control value; down arrow, lower than control value; triple up arrow, greatly higher than control value.

pared with the level in healthy persons. IL-3 is a multiclonal-stimulating factor,⁴⁰ and IL-3 generation in many kinds of cells is stimulated by TNF-α, interleukin-1β, and interferon-γ.^{41, 42} It has been reported that interferons frequently exist in circulating blood in SCC and interferons were generated in bacterial and viral infection.^{34, 43, 44} Therefore enhancement of IL-3 generation from T cells in the presence of interferons seems to be likely in these disorders. It is known that monocytes become candidacidal by treatment with IL-3 or GM-CSF,^{45, 46} and the activated killing activity is preserved for a long time.⁴⁵ Therefore an

increase of serum IL-3 levels seems beneficial for host defense against fungal invasion.

In the oral disorders examined, only a slight increase of serum IL-4 levels was observed in a few cases of OLP, ABI, SCC, and RAU. When such IL-4 roles as promotion of immunoglobulin synthesis,⁴⁷ T cell proliferation,⁴⁸ T cell adhesion to endothelial cells,⁴⁹ and proliferation of helper T2 working with interleukin-10⁵⁰ are considered, IL-4 generation in OLP, SCC, and infectious oral disorders seems advantageous for recovery from the disease condition. However, IL-4 was not detected in most patients. This re-

sult seems to indicate IL-4 does not play any critical role in the disorders examined.

Higher IL-6 levels compared with those in control subjects were detected in the majority of patients; however, only about one fourth of patients with SCC exhibited detectable serum IL-6 levels before treatment. Among the diseases examined, serum IL-6 levels were greatly increased in RAU, PMC, and ABI. The serum IL-6 level in patients with ABI seems to be compatible with IL-6 generation and release in acute inflammation. However, the elevation of the serum IL-6 level in patients with PMC is considered to be contradictory to the cellular immunity that appears to be suppressed. IL-6 is generated by lymphocytes, endothelial cells, fibroblasts, and epithelial cells.⁵¹ Therefore, IL-6 seems to be generated and released into serum even in immunosuppressed patients. In fact, collagen disease and other autoimmune diseases are associated with elevated levels of IL-6.⁵¹

It is not likely that serum IL-6 levels in patients with RAU were generated locally, because aphthous ulcers seem too small and few in number to generate *in situ* detectable IL-6 levels. Perhaps, IL-6 in patients with RAU is generated from leukocytes and other cells. This means leukocytes and other cells in patients with RAU are stimulated to generate IL-6; however, the stimulators are unknown. This hypothesis seems to be supported by the clear elevation of serum sICAM-1 and sIL-2R levels in patients with RAU. Elevation of these molecules in serum appears to be explained only when RAU is not a simple local disease but associated with a systemic disease.^{52, 53}

Even after remission of the oral lesions, IL-6 levels did not rapidly decrease in serum. This result is probably because of the incomplete disappearance of the inflammation. In ABI, PMC, SCC, and HGS, serum IL-6 levels were even increased after treatment. Acute oral ulcerative mucositis induced by chemoradiotherapy seems to be the cause of the increase in SCC. As previously reported, herpes simplex virus-1 induces suppression of lymphocyte function such as lymphocyte blastogenesis and lymphokine-activated killer activity.³⁴ The patients with primary herpes simplex virus-1 infection undoubtedly had immunologic suppression including suppressed cytokine generation. Candidiasis is also induced in immunosuppression. Therefore, the increase of serum IL-6 levels after the disappearance of oral candidiasis appears to be similar to that in HGS.

Like IL-1 and IL-6, TNF- α is also a keystone of the cytokine network.⁵⁴⁻⁵⁶ TNF- α did not disappear from serum even after the OLP lesion improved or SCC was treated. We cannot find any reasonable explanation for this result. As a possibility, the stimulation for

TNF- α production remains even after improvement of OLP because cytokine generation of infiltrated mononuclear cells and peripheral lymphocytes is enhanced in OLP.⁵⁷ In SCC, chemoradiotherapy-induced oral mucositis seems responsible for the continued levels of TNF- α in serum.

Serum GM-CSF levels in patients with HGS and ABI were inferior to those in the control subjects. Because of the inferior GM-CSF production, oral candidiasis and bacterial infection might be induced inasmuch as GM-CSF induces differentiation and activation of granulocytes and macrophages.^{58, 59} GM-CSF productivity of the generating cells correlates with the resistance against bacteria and fungi.⁶⁰ In most SCC cases, GM-CSF activity was detected in serum. Tumor cells can be considered as the GM-CSF producers because normal keratinocytes are prominent GM-CSF producers, and SCC lines are superior to normal keratinocytes in GM-CSF production.^{61, 62} The increased GM-CSF level in serum is therefore considered to be at least partially generated from tumor cells. Of course, stimulation of infiltrated monocytes and macrophages by the tumor antigen and their GM-CSF generation seems to be likely. These ideas are supported by the fact that serum GM-CSF levels were higher in T3 and T4 cases than in T2 cases. Likewise, IL-3 levels were correlated with T stage. The increase of IL-3 levels seems to result from the same mechanism as that for GM-CSF. However, IL-3 production of epithelial cells is not as high.⁶³ Therefore, these increases may be caused by the increase of responders (leukocytes) to the tumor cells or the increased stimulation to the responders for the cytokine generation.

ICAM-1, an important adhesion molecule in antigen presentation and proliferation of cytotoxic T cells, as well as leukocyte activation and adhesion to endothelial cells,⁶⁴⁻⁶⁶ increased in patients with RAU, PMC, and HGS. ICAM-1 molecules are expressed in many kinds of cells. It is known that an increase of sICAM-1 does not depend mainly on an increase of ICAM-1 expression on the cell surfaces, but on splicing.³⁰ Peripheral blood mononuclear cells from cancer patients submitted revealed an increase of ICAM-1 expression (data not shown). Therefore, sICAM-1 molecules released in serum appear to be spliced on the surfaces of mononuclear cells that highly express ICAM-1.

Cell free IL-2R levels in serum increased greatly in patients with OLP, RAU, and PMC. Unlike sICAM-1 levels, high levels of serum IL-2R were observed in many patients with OLP. Increased levels of soluble IL-2R and sICAM-1 have been demonstrated in many disorders. The increase of these mol-

ecules is regarded as an immunosuppressive aspect because soluble IL-2R and sICAM-1 molecules bind IL-2 and lymphocyte function associated antigen-1, respectively, and the lymphocyte actions through these molecules and their ligands become blocked. Perhaps, leukocyte activation via ICAM-1 binding to lymphocyte function associated antigen-1 is impaired in patients with RAU, HGS, and PMC. Likewise, lymphocyte activation by IL-2 binding to its receptors seems to be impaired in patients with OLP, RAU, and PMC.

The pathophysiologic processes of oral disorders were examined from the aspect of serum cytokines. The results obtained appear to indicate the immunologic characteristics of the individual oral disorder. However, detailed analysis remains to be explored.

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Reprint requests:

Tetsuya Yamamoto, DDS
Department of Oral Surgery
Kochi Medical School
Kohasu, Oko-cho, Nankoku-city
Kochi 783, Japan