# Cytokine Expression in Patients Hospitalized for Severe Odontogenic Infection in Brazil

*Augusto C. Sette-Dias, MSc,* Kamilla F. Maciel, PhD,† Evandro N. Abdo, PhD,† Luciana C.N. Brito, PhD,‡ Maria Auxiliadora R. Carvalho, MSc,* Leda Q. Vieira, PhD,§ Luiz M. Farias, PhD,* Antônio P. Ribeiro-Sobrinho, PhD,† and Paula P. Magalhães, PhD**

## Abstract

**Introduction:** Severe odontogenic infections remain an important public health concern and a significant economic burden to public health care facilities. Despite this, several aspects of the disease, such as its immune response profile, remain poorly understood. The aim of this study was to search for an association between mRNA levels of the cytokines interferon-$\gamma$, interleukin (IL)-1$\beta$, tumor necrosis factor-$\alpha$, IL-17A, IL-10, and transforming growth factor-$\beta$ and the chemokines IL-8, CCL2/MCP-1, and CCL5 and odontogenic infection. **Methods:** The case group was composed of 12 patients hospitalized in consequence of severe odontogenic infection, and our control group included 12 individuals with healthy periapical tissues. Clinical samples were taken from the case (drainage site) and control (periapical interstitial fluid) groups with the aid of paper points. Total RNA was extracted, complementary DNA was synthesized, and mRNA levels were determined by quantitative polymerase chain reaction. Data analysis was performed by using SPSS, and the Wilcoxon signed rank test was used to determine statistical significance ($P < .05$). **Results:** Data generated showed a significantly increased expression of proinflammatory cytokines (interferon-$\gamma$, IL-1$\beta$, tumor necrosis factor-$\alpha$, and IL-17A), IL-8, and CCL2/MCP-1 in odontogenic infection patients. The mRNA levels of IL-10, transforming growth factor-$\beta$, and CCL5 were similar in both study groups. **Conclusions:** In general, individuals presenting with odontogenic infections exhibited extraordinary proinflammatory cytokine profiles paralleled with unaltered expression of regulatory mediators. *(J Endod 2016;42:706–710)*

## Key Words

Chemokines, cytokines, immune response, odontogenic infections

Severe odontogenic infections are always a risk to patients. They remain an important public health concern and have become a significant economic burden to public health care facilities. Incorrect or delayed treatment generally leads to serious complications such as compromised airway (1).

Propagation of the infection depends on local and systemic host factors and on the virulence of the pathogen (2, 3). During maturation of the immune response, antigen-presenting cells are responsible for the polarization of T-helper (Th) immune responses. Naive $CD4^+$ Th may undergo polarization into different effector Th cell subtypes. Four T-cell subsets have already been described, Th1, Th2, Th17, and T-regulatory (Treg) cells. Each of them drives a characteristic protective immune response (4, 5).

A type 1 immune response, characterized by the production of interferon-$\gamma$ (IFN-$\gamma$), tumor necrosis factor-$\alpha$ (TNF-$\alpha$), and interleukin-1 (IL-1), is involved in the disease progression, bone destruction, and remodeling of periapical lesions. IL-17A plays a critical role in host defense and inflammation (4). In contrast, immunosuppressive mechanisms mediated by Treg-derived or Th2-derived cytokines are responsible for healing and restricting the inflammatory immune mechanisms (6). Transforming growth factor (TGF)-$\beta$ and IL-10, initially described as a Th2 cytokine, both exhibit strong anti-inflammatory properties. It is now recognized that Th1 and Th17 cells also produce IL-10 in addition to Th2 and Treg cells (7).

Chemokines such as IL-8, CCL2/MCP-1, and CCL5 are a specialized group of cytokines that coordinate movement of leukocytes into and through tissues. They are involved in a multitude of biological processes, including organ development and homeostasis, angiogenesis, and immune activation and regulation. The role of chemokines and their cognate receptors in the immune response is carried out by leukocyte homing and trafficking (8).

Although the immune response in periapical lesions and periodontal disease is well-known, the cytokine response profile in severe odontogenic infections has not been clearly defined. The aim of this study was to assay the mRNA levels of the cytokines IFN-$\gamma$, IL-1$\beta$, TNF-$\alpha$, IL-17A, IL-10, and TGF-$\beta$ and the chemokines IL-8, CCL2/MCP-1, and CCL5 in tissue samples obtained from patients presenting with severe odontogenic infection and to compare the results with those obtained for individuals with healthy periapical tissues.

## Materials and Methods

### Study Group

This study was approved by the Ethics Committee of the Hospital Odilon Behrens and Universidade Federal de Minas Gerais (ETIC 374-08). All subjects recruited to the

From the *Laboratory of Oral Microbiology and Anaerobes, Department of Microbiology, Institute of Biological Sciences; †School of Dentistry, and §Laboratory of Gnotobiology and Immunology, Department of Biochemistry and Immunology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte; and ‡School of Dentistry, Fundação Universidade de Itaúna, Itaúna, Minas Gerais, Brazil.

Address requests for reprints to Dr Paula P. Magalhães, Laboratory of Oral Microbiology and Anaerobes, Department of Microbiology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Av Antônio Carlos 6627, Belo Horizonte CEP 31270-901, MG, Brazil. E-mail address: ppmagalhaes@ufmg.br

0099-2399/$ - see front matter



http://dx.doi.org/10.1016/j.joen.2016.01.018

study signed an informed consent form. The study group was composed of 24 individuals, 12 cases and 12 controls. The case group was recruited from patients hospitalized for severe odontogenic infection in the Hospital Odilon Behrens, Belo Horizonte, MG, Brazil during a 1-year period. Patients presenting with other infectious diseases were excluded. Control group was composed of healthy subjects with prosthetic treatment indications in relation to endodontic treatment selected from those referred to the School of Dentistry at the Universidade Federal de Minas Gerais. All control samples were collected from teeth with vital pulp with no inflammation.

The case group included 8 women and 4 men, ranging in age from 17 to 62 years. The teeth most commonly involved in the origin of the infection (7 of 12 patients) were the lower third and second molars. In the 5 remaining cases 5 different teeth were responsible for the abscesses. Pulpal necrosis was correlated to 7 cases, and postextraction surgery was associated with worsening of the disease in 3 subjects. Periodontitis and pericoronitis were associated to 1 case each. Two patients showed associated systemic diseases (diabetes mellitus and hypertension). The space most frequently affected was submandibular, which was involved in all but 1 case (submandibular plus buccal spaces in 6, submandibular space only in 3, and submandibular plus cervical spaces in 2 patients). In 1 patient only periorbital and canine spaces were affected. Three patients underwent tracheotomy. The main clinical manifestations included submandibular swelling and dysphagia that were associated with trismus in all patients.

## Sample Collection

Clinical samples were taken from extraoral or intraoral drainage sites of patients with odontogenic infection. After antisepsis with 70% alcohol (extraoral) or chlorhexidine 0.12% (intraoral), 2 paper points were introduced into the drainage site for 1 minute. Subsequently they were transferred to microcentrifuge tubes, transported to the laboratory, and stored at −80°C.

In regard to the control group, samples were collected from vital teeth with the aid of #20 ProTaper nickel-titanium files (Dentsply Maillefer, Ballaigues, Switzerland) in conjunction with 5.2% sodium hypochlorite that was used for cleaning and shaping of the root canals. Endodontic treatment was performed according to well-established protocols. After cleaning and drying, 3 paper points were introduced into each root canal passing through the root apex (2 mm) to collect the periapical interstitial fluid. After 1 minute the paper points were cut 4 mm from the tip, placed into a microcentrifuge tube, and stored at −80°C (3, 9, 10).

## Sample Preparation

TRIzol reagent (Gibco/BRL Laboratories, Grand Island, NY) was used for RNA isolation as previously described (3). Briefly, chloroform was added, and the mixture was centrifuged at 12,000*g* at 4°C for 15 minutes. The aqueous phase was collected, and RNA was precipitated by isopropanol. Samples were centrifuged at 12,000*g* at 4°C for 10 minutes. The RNA precipitate was washed once with 75% cold ethanol, dried, dissolved in RNase-free water, and then incubated at 55°C for 10 minutes. Extracted RNA was kept frozen at −80°C.

## Real-time Reverse Transcription Polymerase Chain Reaction

Expression of the cytokines IFN-$\gamma$, IL-1$\beta$, TNF-$\alpha$, IL-17A, IL-10, and TGF-$\beta$ and the chemokines IL-8, CCL2/MCP-1, and CCL5 was quantified by real-time reverse transcription polymerase chain reaction. A total of 1 $\mu$g RNA was used for obtaining complementary DNA (cDNA) by reverse transcription (11). Quantitative polymerase chain reaction was performed in a volume of 25 mL and contained 1 mg cDNA. Primer sequences, melting temperatures, and amplicon sizes are shown in Table 1 (3, 12). Primers were designed by using Primer Express Software (Applied Biosystems, Foster City, CA) on the basis of nucleotide sequences available in the GenBank database. Amplification reactions were conducted according to the following protocol: a holding stage at 95°C for 10 minutes, a cycling stage of 40 cycles at 95°C for 15 seconds followed by 60°C for 1 minute, and a melting curve stage at 95°C for 15 seconds, 60°C for 1 minute, and 95°C for 15 seconds (3, 12). The assays were performed in a Step One Real-time PCR System (Applied Biosystems), and SYBR Green (Applied Biosystems) was used to detect amplification. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene for normalization. All samples were run in duplicate. Sequence Detection Software version 2.0 (Applied Biosystems) was used for data analysis. Results were obtained as threshold cycle values. Expression levels were then calculated by using the comparative threshold cycle method. The values were calculated as the mean value of the duplicates for each patient, and the levels of mRNA in all samples were defined as the ratio of each specific primer to GAPDH expression.

**TABLE 1.** Primer Sequences, Melting Temperatures (Mt), and Amplicon Sizes for Each Target Cytokine or Chemokine

| Target | Primer sequence (5′-3′) | Mt (°C) | Amplicon (base pairs) |
|---|---|---|---|
| GAPDH | F: GCA CCA CCA ACT GCT TAG CA<br>R: TGG CAG TGA TGG CAT GGA GGA | 80 | 96 |
| IFN-$\gamma$ | F: GAA CTG TCG CCA GCA GCT AAA<br>R: TGC AGG CAG GAC AAC CAT TA | 80 | 95 |
| IL-1$\beta$ | F: TGG CAG AAA GGG AAC AGA A<br>R: ACA ACA GGA AAG TCC AGG CTA | 73 | 59 |
| TNF-$\alpha$ | F: TTC TGG CTC AAA AAG AGA ATT G<br>R: TGG TGG TCT TGT TGC TTA AGG | 76 | 73 |
| IL-17A | F: CAA TGACCT GGA ATT ACC CAA<br>R: TGA AGG CAT GTG AAA TCG AGA | 70 | 52 |
| IL-8 | F: GAA TGG GTT TGC TAG AAT GTG ATA<br>R: CAG ACT AGG GTT GCC AG ATT TAA C | 60 | 129 |
| IL-10 | F: GGT TGC CAA GCC TTG TCT GA<br>R: TCC CCC AGG GAG TTC ACA T | 81 | 107 |
| TGF-$\beta$ | F: TCT GCT GAG GCT CAA GTT AAA<br>R: ATC GCC AGG AAT TGT TGC | 54 | 74 |
| CCL2/MCP-1 | F: AAG ACC ATT GTG GCC AAG GA<br>R: CGG AGT TTG GGT TTG CTT GT | 80 | 93 |
| CCL5 | F: CGT GCC CAC ATC AAG GAG TA<br>R: CAC ACA CTT GGC GGT TCT TTC | 80 | 91 |