Case Report

Histological and immunohistochemical features of gingival enlargement in a patient with AML

Norihiro Sonoi, DDS, PhD[1)], *Yoshihiko Soga, DDS, PhD[2)],
Hiroshi Maeda, DDS, PhD[1)], Koichi Ichimura, MD, PhD[3)],
Tadashi Yoshino, MD, PhD[3)], Kazutoshi Aoyama, MD, PhD[4)],
Nobuharu Fujii, MD, PhD[4)], Yoshinobu Maeda, MD, PhD[4)],
Mitsune Tanimoto, MD, PhD[4)], Richard Logan, DDS, PhD[5)],
Judith Raber-Durlacher, DDS, PhD[6)], Shogo Takashiba, DDS, PhD [1)]

1) Department of Patho-physiology - Periodontal Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
2) Division of Hospital Dentistry, Central Clinical Department, Okayama University Hospital, Japan
3) Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
4) Department of Hematology, Oncology and Respiratory Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
5) School of Dentistry, Faculty of Health Sciences, The University of Adelaide, SA, Australia

6) Department of Periodontology, Academic Centre for Dentistry, Amsterdam, The Netherlands

*Corresponding author:

Yoshihiko Soga, D.D.S., Ph.D.

Vice Director

Division of Hospital Dentistry, Central Clinical Department

Okayama University Hospital

2-5-1 Shikata-cho, Okayama 700-8525, Japan

Tel: +81-86-235-6588 Fax: +81-86-235-6588

e-mail: y_soga@md.okayama-u.ac.jp

Key Words

gingival enlargement; acute myelomonocytic leukemia; pathogenesis; histology; immunochemistry

Abstract

Here, we discuss the pathophysiology of leukemia-associated gingival enlargement based on a case of acute myelomonocytic leukemia (AML-M4) with typical gingival enlargement. Uniquely, this patient was well enough to allow full periodontal examination and incisional gingival biopsy to be performed both before and after chemotherapy. The patient was a 39-year-old Japanese woman with AML-M4 showing gingival enlargement. Histological and immunohistochemical features of gingiva and bacterial counts in the periodontal pockets were examined before and after chemotherapy. The results were as follows: 1) infiltration of myelomonocytic blasts in enlarged gingiva; 2) resolution of gingival enlargement with complete remission of AML by anticancer chemotherapy; and 3) the numbers of bacteria in the periodontal pockets were not high and were not altered before or after chemotherapy. In patients with AML-M4, remarkable mucosal enlargement is not generally observed in the body except in the gingiva. We hypothesized that antigens derived from periodontal bacteria, even if they are not present in large numbers, could act as chemoattractants for myelomonocytic leukemic cells.

Introduction

Gingival enlargement is an extramedullary clinical manifestation of myeloblastic leukemia[1-3]. Among the various types of acute leukemia, gingival enlargement is particularly prevalent in French/American/British (FAB) classification acute myelomonocytic leukemia (AML-M4) and acute monocytic leukemia (AML-M5) subtypes[1]. Dreizen et al. evaluated 1076 leukemic patients and found gingival involvement in 66.7% of M5 patients and 18.5% of M4 patients[4].

Remarkable mucosal enlargement is not generally observed in the body except in the gingival tissues. This may be explained by the characteristic and unique anatomy of the periodontal tissues, which are continuously exposed to periodontal bacteria. However, in the majority of cases it is not possible to fully understand the pathology of leukemia-associated gingival enlargement because patients are often so severely medically compromised and associated thrombocytopenia causes difficulty in hemostasis. Thus, it is difficult to perform detailed periodontal examination, including incisional biopsy and subsequent histological examination of tissue.

In our hospital, effective collaboration has been established between hematologists and periodontists. All of the leukemia patients admitted to our hospital are referred to the Department of Periodontology from the Department of Hematology. In daily clinical work, we encountered a typical case of AML-M4 with gingival enlargement. This patient was well enough to allow detailed periodontal examination and incisional gingival biopsy to be performed.

Here, we report the details of the gingival condition in this patient, and discuss the possible pathophysiology of gingival enlargement in leukemia patients.

Case Description

The patient was a 39-year-old Japanese woman who had visited a local hospital because of high fever. Antibiotic treatment did not improve her general condition and leukemia was suspected based on the results of blood examination. Eight days after the initial visit to the local hospital, she was referred to the Department of Hematology, Okayama University Hospital, and a diagnosis of AML-M4 was made. On admission, she was referred to the Department of Periodontics and Endodontics for oral examination. Aside from her leukemia, her medical history was unremarkable.

The first oral examination was performed just prior to induction chemotherapy. The appearance of the gingival tissues is shown in the Figure demonstrating marked gingival enlargement. Periodontal pockets with probing depths ranging from 4 to 6 mm as a result of hypertrophy were observed around the interdental gingiva; no periodontal bone resorption was observed by full-mouth dental x-ray radiographic examination.

The patient's blood data are shown in the Table. On the basis of these results it was considered safe to perform a biopsy of the patient's gingivae. We wished to determine whether the gingival enlargement was caused by inflammation or was the result of leukemic infiltration. Furthermore, a biopsy was indicated to exclude other gingival diseases that can cause gingival enlargement. An incisional biopsy sample was obtained from the interdental gingival region between 23 and 24.

Histological examination of hematoxylin and eosin (H&E)-stained sections showed diffuse infiltration of blasts. These cells had convoluted round or ovoid nuclei and abundant eosinophilic cytoplasm, and showed morphological features of myelomonocytes (Figure). AML-M4 is defined as an acute leukemia with differentiation along both myeloid and monocytic lines. Therefore, we performed

immunohistochemical analysis for the myeloid marker myeloperoxidase (MPO) (polyclonal rabbit anti-human antibody; 1:800 dilution; DAKO #A0398) and the monocytic marker CD68 (monoclonal mouse anti-human antibody; clone number: KP1; dilution 1:200; DAKO #M0814) (Figure).

Immunohistochemical staining was positive in blast cells and showed strong cytoplasmic positivity for MPO, indicating infiltration of the gingival tissue with myeloid cells. CD68-positive cells were also observed in the gingival tissue, indicating infiltration of monocytic cells. Generally, normal macrophages and histiocytes are positive for CD68, but their numbers were elevated. Local pathological diagnosis was extramedullary infiltration of acute myeloid leukemia, while a diagnosis of AML-M4 was made based on corroborating bone marrow biopsy observations.

Bacterial counts in the periodontal pockets in three regions (buccomedial of 21, buccomedial of 37, and palatomedial of 17) were examined. The methods used for isolation and detection of total periodontal bacteria by real-time PCR have been described previously[5]. A total bacterial count of about $10^{3\text{-}6}$ was detected. Our group reported a total bacterial count of about $10^{4\text{-}12}$ in patients with severe periodontitis by the same method[6]. The numbers of bacteria in the periodontal pockets were not high in this case.

After recovery from neutropenia induced by initial chemotherapy (idarubicin, 12 mg/m^2; cytarabine, 100 mg/m^2), the gingival enlargement resolved (Figure) although no periodontal treatment was performed. Periodontal pocket depth was within the healthy range (< 3.0 mm).

A second gingival incisional biopsy was performed to evaluate the effects of chemotherapy on the gingiva. In this biopsy specimen, blast cells were not identified

concomitant with the resolution of gingival enlargement (Figure). MPO-positive cell numbers were also markedly decreased (Figure). Bone marrow biopsy also showed complete remission.

Antibiotics were used for about 1 week (cefozopran) before the first examination, and no antibiotics were used within 2 weeks before the other examinations. The total bacterial counts showed no marked changes within the range of 10^{4-5}.

This patient received hematopoietic cell transplantation following a total of 3 cycles of chemotherapy. Bone marrow biopsy continued to show complete remission. Gingival enlargement as observed at the initial visit has not been seen after the initial round of chemotherapy.

Discussion

The main findings in this case were infiltration of myelomonocytic blasts in hypertrophied gingival tissue, resolution of gingival enlargement with complete remission of AML by anticancer chemotherapy, and the numbers of bacteria in the periodontal pockets were not high and showed no changes before or after chemotherapy.

These observations provide some interesting insights into the pathobiology of AML-associated gingival enlargement. The gingival changes observed in this patient were caused mainly by the infiltration and accumulation of myelomonocytic leukemia cells into the gingival connective tissue. In AML-M4 and M5 subtypes, the leukemic cells are monocytic. Generally, monocytes have the ability to infiltrate tissues with strong chemoattractant ability. We suspect that original periodontitis was mild, but low-level antigens derived from periodontal bacteria acted as chemoattractants for myelomonocytic leukemic cells. Indeed, remarkable mucosal enlargement is not

generally observed except in the gingiva. Gingival enlargement in leukemia may involve accumulation of blast cells in the gingiva by chemoattractants derived from periodontal pathogens. Gingival enlargement, which is often observed in AML-M4 and M5, could be more severe in patients with severe periodontitis. AML-M4 or M5 patients may suffer severe periodontitis, which would result in severe gingival swelling and bleeding, and thus prevent the patients from maintaining good oral hygiene. Poor oral hygiene may then lead to further gingival swelling and bleeding, thus establishing a vicious cycle. Further studies are required to determine the relations between clinical status, oral hygiene, and the severity of gingival swelling and bleeding.

In addition, further studies in larger numbers of cases as well as studies of specific bacterial antigens and receptors on AML cells are required. Some AML-M4 and M5 patients do not show gingival enlargement. The blasts in some patients may show deficiency of the ability of some receptors. The endothelium of gingival vessels and possibly antigen presenting cells, such as Langerhans cells, are also factors worthy of consideration.

The differential diagnosis for gingival hypertrophy should be considered and may include drug-influenced gingival enlargements and hereditary gingival fibromatosis, which are described in the classification system for periodontal diseases and conditions[7]. Based on the patient's responses to the initial questionnaire, these were not considered relevant in this case. Histological examination completely excluded these diagnoses, indicating that the gingival enlargement was caused by remarkable leukemia cell infiltration. Furthermore, the total bacterial count within the periodontal pockets was not high in this case. Based on these results, we could consider anticancer chemotherapy should be the matter of the highest priority.

As the oral cavity may be the primary site revealing clinical findings in leukemia patients, physicians and dentists should be aware of these potential changes and consider leukemia as a diagnosis along with more common inflammatory causes of gingival enlargement. Dentists should be aware that the main treatment for gingival enlargement in leukemia patients without remission of leukemic cells could in fact be anticancer chemotherapy by hematologists, rather than local periodontal treatment.

In conclusion, gingival enlargement observed in a patient with acute myelomonocytic leukemia was caused by remarkable infiltration of myelomonocytic blasts (leukemia cells) into the gingival connective tissue. The characteristic anatomy of periodontal lesions, which are continuously exposed to periodontal bacteria, may explain why remarkable mucosal enlargement is not generally observed in the body except in the gingiva. Antigens derived from periodontal bacteria, even if not present in large numbers, could act as chemoattractants for myelomonocytic leukemic cells.

Acknowledgments

We thank Ms. Mutsumi Okabe (Technical staff, Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences) for pathological diagnosis.

We also thank Drs. Chieko Kudo, Aya Hisaeda, Noriko Kanda, Naoko Koide-Namba, Mayumi Ishii, Shoichi Hongo (All staff belong to the Department of Patho-physiology - Periodontal Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences), and Ms. Yuko Sugiura for daily dental clinical support, and Drs. Katsuyuki Kubo, Atsushi Ikeda, and Daisuke Yamamoto for bacterial data analysis.

This study was supported by a Grant-in-Aid for Young Scientists (B) (22791836 to YS) from the Japan Society for the Promotion of Science. We have no conflicts of interest to report.

References

1. Greer JP, Baer MR, Kinney MC. Acute myeloid leukemia in adults. In: Greer JP, Foerster J, Lukens JN, Rodgers GM., Paraskevas F, Glader B, editors. Wintrobe's Clinical Hematology. Philadelphia: Lippincott Williams & Wilkins; 2004; p. 2097-2142.
2. Mani A, Lee DA. Images in clinical medicine. Leukemic gingival infiltration. N Engl J Med 2008; 358: 274.
3. Tjwa E, Mattijssen V. Images in clinical medicine. Gingival hypertrophy and leukemia. N Engl J Med 2008; 359: e21.
4. Wu J, Fantasia JE, Kaplan R. Oral manifestations of acute myelomonocytic leukemia: a case report and review of the classification of leukemias. J Periodontol 2002; 73: 664-668.
5. Maeda H, Fujimoto C, Haruki Y, Maeda T, Kokeguchi S, Petelin M, Arai H, Tanimoto I, Nishimura F, Takashiba S. Quantitative real-time PCR using TaqMan and SYBR Green for Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, tetQ gene and total bacteria. FEMS Immunol Med Microbiol 2003; 39: 81-86.
6. Iwamoto Y, Nishimura F, Nakagawa M, Sugimoto H, Shikata K, Makino H, Fukuda T, Tsuji T, Iwamoto M, Murayama Y. The effect of antimicrobial periodontal treatment on circulating tumor necrosis factor-alpha and glycated hemoglobin level in patients with type 2 diabetes. J Periodontol 2001; 72: 774-778.
7. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol. 1999; 4: 1-6.

Table

Table. Laboratory data of the patient's blood at the first oral examination

	Patient	Normal range
White blood cell counts	19,590/μL	3,500 – 8,500/μL
Differential		
segmented cell (mature neutrophil)	*9.5%*	*29.0 – 70.0%*
stab cell (immature neutrophil)	*1.5%*	*0.0 – 13.0%*
lymphocyte	*13.0%*	*20.0 – 52.0%*
monocyte	*36.0%*	*0.0 – 13.0%*
eosinophil	*0.0%*	*0.0 – 11.0%*
basophil	*0.0%*	*0.0 – 2.0%*
blast	*36.5%*	
Platelets	336,000/μL	150,000 – 350,000/μL
PT	69%	80 – 120%
APTT	33.8 s	25.0 – 36.0 s
CRP	1.76 mg/dL.	0.0 – 0.3 mg/dL.

Figure legend

Figure

Clinical and microscopic appearance at the interdental gingiva between maxillary left canine and first premolar before and after induction chemotherapy.

HE: Hematoxylin & eosin staining; MPO: Immunohistochemical staining for myeloperoxidase; CD68: Immunohistochemical staining for CD68. All immunohistochemistry was performed with a diaminobenzidine (DAB) chromogen and hematoxylin counterstaining.

Before commencement of induction chemotherapy, gingival hyperplasia was remarkable. The histopathological findings on H&E staining showed many blasts with high N/C ratios in the lamina propria. The same region was strongly stained with MPO, indicating the presence of many myeloid cells. CD68 staining showed that the cells were monocytic in nature.

Just after induction chemotherapy, gingival hyperplasia disappeared. A second biopsy of the same area showed that blasts with high N/C ratio disappeared from the lamina propria on H&E examination and immunohistochemistry showed numbers of MPO-positive cells were also markedly decreased. Thus, repeat immunohistochemistry for CD68 was not performed.

Fig. 1