

Producing Capabilities of Interferon-gamma and Interleukin-10 in Peripheral Blood from Oral Squamous Cell Carcinoma Patients

Kosuke Naganawa^{1,2,#}, Eiji Takayama^{1,#}, Makoto Adachi^{2,3,#}, Kenji Mitsudo^{3,#}, Masaki Iida^{3,#}, Masako Kamiya-Mizuno¹, Harumi Kawaki¹, Masao Ichinose⁴, Masayuki Motohashi², Yasunori Muramatsu², Iwai Tohnai³, Shin-Ichiro Sumitomo², Michio Shikimori² and Nobuo Kondoh^{1,*}

¹Department of Oral Biochemistry, School of Dentistry, Asahi University, 1851 Hozumi, Mizuho, Gifu 501-0296, Japan;

²Department of Oral and Maxillofacial Surgery, School of Dentistry, Asahi University, 1851 Hozumi 1851-1, Mizuho-shi, Gifu 501-0296, Japan; ³Department of Oral and Maxillofacial Surgery, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Yokohama, Kanagawa 236-0004, Japan; ⁴Second Department of Internal Medicine, Wakayama Medical University, 811-1 Kimiidera, Wakayama 641-0012, Japan

Abstract: In order to evaluate the Th1 and Th2 responses of Oral Squamous Cell Carcinoma (OSCC) patients, we investigated the cytokine producing capability of peripheral blood (PB), and compared it with clinicopathological appearances of OSCC patients. The production of a Th1-type cytokine, interferon (IFN)- γ , from lipopolysaccharide (LPS)-stimulated PB correlated positively with the frequency of lymph node metastasis. We also investigated the production of a Th2-type cytokine, IL-10, however, no significant correlation was observed with the clinicopathological appearances. Our results suggested that the IFN- γ producing capability was specifically regulated and dependent on the regional metastatic potencies of OSCCs.

Keywords: Cytokine-producing capability, interferon-gamma (IFN- γ), Oral squamous cell carcinoma, peripheral blood, tumor immunology.

INTRODUCTION

OSCC is the most frequent malignant tumor of the oral cavity, representing more than 80 % of all oral malignancies [1]. For patients with locally advanced OSCC, surgery, with or without radiotherapy, has been widely accepted as the standard treatment and is thought to be the most curative therapy. However, the disease still has a low overall survival rate of about 60% [2], and for many OSCC patients, it remains difficult to predict the risk of metastasis and offer a reliable prognosis.

Depending on the cytokine profile, the immune responses have been, at least, classified into 2 functionally distinct helper T cell subsets. The cytokines produced by the Th1 and Th2 cell subsets are important for the effective function and immune response of T lymphocytes to regulate the distinct differentiation of these cells. The cytokine products of Th1 and Th2 cells are mutually inhibitory for the differentiation and effect or function of the reciprocal phenotype. This regulation may explain the biases of the Th1 and Th2 responses [3, 4]. It has been reported that an imbalance

between Th1 and Th2 immune response has a correlation with immune dysregulation in various malignancies. The Th1 and Th2 balance has been evaluated in bladder carcinoma [5], colorectal cancer [6, 7], breast cancer [8] and melanoma [9] patients. These studies comparing the Th1/Th2 balance of cancer patients demonstrated that there was a Th1 deficit in cancer patients, while Th2 cytokine was found to be increased. In line with these observations, we have also demonstrated that the IFN- γ producing capability from LPS-stimulated PB is significantly reduced in gastric cancer [10] and hepatocellular carcinoma patients [11].

In this study, we examined the Th1/Th2 cytokine producing capability of PB, and evaluated the diagnostic significance for OSCC patients.

MATERIALS AND METHODS

Patients with Oral Squamous Cell Carcinoma

Seventy-two patients with OSCC treated at Yokohama City University Hospital and Asahi University School of Dentistry between April 2010 and March 2013 were enrolled in the present study (44 men, 28 women; mean (standard deviation / SD) age 65.0 (15.4) years, range 32-92 years) (Table 1). The histopathological characteristics were diagnosed with a biopsied tumor specimen. Clinical staging, including information of tumor sizes, lymph node status and

*Address correspondence to this author at the Department of Oral Biochemistry, School of Dentistry, Asahi University, 1851 Hozumi, Mizuho, Gifu 501-0296, Japan; Tel: +81-058-329-1416; Fax: +81-058-329-1417; E-mail: kondoh@dent.asahi-u.ac.jp

#These authors contributed equally to this work.

Table 1. Clinicopathologic parameters of the enrolled patients with OSCC.

Stage	Total	I	II	III	IV	P-value
Number of patients	72	7	16	14	35	
Age (years)	65.0 ± 15.4	70.5± 16.1	58.3± 16.0	71.9± 8.7	64.2± 15.5	0.3842
Gender						
Male	44	3	9	9	23	0.2146
Female	28	4	7	5	12	
Location						
Tongue	40	5	12	8	15	0.8471
Lower gingival	11	0	2	2	7	
Upper gingival	10	2	2	2	4	
Oral floor	5	0	0	1	4	
Buccal mucosa	4	0	0	1	3	
Plate	1	0	0	0	1	
Lip	1	0	0	0	1	
Tumor size						
T1	7	7	0	0	0	0.0001**
T2	22	0	16	2	4	
T3	18	0	0	12	6	
T4	25	0	0	0	25	
LN-metastasis						
N0	41	7	16	11	7	0.0001**
N1	8	0	0	3	5	
N2	23	0	0	0	23	
Distal metastases						
Negative	72	7	16	14	35	1.0000
Positive	0	0	0	0	0	

The clinical staging and TNM classification were based on the UICC staging system as described (Sobin L, *et al.*). (* $p < 0.05$, ** $p < 0.01$)

histopathological grading was determined according to the Union Internationale Contre le Cancer TNM Classification [12]. The ethics committees of Asahi University School of Dentistry (20071) and Yokohama City University Hospital (B10010728) approved the study protocols, and informed consent was obtained from all patients prior to initiation of the study.

Cytokine-producing Capabilities of PB

The established method for determining the cytokine-producing capabilities of PB has already been described in previous reports [11]. Briefly, less than 1 milliliter (> 0.1 ml) of peripheral blood was stored in a tube containing sodium heparin (Terumo Medical Products, Japan) at room temperature and was used for the subsequent assay within 24 h. Peripheral blood (0.1 ml) was added to 0.1 ml of serum-free RPMI-1640 medium (R8758, Sigma-Aldrich, USA), placed

in a 96 well plate (3599, Costar, USA), then incubated with 0.5 µg/ml of LPS (L2654, Sigma-Aldrich) for 24 h under 5% CO₂ at 37°C. The supernatant was harvested, and then stored at -80°C until ELISA analysis. Cytokine production (IFN-γ and IL-10) was measured using ELISA kits (OptEIA ELISA Set, BD Bioscience, USA).

Statistics

All analyses were performed using statistical software (Stat Mate Version 4.0 ATOMS; JAPAN). Data are expressed as means ± standard deviation (SD) and standard deviation error. Welch's t-test was applied to determine the significance of differences between two groups. One-way ANOVA was applied to reveal the significance of overall differences among the groups. *P* values less than 0.05 were considered to be statistically significant.

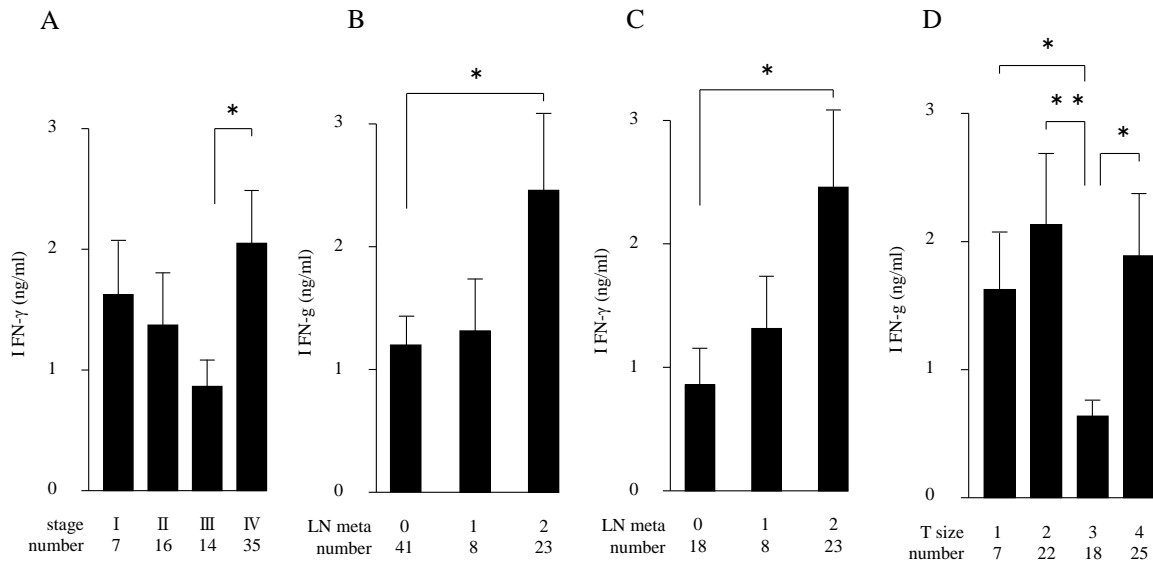


Fig. (1). LPS-induced IFN- γ production in PB from 72 OSCC patients (A, B, D), or from the 49 patients in stages III and IV (C). Amounts of IFN- γ (ng/ml) are in ordinate, and samples classified with corresponding tumor stages (A), lymphnode metastases (B, C) and tumor sizes (D) are denoted in abscissa, respectively. (* $p < 0.05$, ** $p < 0.01$). Error bar shows SD.

RESULTS

Clinicopathological Appearances

The clinicopathological appearances of 72 OSCC patients were summarized in Table 1. The median age of the patients was 68 (range, 32-92) yr. Lymph node metastasis and tumor sizes correlated with clinical stages, which were determined according to the TNM classification [12]. On the other hand, age and gender showed no prognostic significance in the clinical stages (Table 1).

Cytokine-producing Capability of PB from OSCC Patients

We attempted to examine plasma cytokine levels, however, the levels of IFN- γ and IL-10 in our group of OSCC patients were unchanged (or undetected) at all measured stages (data not shown). We determined the production of Th1 cytokine IFN- γ from LPS-stimulated PB using ELISA analysis. As shown in Fig. (1A), the IFN- γ -producing capability was high in stage I patients, but was somewhat decreased in stage II and III tumor progressions. However, the highest level was seen in stage IV patients. Since the clinical staging mainly comprises parameters including primary tumor sizes (from grade T1 through T4) and local metastases (from N0 to N3 in Table 1) [12], we separately compared the IFN- γ -producing capability with each parameter. As shown in Fig. (1B), the IFN- γ -producing capability was significantly increased according to the severity of lymph node metastases among the 72 patients; this tendency was significant in 49 patients in the advanced stages, III and IV (Fig. (1C)). On the other hand, the IFN- γ -producing capability fluctuated among the samples of different T-grades, in which no particular propensity was observed Fig. (1D).

We also examined the production of a Th2 cytokine, IL-10, in LPS-stimulated PB from the 72 patients. The IL-10-

producing capability was unchanged among patients with different stages, lymph node status or T-grades (Fig. 2A, B and C).

DISCUSSION

IFN- γ and IL-10 are secreted by various leukocytes and modulate immune function in the tumor micro environment. IFN- γ has been shown to promote host antitumor immunity since the mice in these studies that were deficient in IFN- γ signaling proved susceptible to carcinogen-induced tumor formation [13]. In this study, we demonstrated that IFN- γ -producing capability increased according to the severity of lymph node metastasis; this tendency was significant in advanced stages, III and IV, in which all of the metastatic cases are included. IFN- γ is produced by Th1, as well as natural killer (NK) cells, and stimulates inflammatory cells including NK cells to induce functional and morphological changes [14]. LPS primarily activates dendritic cells or macrophages through Toll-like receptor 4 (TLR4), triggering cytokines and stimulating surface ligands, leading to NK cell activation [15], however, it can also induce IFN- γ that is distinct from TLR4-mediated signaling pathways [16]. It has been reported that head and neck cancer patients with advanced lymphnode metastases accompanied by extranodal disease show high levels of NK cell cytotoxicity [17]. Therefore, our observations upon IFN- γ may in part be associated with the NK cell-mediated activity.

It has been reported that IL-10 is also produced by OSCC as well as dysplastic cells, while IFN- γ and IL-10 are primarily produced by inflammatory cells. These reports demonstrated that a combination of low IFN- γ and high IL-10 levels were associated with a poor prognosis [18]. In cervical cancer, important regulatory roles in cancer-mediated immunosuppression are mainly mediated by IL-10 and TGF- β [19]. However, our results demonstrated that the IL-10 producing capability by PBCs was not significantly changed

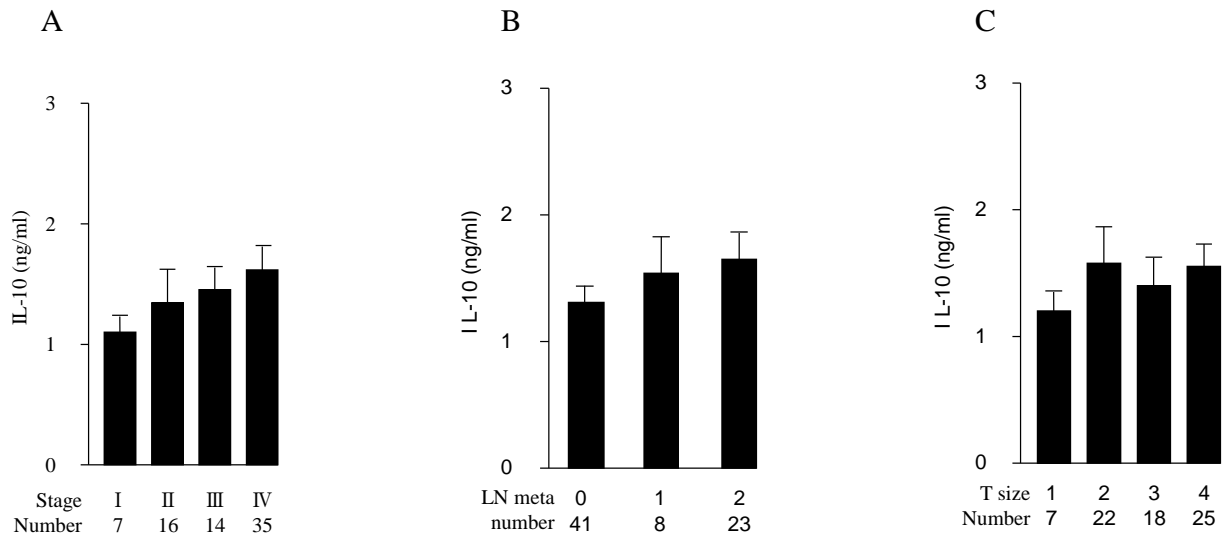


Fig. (2). LPS-induced IL-10 production in PB from 72 OSCC patients. Amounts of IL-10 (ng/ml) are in *ordinate*, and samples classified with corresponding tumor stages (A), lymphnode metastases (B) and tumor sizes (C) are denoted in *abscissa*, respectively (* $p < 0.05$, ** $p < 0.01$). Error bar shows SD.

among OSCC patients even though they were in different stages, and had varying tumor sizes and lymph node status.

So far, we have been unable to explain the regulatory mechanisms of IFN- γ -producing capability in tumor milieu. However, since the NK cell activity correlates well with the disease free interval after definitive therapy [17], the IFN- γ -producing capability of PB could also provide a promising prognostic significance for OSCC patients.

ABBREVIATIONS

OSCC = Oral Squamous Cell Carcinoma
 IFN- γ = interferon-gamma
 PB = peripheral blood
 LPS = lipopolysaccharide
 IL-10 = Interleukin-10

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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