A Murine Model of Lipopolysaccharide-Induced Peri-Implant Mucositis and Peri-Implantitis

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Dental implants are a widely used treatment option for tooth replacement. However, they are susceptible to inflammatory diseases such as peri-implant mucositis and peri-implantitis, which are highly prevalent and may lead to implant loss. Unfortunately, the understanding of the pathogenesis of peri-implant mucositis and peri-implantitis is fragmented and incomplete. Therefore, the availability of a reproducible animal model to study these inflammatory diseases would facilitate the dissection of their pathogenic mechanisms. The objective of this study is to propose a murine model of experimental peri-implant mucositis and peri-implantitis. Screw-shaped titanium implants were placed in the upper healed edentulous alveolar ridges of C57BL/6J mice 8 weeks after tooth extraction. Following 4 weeks of osseointegration, Porphyromonas gingivalis-lipopolysaccharide (LPS) injections were delivered to the peri-implant soft tissues for 6 weeks. No-injections and vehicle injections were utilized as controls. Peri-implant mucositis and peri-implantitis were assessed clinically, radiographically (microcomputerized tomograph [CT]), and histologically following LPS-treatment. LPS-injections resulted in a significant increase in soft tissue edema around the head of the implants as compared to the control groups. Micro-CT analysis revealed significantly greater bone loss in the LPS-treated implants. Histological analysis of the specimens demonstrated that the LPS-group had increased soft tissue vascularity, which harbored a dense mixed inflammatory cell infiltrate, and the bone exhibited noticeable osteoclast activity. The induction of peri-implant mucositis and peri-implantitis in mice via localized delivery of bacterial LPS has been demonstrated. We anticipate that this model will contribute to the development of more effective preventive and therapeutic approaches for these 2 conditions.

Key Words: peri-implant mucositis, peri-implantitis, implants, mouse, dental implants, lipopolysaccharide

INTRODUCTION

Dental implants have become an increasingly popular and reliable treatment option for tooth replacement. However, periodontal tissues surrounding dental implants are susceptible to inflammatory diseases such as peri-implant mucositis and peri-implantitis, which resemble gingivitis and periodontitis.

Peri-implant mucositis involves inflammation of the soft tissues surrounding a dental implant. It is present in 80% of subjects who received dental implants and in 50% of functional implants. Peri-implant mucositis is a reversible process and does not result in peri-implant bone loss. However, if left untreated, peri-implant mucositis can progress to peri-implantitis.

Peri-implantitis, an inflammatory condition of the soft and hard tissues around implants, includes peri-implant bone destruction and can result in implant loss. Approximately 11% to 47% of implants have some degree of peri-implantitis, and such high prevalence poses a significant clinical problem given the cumulative number of implants delivered over time. Unfortunately, there are no established effective treatment protocols for peri-implantitis, primarily because the pathogenesis of the disease is not fully understood.

Peri-implantitis shares some common features with periodontitis. Clinically, implant fixtures affected by peri-implantitis present with increased pocket depth, inflammatory soft tissue changes including bleeding on probing, clinical attachment loss, and radiographic bone loss. In addition, the biofilm in deep pockets around implants is similar to the microbiota associated with periodontitis. From the macroscopic and histopathological standpoints; however, periodontitis and peri-implantitis lesions are markedly different. Clinically and radiographically, peri-implantitis is associated with significant bone destruction whereas peri-implant mucositis is a reversible process.
implantitis is often symmetric, occurring around the whole fixture perimeter, as opposed to surface-specific periodontitis lesions around natural teeth. Histopathologically, inflammation in peri-implantitis penetrates deep and beyond the soft tissue around the pocket, often onto bone and its alveolar spaces.\textsuperscript{16} In contrast, inflammation in periodontitis is consistently separated from the alveolar bone from a zone of inflammation-free connective tissue that is about 1 mm wide. One of the most interesting and puzzling differences between periodontitis and peri-implantitis has been revealed in a dog study.\textsuperscript{17} Plaque-induced lesions were initiated around natural teeth and implants via installation of ligatures, which were subsequently removed. Upon ligature removal, periodontitis and peri-implantitis lesions responded differently: Periodontitis lesions tended to stabilize and present with no further progression while peri-implantitis lesions almost invariably progressed and were not self-limiting. Moreover, little biochemical and molecular information exists about the possible role of pro-inflammatory and other mediators in the pathogenesis of peri-implantitis.

Therefore, understanding the pathogenesis of peri-implant mucositis and peri-implantitis and developing a systematic approach to prevent the onset and progression of both conditions is crucial to maximize the longevity of dental implants.\textsuperscript{5,7,18} One of the main limiting factors in dissecting the pathogeneses of peri-implant mucositis and peri-implantitis is the absence of an easily accessible, reproducible, and inexpensive animal model.

Rodent models are preferred when studying the pathogenic phenomena and biological pathways associated with various diseases due to the similarities they share with humans. Mouse models, in particular, present with the additional advantage of the availability of genetically manipulated animals, which can serve as unique and sophisticated tools to studying disease pathogenic mechanisms. This study describes a murine model of peri-implant mucositis and peri-implantitis.

\textbf{Materials and Methods}

\textbf{Mice}

Four-week old C57BL/6J (The Jackson Laboratories, Bar Harbor, ME) male mice were utilized in this study adhering to the approved protocol and guidelines of the Chancellor’s Animal Research Committee at the University of California, Los Angeles. Mice were fed a soft diet (BioServe, Frenchtown, NJ) ad libitum for the duration of the experiments.

\textbf{Tooth extraction and implant placement}

Under inhalation anesthesia with 3% isoflurane, maxillary left 1st, 2nd, and 3rd molars were extracted and sockets were allowed to heal for 8 weeks. Animals were given antibiotics (sulfamethoxazole and trimethoprim oral suspension, USP; 850\(\mu\)g/170\(\mu\)g/mL) orally by incorporating the medications into the drinking water for 4 weeks after extractions.

Custom-designed screw-shaped implants 0.5 mm in diameter by 1 mm in length were fabricated from 6AL4V titanium rods (DP Machining Inc., Inc, La Verne, Calif; Figure 1). Eight weeks after extractions, the mice were put under general anesthesia and a mesial-distal incision was made with a 12D blade in the edentulous left alveolar ridge. Buccal and palatal full-thickness flaps were elevated with a #5 dental explorer as it exposed the healed alveolar ridge. Implant fixture osteotomy was performed with a 0.03-mm carbide micro hand drill (BIG Kaiser Precision Tooling Inc, Hoffman Estates, Ill) in the first/second molar area, using the teeth in the opposite side as spatial reference. One implant was placed in each mouse. Using a clockwise screwing motion, implant fixtures were inserted so that the implant head was leveled with the bone. Antibiotics as described previously were given to the animals for 4 weeks after implant placement.

\textbf{Induction of peri-implant mucositis and peri-implantitis}

The induction of peri-implant mucositis and peri-implantitis was initiated 4 weeks after implant placement via lipopolysaccharide (LPS) injection into the peri-implant mucosa. Mice were divided into 3 groups: (1) noninjected control, (2) vehicle-injected control (2\(\mu\)l injection of phosphate-buffer saline: PBS), and (3) LPS-injected, experimental group (2 \(\mu\)l of 10 mg/mL ultrapure LPS from \textit{Porphyromonas gingivalis} [InvivoGen, San Diego, Calif]), as described in a previous study.\textsuperscript{24}

Injections were administered in the disto-palatal aspect of the peri-implant mucosa twice a week for a period of 6 weeks with the use of a Hamilton syringe and a 33-gauge needle. At the end of the treatment period, mice were humanely killed, their maxillae harvested and fixed in 4% paraformaldehyde for 48 hours. Maxillae were then stored in 70% ethyl alcohol.
Peri-Implant Mucositis and Peri-Implantitis Models

Clinical assessment of peri-implant mucositis

Macroscopic images of the specimens were obtained using a Keyence VHX-1000 (Osaka, Japan) digital optical microscope. Clinical images were analyzed with Aperio Image Scope software V11.1.2.752 (Vista, Calif) to determine the amount of soft tissue coverage over the implant head. Soft tissue coverage was assessed by the evaluation of the exposed implant head surface area (μm²). If the head of the implant had complete soft tissue coverage, the exposed surface area was considered to be 0 μm².

Micro-CT analysis of peri-implantitis

Mouse maxillae were imaged by microcomputerized tomography (micro-CT) scanning (SkyScan 1172; SkyScan, Kontich, Belgium) at 10-μm resolution and X-ray energy of 55 KVP and 181 μA. Volumetric data were converted into Digital Imaging and Communications in Medicine (DICOM) format and imported into Dolphin Imaging software (Chatsworth, Calif) to generate 3-dimensional/multiplanar reconstructed images. Through the use of sagittal and coronal images, bone levels were assessed using the Dolphin software by measuring from the outermost edge of the head of the implant to the alveolar bone in the palatal aspect of the implant.

Histology

For histological evaluation of implant osseointegration and peri-implant bone levels, nondecalcified maxillae were embedded in methyl methacrylate and ground to 20 μm-thick sections in the sagittal plane, parallel to the long axis of the implant (EXAKT Cutting & Grinding System, Exakt Apparatebau, Norderstedt, Germany). Toluidine blue staining of the sections was performed according to standard protocols. Additional samples were decalcified in 15% EDTA. At the end of the decalcification period, implants were removed via unscrewing motion and the specimens embedded in paraffin. Five μm-thick paraffin sections were generated. Sections were cut sagittally and stained with hematoxylin and eosin (H&E) using standard protocols.

Statistics

Data were represented as group means plus/minus the standard error of the mean (SEM). Comparisons between groups were conducted by Student t tests utilizing GraphPad InStat software (La Jolla, Calif).

Results

Implant placement and osseointegration

Healing of the extraction sockets was uneventful in all animals (Figure 1a). To determine implant osseointegration (Figure 1b), implant mobility was evaluated 4 weeks after implant placement by observing a wiggling movement between 2 periodontal probes; results indicated that 84.6% (22/26) implants were clinically osseointegrated. Additionally, the soft tissues around implant fixtures appeared to be healthy with no swelling or any other inflammatory signs (Figure 2a).

Induction of peri-implant mucositis and peri-implantitis

Clinically, the experimental group presented with significantly more edema around the head of the implant than the control groups (noninjected and vehicle-injected). The mean implant head surface was 29 518 μm². The mean exposed surface was 12 076 μm² in the noninjected, 13 743 μm² in the vehicle-injected and 3191 μm² in the LPS-injected group, respectively (Figure 2b through e). There was a statistically significant increase in implant coverage in the LPS group compared to the control groups (noninjected and vehicle-injected). There was no statistical difference in implant head coverage in the noninjected group compared to the vehicle-injected group.

Micro-CT measurements of the distances between the implant head and the bone (Figure 3) revealed that the measurements were significantly increased in the LPS-injected group (320 μM) as compared to the control groups, noninjected (217 μM) and vehicle-injected (230 μM), suggesting increased bone loss. No significant differences were detected between the noninjected versus vehicle-injected groups.

Histology

Toluidine blue staining showed that all implants in the 3 study groups were osseointegrated at the end of the experimental period (Figure 4). The peri-implant mucosa of the experimental group exhibited increased vascularity and harbored a dense mixed inflammatory cell infiltrate. The experimental group had vertical defects on the buccal alveolar process of the implant. The surrounding bone was porous and exhibited noticeable osteoclast activity. The porous nature of the bone was responsible for bone fragmentation after removing the implant (as shown in the H&E section; Figure 4).

Discussion

A substantial body of scientific knowledge about peri-implant mucositis and peri-implantitis has emerged through human studies. While human studies are essential in framing a problem at the clinical and epidemiological levels, they present with limitations. Human studies do not allow for the conduction of detailed specimen histological examination. Additionally, experimental protocols cannot be established with respect to disease initiation and progression. Oftentimes, human study protocols cannot avoid confounding variables such as the existence of systemic conditions (ie, diabetes), smoking habits, oral hygiene habits, small sample cohorts, or genetic heterogeneity. Together, researchers cannot solely rely on human studies to understand the pathogenesis of disease and to conduct initial treatment protocol testing for diseases. Therefore, much can be achieved by combining data obtained from human and animal studies.

Therefore, animal models where genetic and environmental factors can be tightly controlled offer an attractive and essential complement to human studies in the process of understanding a disease or condition. As an example, mice share structural, functional, and genetic traits with humans, which make them a convenient starting point in dissecting disease pathways at the tissue and cell levels. Moreover, powerful molecular and
genetic tools developed in the past two decades make mice an ideal animal model to study diseases that may be multifactorial and complex in etiology. A major advantage of murine models is that the genetic mechanisms of disease can be dissected, facilitating the functional characterization of genes that may be important in disease initiation and development.20,21 Hence, establishing an inflammatory-mediated peri-implant mucositis and peri-implantitis mouse model is essential for understanding disease initiation and progression.

Peri-implant mucositis and peri-implantitis are multifactorial diseases. They are initiated by bacterial biofilm accumulation, which leads to the development of an inflammatory response. This inflammatory response activates multiple signaling pathways that lead to inflammation of the peri-implant soft tissues, which has been termed peri-implant mucositis. It is believed that some cases of chronic peri-implant mucositis progress into peri-implantitis, which includes bone destruction and clinical attachment loss.9 While some basic understanding of peri-implant mucositis and peri-implantitis...
Implant mucositis and peri-implantitis is available, including the fact that they appear to be conditions initiated by bacteria, details of their pathogenesis are still elusive.

Based on the similarities that are evident between inflammatory diseases around natural teeth and dental implants, our peri-implant mucositis/peri-implantitis murine model derived from a well-characterized model of periodontal bone loss, which involved localized P. gingivalis LPS delivery to the gingival tissues around a maxillary implant. This method was chosen because it bypasses the bacterial colonization process, allowing to focus on the inflammatory components of the disease, and avoiding variations that are inherently associated with bacterial colonization disease models.

**Figure 4.** (a) Representative images of toluidine blue stained sections 6 weeks after treatment (noninjected, vehicle-injected, and LPS-injected groups); magnification ×100. Notice different bone levels between the experimental and the control/vehicle groups. Black arrows represent osteoclasts. (b) Representative images of hematoxylin and eosin stained sections 6 weeks after treatment (control, vehicle, and experimental groups), magnification ×100. Notice the presence of a dense mixed inflammatory cell infiltrate in the experimental group as compared to the control/vehicle groups.
In this study, we utilized *P. gingivalis*-derived LPS because *P. gingivalis*—a gram-negative anaerobic rod and member of the “red complex,”—is widely recognized as a main contributor to periodontitis in humans, and has also been implicated in human peri-implantitis.\(^1,13,35\) Our data clearly show that the injection of *P. gingivalis*-derived LPS not only causes peri-implant bone loss, but also results in inflammatory changes in the peri-implant soft tissues, resembling the inflammatory sequential processes that are observed around dental implants placed in humans.

In the model presented here, threaded implants were placed in postextraction sockets. Becker et al. also developed a murine model of periodontitis. However, Becker et al. placed non-threaded implants in the palate. Although information can be gained through Baker’s model, it does not resemble clinical implant placement in humans. Nonetheless, our results corroborate those of Becker et al. in that they confirm that a mouse model can be effective in the investigation of the pathogenesis of peri-implant mucositis and peri-implantitis.\(^36\) Other disease models, such as ligature-induced peri-implantitis are under investigation by our research group. Altogether, these models can be a valuable tool for the study of peri-implant mucositis and peri-implantitis.

In conclusion, a peri-implantitis model that focuses on understanding the inflammatory component of the disease may allow us to prevent and treat conditions that are highly prevalent\(^4,13\) in human kind. Despite the fact that there are treatment options for these conditions such as nonsurgical and resective/reconstructive surgical therapies,\(^37\) the effectiveness of these treatment methods has not been determined. We anticipate this model will contribute to the development of more effective preventive and therapeutic approaches for inflammatory conditions around dental implants.

**Abbreviations**

H&E: hematoxylin and eosin
LPS: lipopolysaccharide
micro-CT: micro-computerized tomography

**References**


