Immunology, Microbiology, and Virology Following Placement of NobelPerfect™ Scalloped Dental Implants: Analysis of a Case Series

Hessam Nowzari, DDS, PhD;* Klaus Yi, DDS;† Winston Chee, DDS, FACP;‡ Sandra K. Rich, MPH, PhD§

ABSTRACT

Background: Cytokine–microbiology–virology monitoring after implant placement may help to develop profiles of variables that can help to explain interaction between the immune system and alveolar bone. Descriptive information at the molecular and cellular levels after implant placement is important in the emerging field of osteoimmunology and may help to formulate hypotheses and intervention strategies in periodontology and implantology.

Purpose: The purpose of this study was to determine the presence or absence of selected cytokines in association with periodontopathogens and human cytomegalovirus (HCMV) after placement of dental implants.

Materials and Methods: Charts of seven consecutive patients with 19 NobelPerfect™ (Nobel Biocare, Yorba Linda, CA, USA) implants were reviewed for crevicular fluid sample outcomes. Anaerobic culture determined periodontopathogens 2 to 5 days, 3 and 6 months postimplant insertion. At 3, 6, and 12 months, real-time reverse transcriptase–polymerase chain reaction (RT–PCR) was performed to detect active HCMV, interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (INF-γ).

Results: Four of five, and six of seven patients harbored no periodontopathogens at 3- or 6-month intervals, respectively. In spite of absence of a bacterial challenge, IL-1β and TNF-α activity was significant. INF-γ was not detected, and HCMV was present at one time interval only.

Conclusions: TNF-α is produced mainly in the early stages of acute inflammation, and high levels of this cytokine at 3 and 6 months postimplant placement may be related to a repetitive acute-phase inflammatory response. Lack of INF-γ and a high cytokine presence without significant corresponding periopathogens or viruses raise a concern that inflammation and, thus, inflammatory bone destruction, is possible outside of these variables. Inflammation and bone loss around this same group of scalloped implants, reported by our previous study, may have been initiated by local factors, such as particular implant design features.

KEY WORDS: host mechanisms, interferon-gamma, interleukin-1beta, periodontopathogens, scalloped dental implants, tumor necrosis factor-alpha

*Professor, clinical dentistry, and director, advanced periodontics, University of Southern California School of Dentistry, Los Angeles, CA, USA; †assistant professor, clinical dentistry and advanced periodontics, University of Southern California School of Dentistry, Los Angeles, CA, USA; ‡Ralph & Jean Bleak professor of restorative dentistry and director, implant dentistry, University of Southern California School of Dentistry, Los Angeles, CA, USA; §associate professor, advanced periodontics, University of Southern California School of Dentistry, Los Angeles, CA, USA

Reprint requests: Dr. Hessam Nowzari, University of Southern California Dental Science Center-DEN, 925 W. 34th Street, Room 119, Los Angeles, CA 90089-0641, USA; e-mail: nowzari@usc.edu

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Bone resportion around dental implants, with resultant loss of papillary soft tissue, may be driven by an excess of cytokines, which stimulate osteoclasts or inhibit osteoblasts.[1,2] Increases of cytokines may lead to bone loss or failure of dental implants.[3,4] Initiation of the inflammatory response by bacterial or viral invasion will elicit an immune response by key cytokines for progression and/or suppression of the inflammatory response. Proinflammatory cytokines, released by the host's monocytes and macrophages in response to bacterial products such as lipopolysaccharide (LPS), can be responsible for the breakdown of the peri-implant tissues. LPS of periodontal pathogens, such as

Porphyromonas gingivalis, is known to induce different cell types to produce cytokines.[5] Additionally, surgical trauma is known to induce an early hyper-inflammatory response, which is characterized by both tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) cytokine release.[6,7]

Cytokines and their receptors form a complex network that is biologically controlled to include positive and negative feedback from the cytokines themselves.[8] Periodontal stability and osseointegration may depend on the local balance of reactive and suppressor immune cells, their cytokines, and mediators. Bone is continuously remodeled through the dynamic processes of resorption and formation and is controlled by local production of cytokines, small polypeptides with a wide range of inflammatory, hemopoietic, metabolic, and immunomodulary properties.[9,10] IL-1β and TNF-α appear to play major roles in mediating the inflammatory response and in the pathogenesis of many chronic inflammatory diseases.[11–13] IL-1β is present at elevated levels in the gingival crevicular fluid during periodontitis.[4,14] It is produced primarily by monocytes, but may be produced by other nucleated cells in response to injury.[15] TNF-α, a cytokine with functions similar to those of IL-1β, has been detected in sites affected by periodontitis.[16]

Interferon gamma (INF-γ) is produced mainly by T_H1 cells and natural killer cells in both innate and adaptive cell-mediated immune response. Although it is pleiotropic, INF-γ is considered a proinflammatory cytokine because it assists TNF activity and induces nitric oxide.[17] INF-γ is known to inhibit osteoclast formation and bone resorption and can act to restore the balance between proinflammatory and anti-inflammatory forces.[6] Additionally, INF-γ can exhibit antiviral activity, as it is an activator of the pathway that leads to cytotoxic T cells.

Dental implants are not physiologically inert and can stimulate peripheral blood mononuclear cells to produce IL-1β and TNF-α in vitro.[15] Other investigators found that implant surfaces of pure titanium and titanium alloys stimulated in vitro production of IL-1, IL-6, and IL-18.[18] Many investigators have examined natural teeth for cytokine activity in crevicular fluid and/or serum and found greater numbers of cytokines in diseased versus healthy sites.[8,19] However, peri-implant bone and resultant papillae loss have not been studied to assess influence of a combination of cytokine, bacterial, and viral variables in crevicular fluid, particularly with use of current reverse transcriptase–polymerase chain reaction (RT–PCR) laboratory testing methods. In order to provide descriptive data for further development and testing of hypotheses related to implant designs and peri-implant bone and papillae loss, the purpose of this study is threefold: (1) to describe peri-implant proinflammatory activity with regard to selected cytokines; (2) to examine whether cytokine levels may be related to specific bacterial pathogens or viral activity; and (3) to consider the relationship between these variables and our previously published data on bone and papillae loss following placement of NobelPerfect™ (Nobel Biocare, Yorba Linda, CA, USA) scalloped implants.[20]

MATERIALS AND METHODS

Our previous chart review study[20] revealed greater than expected bone and papillae loss for six patients (nonsmokers, systemically healthy) monitored for 18 months following placement of the newly designed scalloped implant. In this follow-up chart review, data will be interpreted in relation to bone and papillae loss for the six subjects previously studied, plus one additional, consecutive patient whose data were not available for analysis at the time of the first chart review study. This chart review protocol was approved by the University of Southern California Institutional Review Board, #05-07-208.

In seven consecutively treated patients (19 scalloped dental implants, 5 women, 2 men, 26 to 71 years old), anaerobic bacterial culture was used to determine the occurrence of potential periodontal pathogens 2 to 5 days after placement and at 3 and 6 months postimplant insertion.[21,22] At 3, 6, and 12 months, RT–PCR was performed to detect active human cytomegalovirus (HCMV), IL-1β, TNF-α, and INF-γ. Cytokine activity will reach a high level immediately after implant placement because of the trauma and cellular injury imposed by surgery; therefore, no cytokine measurement was taken until 3 months postimplant placement.[23] Finally, radiographs and intraoral photography from our previous study provided the basis for analysis of bone and papillary response after implant placement.[20] Radiographic bone loss was measured as the distance from the interproximal shoulder of the scalloped implant to the crest of the bone with ImageJ, a public domain, computer software.[24,25]

RT–PCR Measurement

Pooled subgingival crevicular fluid samples were collected from the mesial and distal interproximal sulci of two scalloped implants for each patient. Samples were collected by gently inserting endodontic paper points (Johnson & Johnson, East Windsor, NY, USA) into the gingival crevices for 10 seconds. The paper points were placed into a labeled centrifuge and immediately stored at −70°C until real-time RT–PCR could be performed. Specific patient information was not included with the samples when they were submitted for cytokine analysis to ensure blinding of laboratory personnel. Copies per milliliter of measured cytokines at a level of 200 were considered significant. The primers and probe designs were:

HCMV: pp65 gene, 207 bp
Forward; 5′-ACGCGCTGCCGCTCAAGAT-3′
Reverse; 5-TGTAGTAGACGTCGGGCTCTTT-3′
Probe; 5′-CCGTCGGCGGCCGAGCGCAAACA-3′

IL-1β
200 bp
Forward; 5′-CGACACATGGGATAAGAG-3′
Reverse; 3′-CCAAGGCCACAGGTATTTTG-3′
Probe; 5′-CACTGAACTGCACGCTCCGGGACTC-3′

TNF-α
180 bp
Forward; 5′-CTTCAAGGGCCAAGGCTGC-3′
Reverse; 3′-CCAGATAGATGGGCTCATACCA-3′
Probe; 5′-TCCACCCATGTGCTCCTCACCCACAC-3′

β-globulin
124 bp
Forward; 5′-TGGCAAGAAAGTGCTCGGTG-3
Reverse; 5′-CCTGAAGTTCTCAGGATCCAC-3
Probe; 5′-TAGTGATGGCCTGGCTCACCTGGAC

Microbial Examination

Three fine endodontic paper points were inserted to the depth of each inproximal site for 10 seconds (mesial and distal surfaces of two scalloped implants for each patient) and transferred to VMGA III transport medium.[26] Samples were processed within 2 hours of collection. Specific patient information was not included with the samples when they were submitted for microbiological analysis to ensure blinding of the laboratory personnel. Anaerobic microbiological isolation and identification of putative periodontal pathogens were carried out following established procedures. Samples were dispersed on a vortex mixer at the maximal setting for 45 seconds and then 10-fold serially diluted in VMG I anaerobic dispersion solution.[26] Using a sterile bent glass rod, 0.1 mL aliquots from 10^3 to 10^5 dilutions were plated onto nonselective 4.3% brucella agar (BBL Microbiology Systems, Cockeysville, MD, USA) supplemented with 0.3% bactoagar, 5% defibrinated sheep blood, 0.2% hemolyzed sheep red blood cells, 0.0005% hemin, and 0.00005% menadione. Total viable counts and proportions of specific bacteria in relationship to the total viable counts were determined. Aliquots diluted in VMGA III medium were plated onto TSBV medium for the culture of *Actinobacillus actinomycetemcomitans*, enteric Gram-negative rods and yeasts.[27] The nonselective blood agar was incubated at 35°C in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI, USA) containing 85% N_2-10% H_2-5% CO_2 for 10 days. TSBV medium was incubated in 10% CO_2 in air at 35°C for 4 days. Presumptive identification of representative colonies of each group of organism that morphologically resembled the study species was performed according to methods described by Slots[28] and by use of a micromethod system (API 20°, bioMérieux, Marcy l'Etoile, France). Organisms examined included *A. actinomycetemcomitans*, *Prevotella intermedia*, *Prevotella nigrescens*, *P. gingivalis*, *Dialister pneumosintes*, *Tannerella forsythensis*, *Campylobacter* species, *Fusobacterium* species, *Micromonas micros*, enteric Gram-negative rods, and *Candida* species. Bacteria designated as major periodontal pathogens included *A. actinomycetemcomitans*, *P. gingivalis*, *D. pneumosintes*, and *T. forsythensis*. The percentage recovery of periodontal pathogens was determined by the colony count of each microbial taxon in relation to total viable count.

Data Analysis

Descriptive data are presented in tables and summarized in the narrative. Raw data for all variables, averages of peri-implant crestal bone loss from our previous report on this same group of implants,[20] and frequency data are shown. Radiographs and clinical photographs from one selected representative case are presented.

RESULTS

Table 1 presents raw data for microbiology, virology, and immunology for each of the seven subjects. Patient

TABLE 1 Microbiology, Immunology, and Virology Measured at 0, 3, 6, and 12 Months

	T_0 (placement)	T_1 (3 months)	T_2 (6 months)	T_3 (12 months)
Subject 1 – ASA II, woman, 49 years old, one stage, implant sites: 7, 8, 9, 10				
Microbiology	*Campylobacter*, *Fusobacterium*, *Eubacterium*, *Dialister pneumosintes*	Campylobacter	ND	*
IL1-β copies/mL	*	5,900	3,200	ND
TNF-α copies/mL	*	4,200	1,000	ND
INF-γ copies/mL	*	ND	ND	ND
HCMV copies/mL	*	ND	ND	ND
Subject 2 – ASA I, woman, 37 years old, one stage, implant sites: 7, 8				
Microbiology	*Tannerella forsythensis*, *Campylobacter*, *Fusobacterium*, *Peptostreptococcus micros*	ND	ND	*
IL1-β copies/mL	*	3,800	2,000	ND
TNF-α copies/mL	*	5,600	1,700	ND
INF-γ copies/mL	*	ND	ND	ND
HCMV copies/mL	*	ND	ND	ND
Subject 3 – ASA I, woman, 26 years old, one stage, implant sites: 8, 9				
Microbiology	ND	ND	ND	*
IL1-β copies/mL	*	4,000	2,000	ND
TNF-α copies/mL	*	5,400	1,800	ND
INF-γ copies/mL	*	ND	ND	ND
HCMV copies/mL	*	ND	ND	ND
Subject 4 – ASA II, man, 54 years old, one stage, implant sites: 8, 9, 10				
Microbiology	ND	ND	ND	*
IL1-β copies/mL	*	6,200	5,000	1,800
TNF-α copies/mL	*	5,400	1,500	ND
INF-γ copies/mL	*	ND	ND	ND
HCMV copies/mL	*	ND	ND	ND
Subject 5 – ASA II, man, 36 years old, one stage, implant sites: 8, 9				
Microbiology	ND	ND	ND	*
IL1-β copies/mL	*	8,000	4,200	2,000
TNF-α copies/mL	*	6,000	2,300	ND
INF-γ copies/mL	*	ND	ND	ND
HCMV copies/mL	*	530	ND	ND
Subject 6 – ASA I, woman, 41 years old, two stage, implant sites: 9, 12, 13, 14				
Microbiology	*Actinobacillus actinomycetemcomitans* (Aa), *Porphyromonas gingivalis*, *Campylobacter*, *Eubacterium*	*	Aa, P. gingivalis	*
IL1-β copies/mL	*	*	ND	ND
TNF-α copies/mL	*	*	ND	ND
INF-γ copies/mL	*	*	ND	ND
HCMV copies/mL	*	*	ND	ND
Subject 7 – ASA II, woman, 71 years old, two stage, implant sites: 8, 9				
Microbiology	*	*	ND	*
IL1-β copies/mL	*	*	3,700	*
TNF-α copies/mL	*	*	2,000	*
INF-γ copies/mL	*	*	ND	*
HCMV copies/mL	*	*	ND	*

*No sample taken.
Red = implants used for sampling sites.
HCMV = human cytomegalovirus; ND = sample taken, no periodontopathgens or cytokines detected.

TABLE 2 Radiographic Analysis Comparing Implant–Implant and Implant–Tooth Peri-implant Mean Bone Level at 0, 6, 12, and 18 Months[20]

Mean peri-implant bone level (mm)	T_0 (placement)	T_1 (6 months)	T_2 (12 months)	T_3 (18 months)
Implant–implant (16 peri-implant sites $T_0 - T_2$)	−1.0	−3.6	−4.3	−4.4*
Implant–tooth (10 peri-implant sites $T_0 - T_2$)	−1.7	−3.5	−3.8	−3.9**

*Ten peri-implant sites at T_3.
**Eight peri-implant sites at T_3.
$n = 13$ one-stage scalloped implants for five subjects.

records revealed that most subjects harbored no periodontopathogens at peri-implant sites. Four of five subjects tested negative for selected pathogens at 3 months, and six of seven subjects tested negative at 6 months. Yet, our previous study[20] demonstrated excessive bone and soft tissue/papillae loss around the scalloped dental implants (Table 2). When scalloped implants were placed adjacent to other scalloped implants, the average bone loss from baseline to 18 months was −3.4 mm, and mean crestal bone height at 18 months measured −4.4 mm. Considering the same time intervals, bone loss measured from implants placed adjacent to existing natural dentition averaged −2.2 mm, and mean crestal bone height at 18 months measured −3.9 mm.

In spite of the absence of a major bacterial challenge, cytokine activity of IL-1β and TNF-α was substantial at peri-implant sites at both 3- and 6-month intervals (Table 1). Table 3 illustrates the frequency of high levels of cytokine activity while an absence of putative periodontal pathogens predominated. At 12 months, there was notable reduction in cytokine levels; however, two of six subjects continued to exhibit excessive copies per milliliter of IL-1β. No microbiology samples were taken at the 12-month interval, so any relationship of periodontopathogens to cytokines for these two subjects at this time interval cannot be assessed. INF-γ was not detectable at any time interval; therefore, this cytokine could not be identified as a possible factor in the degeneration of peri-implant structures.

TABLE 3 Comparison of Frequency of Crevicular Fluid Measurements Showing Significant (+) and Nonsignificant (–) Copies/mL of Cytokine Activity with Presence (+) or Absence (–) of Putative Periodontopathogens*

		Culture for periodontal pathogens		
RT–PCR		(+)	(–)	Total
IL-1β	(+)	1	10	11
TNF-α	(+)	1	10	11
IL-1β	(–)	1	0	1
TNF-α	(–)	1	0	1
	Total	4	20	24

*Table includes summary of combined 3- and 6-month measurements.
$n = 24$ measurements.

HCMV was detected only at one time interval for one subject (Table 1). The virus was detected at the 3-month interval for subject 5, who exhibited no periodontopathogens and significant cytokine activity throughout the sampling period.

Figures 1–3 are radiographs and intraoral photographs from one selected representative case (subject #4, Table 1).

DISCUSSION

Significant levels of cytokines IL-1β and TNF-α were detected 3 to 6 months after implant placement in six of seven patients. Findings of Perala and colleagues[3] indicated that dental implant surfaces may lead to an activation of human peripheral blood mononuclear cells for secretion of IL-1β and TNF-α. Active IL-1β and TNF-α mediate a variety of biological functions including bone resorption. Both are proinflammatory cytokines that can lead to pathologic tissue breakdown by inducing the expression of matrix metalloproteinases (MMPs) or programmed cell death of fibroblasts. IL-1β is a cytokine activated by mononuclear phagocytes whose main function is to mediate the host inflammatory response in innate immunity. A significant property of IL-1β is the stimulation of the production of MMPs including collagenase, gelatinase A (MMP-2), and stromelysin 1 (MMP-3), which are able to degrade the extracellular matrix macromolecules.[29–31] Continuous production of IL-1β plays a key role in chronic leukocyte recruitment and excessive production of MMPs. The imbalance

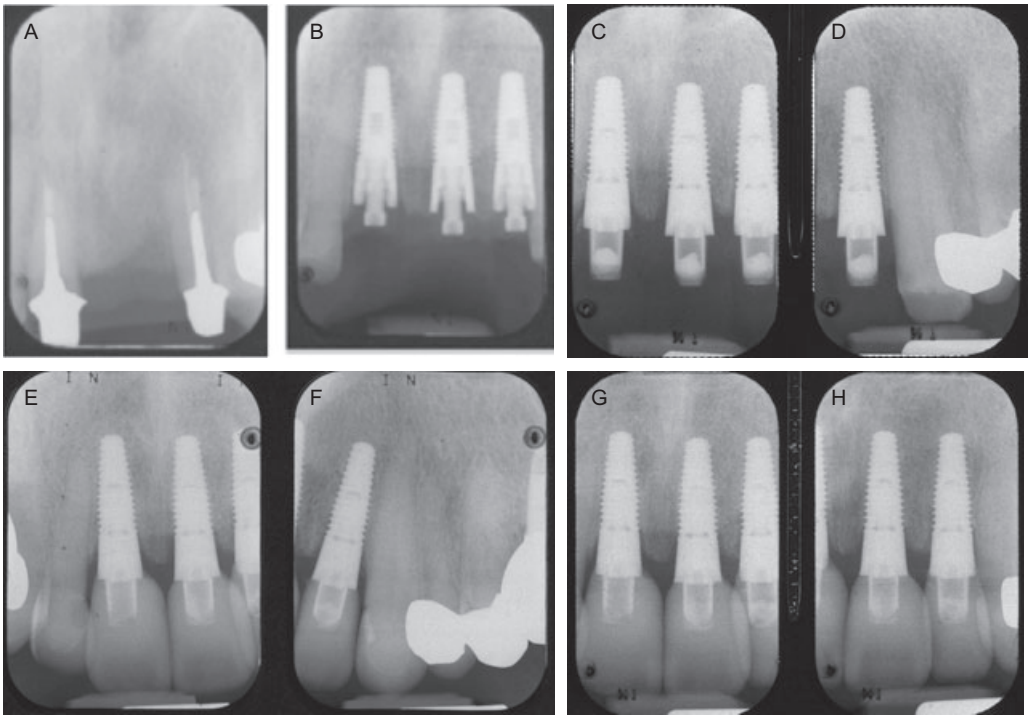

Figure 1 Apical radiographs at intervals: (A) presurgical, (B) day of surgery, (C,D) 6 months, (E,F) 12 months, and (G,H) 18 months postsurgical. Interproximal bone peaks are evident between implants at (B) day of surgery. Views (E,F) at 12 months and (G,H) at 18 months postsurgical show progressive resorption of interproximal supporting bone.

between activated MMPs and their endogenous inhibitors leads to pathologic breakdown of the extracellular matrix.[32]

Further, the proinflammatory cytokines examined in this study decrease the expression of transforming growth factor TGF-β1, in both human periodontal ligament and alveolar bone cells. TGF-β1 is expressed in the developing alveolar bone and periodontal ligament at all stages of tissue development, which suggests its involvement in the modulation of tissue formation.[33] IL-1β and TNF-α not only stimulate the expression of MMPs and the subsequent tissue degradation, but also inhibit the synthesis of the matrix by down-regulating TGF-β1 expression. TGF-β1 expression is not detectable in chronically inflamed periodontal tissues.[34] Bone morphogenetic proteins (BMPs) are osteoinductive agents and belong to the TGF-β superfamily, crucial factors in osteogenesis.[35] In alveolar bone cells, IL-1β significantly

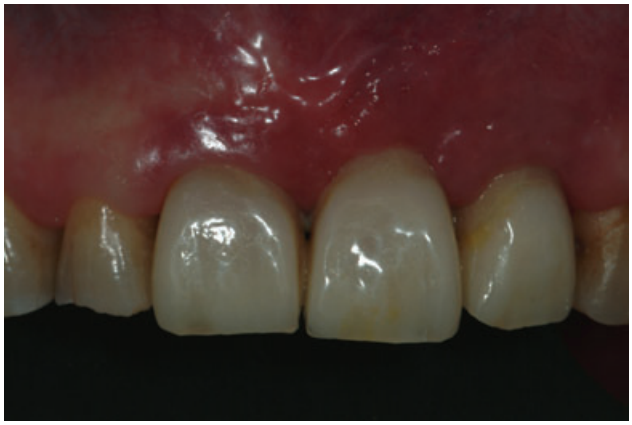

Figure 2 Clinical view of provisional teeth at 3 months postoperatively.

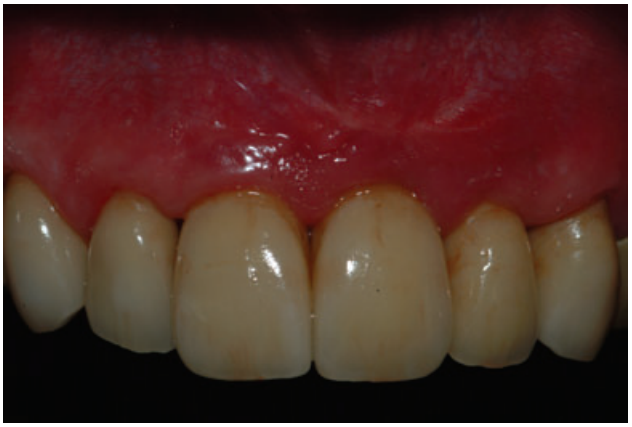

Figure 3 Clinical view of definitive restorations at 18 months postoperatively.

reduces the expression of BMP-2 mRNA.[36] This effect could contribute to the loss of bone.

Inflammatory cytokines are present in low levels in clinically healthy tissues.[8,19] Therefore, in practice and in the present study, excessive or continuous production of cytokines may be a clinical marker of potential tissue destruction around natural teeth as well as around dental implants. However, although studies have reported that cytokine activity seems to be relevant for alveolar bone resportion and destruction of collagen, periodontal research to date has not established any particular cytokine profiles that could be of predictive value for disease progression.[37–39]

Because TNF-α is produced mainly in the early stages of acute inflammation, high detection level of this cytokine at 3 and 6 months suggests that bone loss around scalloped implants may be characterized by a repetitive acute-phase inflammatory response. INF-γ is activated by T lymphocytes that prevent bone resorption by inhibiting the differentiation of precursors of osteoclastic cells to mature cells. The principal role of T cells is to respond against microorganisms. INF-γ can be evaluated as outcome variable and marker of a Th1 response.[40] INF-γ was not detectable in the present patient population. This implies that T cells were not the most relevant cells associated with tissue destruction around the implants.

The design of the implant placed was of a scalloped type, which is purported to help maintain bone height during remodeling by the biological guidance of its scalloped regions.[41] TNF-α and IL-1β potentiate osteoclastogenesis and alveolar bone destruction. And, because the release and destructive impact of these cytokines are most likely to be diffuse, there should be no expectation that the cytokines will be influenced by a scalloped outline design in their impact. Gingival height will follow crest of the bone; however, determining factors in inter-implant papillae development are complex and cannot likely be controlled by custom design features. Although bone height is a major determinant of soft tissue height, other factors such as the bone thickness; tooth morphology; location of the contact point; arrangement and quality of soft tissue fibers; including the dento-gingivo-alveolar, circular, semicircular, transeptal, interpapillary, and intergingival fibers have significant developmental influence.

Immune response to titanium and titanium alloys has been examined in vitro.[3,18] These studies and others help lend support to a dental implantology theory that a repetitive acute-phase inflammatory response could be responsible for bone loss and, therefore, a black triangle condition after implant placement. Titanium particles in vitro have been shown to influence release of Il-2.[42–45] Both the composition and design of the dental implant must be considered as possible contributing factors to immune response and bone loss when a bacterial challenge cannot be identified or confirmed.

CONCLUSION

These findings from a sample of seven patients and 19 scalloped implants revealed that significant cytokine activity, largely unrelated to a bacterial challenge, occurred after dental implant placement. Because the cytokines studied are potent stimulators of bone resorption, they are likely to be involved in an inflammatory response that can lead to early bone loss following implant placement. In our previous study, this same group of implants exhibited bone loss 6 months after placement.[20] This further analysis implies that implant design, independent of a bacterial challenge, may contribute to a destructive, repetitive, acute inflammatory response. Data from the present study provide a basis for further developing and testing hypotheses related to cytokine–microbiology–virology profiles, implant designs, and peri-implant bone and papillae loss.

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