Effect of ancestry on interleukin-10 haplotypes in chronic periodontitis

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1. ABSTRACT

Chronic periodontitis is caused by an inflammatory reaction of the periodontal tissues and alveolar bone. This inflammation is caused by periodontopathic bacteria located in the subgingival biofilm, resulting in inflammatory reactions that may lead to loss of attachment. This tissue destruction is a consequence of host immune and inflammatory responses to specific periodontal pathogens and their metabolic products. Cytokines modulate the immune response, altering its efficiency in the competition against pathogens and increasing periodontal susceptibility. This study investigated genetic polymorphisms in Interleukin 10 (A-1082G, C-819T and C-592A) in 205 individuals from an admixed Brazilian population. A significantly increased risk of developing chronic periodontitis was observed in individuals with low IL-10 production and Amerindian ancestry. These results suggest that the polymorphisms A-1082G, C-819T, and C-592A, which are associated with ancestry, are involved in the susceptibility to the development of chronic periodontitis in an admixed northern Brazilian population.

2. INTRODUCTION

Chronic periodontitis (CP) is complex disease with a pathology that includes microbiological and inflammatory responses and genetic, epigenetic and environmental factors (1, 2). Pathogenic microorganisms colonize and proliferate in the gingival crevice, leading to local infection and periodontal pockets in a susceptible host. These factors cause the destruction of the supporting tissues of the teeth, progressive loss of attachment, and bone loss. CP is characterized by periodontal pocket formation and/or recession of the gingiva (3). The progression of this destructive disease seems to depend on an abnormal host response to pathogenic microorganisms within plaque biofilm (4). The host immune system releases inflammatory cytokines and enzymes, which may act in the degradation of periodontal tissues and lead to the clinical signs of the disease (4, 5).

Chronic inflammation and cytokine levels play a pivotal role in the destructive processes occurring in periodontitis (6). Several inflammatory mediators,
such as pro-inflammatory and anti-inflammatory interleukins, are involved in periodontal diseases (7, 8). Interleukin-10 (IL-10) is an anti-inflammatory cytokine and is a key modulator of inflammation. IL-10 stimulates the production of protective antibodies and down-regulates pro-inflammatory cytokines; therefore, a decrease in the normal levels of IL-10 or its receptor results in inflammatory disorders in mouse and humans (9, 10).

Polymorphisms of the IL-10 gene that may reduce IL-10 production are significantly associated with reduced bone mineral density in women who are prone to osteoporosis (11, 12). Low levels of IL-10 may result in an insufficient inhibition of pro-inflammatory cytokines and collagenase, which may impact bone loss diseases, such as osteoporosis and periodontitis (13). However, IL-10 is an important regulator of alveolar bone homeostasis during development (13).

Polymorphisms in cytokine genes often affect cytokine expression profiles and may play an important role in resistance or susceptibility to CP (8, 14). Specifically regarding the IL-10 gene, single nucleotide polymorphisms (SNPs) (A-1082G (rs1800896), C-819T (rs1800871) and C-592A (rs1800872)) are associated with diseases with different pathologies, such as leprosy and type 2 diabetes mellitus (15, 16). The literature reveals a strong correlation of the allele IL-10*-1082G with the overexpression of IL-10; in contrast, the allele IL-10*-1082A is associated with the low expression of IL-10. For example, the genotype IL-10*-1082GG confers overexpression of IL-10, whereas the IL-10*-1082GA and IL-10*-1082AA genotypes confer intermediate and low expression of IL-10, respectively (17, 18, 19). IL-10 modulates the immune response for antibody production; thus, reduced IL-10 production could be a risk factor for the development of CP (7, 20).

In addition, these three SNPs can generate haplotypes that also change the level of IL-10 production (9, 17, 18, 19, 21). The modulation of serum IL-10 levels from an individual’s haplotype depends on polymorphism A-1082G; ergo, genotypes with two IL-10*-1082G alleles (GCC/GCC and other combinations) exhibit increased IL-10 production, genotypes with one IL-10*-1082G allele (GCC/ATA and other combinations) exhibit average IL-10 production, and genotypes without an IL-10*-1082G allele (ATA/ATA and other combinations) exhibit low IL-10 production (9, 17, 18, 19, 21).

Brazil is one of the most genetically heterogeneous populations in the world, with influential genetic contributions from three main continental groups: Europeans, Africans and Amerindians (22). The relative proportions of these three ancestral roots within the Brazilian population have changed considerably over time (23). Therefore, the modern Brazilian population is genetically diverse and considered very heterogeneous (22). Consequently, the analysis of genetic markers in complex diseases within this population may result in misinterpretation due to the existence of population substructure (24). Furthermore, it is important to assess the control of genomic ancestry in association studies using Ancestry Informative Markers (AIMs), especially in populations with a high degree of interethnic admixture (25).

Within this context, we investigated the haplotypes formed by three SNPs within the IL-10 gene (A-1082G (rs1800896), C-819T (rs1800871) and C-592A (rs1800872)) to better understand how genetic mechanisms of a host can affect how factors of susceptibility to chronic periodontitis in an admixed Brazilian population.

3. MATERIALS AND METHODS

3.1. Cases and controls

A total of 205 individuals were included in this study: 55 with chronic periodontitis (CP) who attended dental clinics in Belém (PA, Brazil) and 150 controls subjects (HC) without CP or any other chronic disease were selected from the same geographic area. The diagnosis diagnosed and classified based on dental exams with the following clinical description: probing depth, bleeding upon probing, gingival inflammation, tooth mobility and clinically detected attachment loss >5 mm in at least ≥30% of total sites. Measurements of probing depth and attachment level were recorded at six points around each tooth. The HC group included subjects presenting healthy gingival tissues without evidence of attachment loss (clinical attachment loss ≤1 mm) at more than one site, a probing depth <3 mm and no history of previous periodontal disease. Individuals meeting these criteria were defined as periodontally HC subjects.

3.2. Ethical aspects

All patients were informed about the research and signed informed consent forms. This study was approved by the Ethics Committee in Research (ECR) of the Instituto de Ciências da Saúde (ICS) of the Universidade Federal do Pará, protocol No. 186/97 CEP-ICS/UFPA.

3.3. Sample collection and DNA extraction

Buccal mucosa samples were collected with a cotton swab and stored in a microtube with buffer at -20°C until analysis. The genetic material was extracted from the cell fraction using phenol-chloroform and precipitated using ethanol (26).
3.4. Analysis of polymorphisms

The IL-10 SNPs were investigated using the TaqMan genotyping assay with TaqMan® probes conducted in a 7500 Real-Time PCR System (Life Technologies, CA, USA). PCR genotyping of all three polymorphisms (A-1082G (rs1800896), C-819T (rs1800871) and C-592A (rs1800872)) was performed with 3.5 μL of master mix, 0.17 μL of probe TaqMan 40X, 3.32 μL of water and 1.0 μL of DNA. The final mix was amplified using the following cycling conditions: 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 92°C and 1 minute at 60°C.

3.5. Analysis of genetic ancestry

Population substructure was investigated using 48 AIMs as previously described (25). Three multiplex PCR reactions were performed, each with 16 markers, followed by electrophoresis on an ABI-PRISM 3130 (Life Technologies, CA, USA) sequencer and analysis using GeneMapper ID version 3.2 (Life Technologies, CA, USA).

3.6. Statistical analysis

Allele frequencies were estimated by gene counting, and deviation from Hardy-Weinberg equilibrium was tested by Chi-square with Benjamini and Hochberg False Discovery Rate (FDR) correction. Haplotype frequencies were obtained using PHASE 2.1. software (27, 28, 29). The p-value calculated from a permutation test of the null hypothesis (that the case and control haplotypes were a random sample from a single set of haplotype frequencies) was also obtained using this software.

Haploview version 4.2. (https://www.broadinstitute.org/haplovie) was used to analyze the linkage disequilibrium (LD) structure among polymorphisms (30). Pairwise LD between SNPs was estimated using Lewontin’s standardized coefficient D’ and LD coefficient r² (31), and haplotype blocks were defined according to the method of Gabriel et al., 2002 using Haploview 4.2. with default settings. Haplotypes were used to estimate the maximization algorithm (32).

Differences in the demographic, clinical and biochemical characteristics between CP and HC samples were compared by Student’s t-test for quantitative variables, Fisher’s exact test for qualitative variables and the Mann–Whitney test for estimates of differences in genetic ancestry. Multiple comparison corrections and adjusted ORs (and 95% CI) for the association between genetic markers were obtained by logistic regression. These tests were performed with PASW Statistic (formerly SPSS) version 18.0. Statistical significance was accepted if the p-value <0.05.

The individual proportions of European, African, and Amerindian genetic ancestry were estimated using STRUCTURE software 2.3.3., assuming three parental populations (Europeans, Africans, and Amerindians) and using 200,000 runs for the burn-in period and 200,000 Markov chain Monte Carlo repetitions after burning (33, 34, 35, 36). An association test between CP and HC subjects following an adjustment for population stratification was performed using the STRAT software program with 10,000 simulations (33, 34, 35, 36).

4. RESULTS

The clinical and demographic variable parameters of the CP and HC samples are provided in Table 1. The CP and HC samples included in this study were mainly composed of females. The average age was 37.0.7 ± 2.1.7 years for the CP group and 24.2.8 ± 0.4.2 years for the HC group, and the difference between these groups was significant (p=0.0.3).

Regarding genomic ancestry, the ethnic composition of the CP group was 23% African, 38% European and 39% Amerindian, and the ethnic composition of the HC group was 18% African, 57% European and 25% Amerindian. These results revealed significant differences in African (p=0.0.04), European (p<0.0.01) and Amerindian (p<0.0.01) ancestry between the CP group and the HC group (Table 1). Figure 1 shows the individual estimates of the interethnic mixture between cases and controls.

Table 2 presents the allele and genotype frequencies for three IL-10 SNPs and the multiple logistic regression analyses after correction for age and ethnicity between the HC and CP groups. There were no significant differences between IL-10*-1082G>A and IL-10*-819C>T. The IL-10*-819C>A SNP was significantly different for genotype IL-10*-819CA between the HC and CP groups (p=0.0.1; OR=4.2.4), whereas the allele frequencies were corrected by structure populations. In addition, the differences were statistically significant (p<0.0.01). However, this locus did not meet Hardy–Weinberg equilibrium criteria in the CP group and was not included in the analysis.

In the analysis of the three polymorphisms of the anti-inflammatory cytokine IL-10 (IL-10*-1082G>A, IL-10*-819C>T and IL-10*-592C>A), seven haplotypes (ATA, ACT, ACC, GTC, GCA, GTA, and GCC) and the risk haplotype ATA were more frequent in the CP group (0.4.23) than in the HC group (0.1.31). The wild-type haplotype was more frequent in the HC group (0.3.26) than in the CP group (0.1.16). The haplotypes showed a block of LD (Figure 2; Table 3) in the CP patients and the HC group for the loci G-1082A/C-819T (D'=0.9.5, p<0.0.01) and C-819T/C-592A (D'=0.9.5, p<0.0.01).

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This result suggests that the haplotypes may explain more of the association than the individual SNPs. Permutation analysis of the global differences between haplotype frequencies between the two groups (CP and HC) showed that a set of haplotypes was differentially distributed and indicates that these differences were not random ($P_{\text{global}}=0.05$ - Table 3). Additionally, the multiple comparisons corrected test revealed that carriers of the $\text{IL10}^{*-1082}\text{A}$-containing haplotypes (ATA, ACT, and ACC) were associated with risk factors for CP ($p=0.0.46$, OR=3.459). This finding remained true after correction for population stratification ($p_{\text{strat}}=2.4E^{-15}$) (Table 3).

Table 4 summarizes the distribution of the genotypes and IL-10 production profiles with their respective frequencies. The distribution of the IL-10 production profiles with their respective frequencies is summarized in Table 5. In multiple logistic regression analyses, genotypes significantly associated with a low-production profile ($p=0.0.4$; OR=2.7.7) exhibited an increased risk of CP, whereas genotypes associated with a high-production profile ($p=0.0.3$; OR=0.2.8) were associated with a decreased risk of CP.

Figure 3 presents the OR (odds ratio) values obtained between the CP and HC groups in relation to the distinct level of ancestry composition. Figure 3a shows that the greater the European contribution is between groups, the lower is the risk of developing CP. These results are expected because this ethnic group is more frequent in the HC group (Table 1). Additionally, Figure 3b shows that the higher the Amerindian contribution is, the higher is the risk of developing CP. These results are expected because this ethnic group is more frequent in the CP group (Table 1). These analyses were performed for the two ethnic components with differences greater than 10% between the HC and CP groups.

5. DISCUSSION

The ancestry data revealed significantly different distributions of African ethnicity (23.7% versus 18.4%), European ethnicity (38% versus 58.2%) and Amerindian ancestry (38.3% versus 23.4%) between the CP and HC groups. These results suggest a loss of contribution from European genes and an increased contribution from African ancestry genes in the case group compared with the control population.
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Table 2. Genotype distributions between cases and controls

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Case N (%)</th>
<th>Control N (%)</th>
<th>p-value a</th>
<th>( p_{\text{strat}} ) b</th>
<th>OR (IC95%) a</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL10 G-1082A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>5 (9.1)</td>
<td>18 (12.0)</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>GA</td>
<td>14 (25.5)</td>
<td>61 (40.7)</td>
<td>0.85</td>
<td></td>
<td>1.10 (0.37 - 3.22)</td>
</tr>
<tr>
<td>AA</td>
<td>36 (65.5)</td>
<td>71 (47.3)</td>
<td>0.37</td>
<td></td>
<td>0.58 (0.18 - 1.88)</td>
</tr>
<tr>
<td>allele G</td>
<td>0.21</td>
<td>0.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>allele A</td>
<td>0.79</td>
<td>0.67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HWE</td>
<td>5.355</td>
<td>1.617</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL10 C-819T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>19 (34.5)</td>
<td>72 (48)</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>CT</td>
<td>25 (45.5)</td>
<td>59 (39.3)</td>
<td>0.72</td>
<td></td>
<td>0.84 (0.34 – 2.09)</td>
</tr>
<tr>
<td>TT</td>
<td>11 (20)</td>
<td>19 (12.7)</td>
<td>0.09</td>
<td></td>
<td>0.31 (0.08 - 1.21)</td>
</tr>
<tr>
<td>allele C</td>
<td>0.57</td>
<td>0.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>allele T</td>
<td>0.43</td>
<td>0.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HWE</td>
<td>4.186</td>
<td>1.855</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL10 C-592A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>0 (0.0)</td>
<td>48 (32.0)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA</td>
<td>42 (76.4)</td>
<td>83 (55.3)</td>
<td>0.01</td>
<td>7E-32</td>
<td>4.24 (1.33 – 13.52)</td>
</tr>
<tr>
<td>AA</td>
<td>13 (23.6)</td>
<td>19 (12.7)</td>
<td>0.14</td>
<td></td>
<td>0.37 (0.1 - 1.38)</td>
</tr>
<tr>
<td>allele C</td>
<td>0.38</td>
<td>0.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>allele A</td>
<td>0.62</td>
<td>0.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HWE</td>
<td>5.32E-8</td>
<td>2.52E-31</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Multiple logistic regression adjusted for independent variables age and ethnicity; \( ^{b} \) p-value adjusted by population structure using strat.

Figure 2. Pairwise linkage disequilibrium (LD) structure of the IL-10 gene SNPs. Panel (A) represents the LD of the IL-10 SNPs in the CP group. Panel (B) represents LD of the IL-10 SNPs in the HC group.

from northern Brazil (25). Furthermore, individuals with higher proportions of European ancestry had a decreased risk of developing the disease, whereas individuals with a higher contribution of Amerindian ancestral genes exhibited a disease susceptibility risk (Figure 2).

Due to the proportional differences in ancestry between the CP and HC groups (Table 1), haplotype association analyses corrected by population stratification were performed using STRAT software (Table 3). After population structure correction, the IL-10 haplotype that conferred a low production profile exhibited a significant risk for developing CP. Similarly, the IL-10 haplotypes that conferred a high production profile were associated with protection in the CP group. These results suggest that IL-10 haplotypes formed by the polymorphisms A-1082G, C-819T and C-592A are
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Genetic polymorphisms may modify the biological pathways of periodontitis. The action of multiple genes can contribute to the relative risk of susceptibility and severity, but these effects may be confounded by the influence of population substructure (7, 15, 37). We analyzed the genetic contribution of Africans, Europeans and Amerindians of an admixed population using a panel of 48 AIMs that are capable of precisely distinguishing with low statistical error and cost (25). This control was important for analyses in our admixed population samples and for consolidating the susceptibility and severity of CP in relation to the SNPs investigated.

The three IL-10 SNPs (-592/-819 and -819/-1082) are in LD (Figure 1) and represent three major haplotypes (GCC, ACC and ATA) (15, 19). In our study, we identified seven haplotypes: ATA, ACT, ACC, GTA, GCA, GCC and GTC. Scarl-caminaga et al. 2004 (7) showed the ATA haplotype was predominant in individuals with CP, whereas the ACC haplotype was

<table>
<thead>
<tr>
<th>SNP</th>
<th>Frequency of haplotypes</th>
<th>P_{phase}</th>
<th>P_{str}</th>
<th>p-value</th>
<th>OR (IC95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1082</td>
<td>819</td>
<td>592</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case % (N)*</td>
<td>Control % (N)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>T</td>
<td>C</td>
<td>0.010 (1)</td>
<td>-</td>
<td>0.005</td>
</tr>
<tr>
<td>G</td>
<td>C</td>
<td>A</td>
<td>0.000 (13)</td>
<td>0.037 (7)</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>T</td>
<td>A</td>
<td>-</td>
<td>0.192 (71)</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>C</td>
<td>C</td>
<td>0.116 (10)</td>
<td>0.326 (89)</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>T</td>
<td>A</td>
<td>0.423 (47)</td>
<td>0.131 (26)</td>
<td>2.4E-16</td>
</tr>
<tr>
<td>A</td>
<td>C</td>
<td>T</td>
<td>0.103 (8)</td>
<td>0.045 (17)</td>
<td>(1.025-11.677)</td>
</tr>
<tr>
<td>A</td>
<td>C</td>
<td>C</td>
<td>0.258 (31)</td>
<td>0.269 (90)</td>
<td></td>
</tr>
</tbody>
</table>

* Counts of haplotypes; ** p-value obtained by permutation test in software PHASE v2.1.1 for global differences between haplotypic frequencies; * Multiple logistic regression adjusted for independent variables age and ethnicity; b p-value adjusted by population structure using strat.
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Table 5. Analysis of haplotype of the IL-10 production profiles in case versus control

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Case % (N)*</th>
<th>Control % (N)*</th>
<th>p</th>
<th>OR (IC95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average production</td>
<td>14 (25.5)</td>
<td>61 (40.7)</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Low production</td>
<td>36 (65.5)</td>
<td>36 (24.0)</td>
<td>0.05</td>
<td>2.77 (1 - 7.88)</td>
</tr>
<tr>
<td>High production</td>
<td>5 (9.1)</td>
<td>53 (35.3)</td>
<td>0.04</td>
<td>0.28 (0.09 – 0.90)</td>
</tr>
</tbody>
</table>

*Counts of haplotypes ;  a Multiple logistic regression adjusted for independent variables age and ethnicity;  b p-value adjusted by population structure using strat

more common in the control group using a Brazilian population similar to our data. The authors also found non-significant, rare haplotypes: GTA, ATC, GTC and ACA. On the other hand, Cullinan et al. (38) investigated the association between IL-10 gene polymorphisms and periodontal disease progression in an Australian population. They reported that individuals having either the ATA/ACC or the ACC/ACC genotype experienced less periodontal disease progression after 5 years than did individuals with other genotypes. In European populations, the IL-10 haplotype ATA frequency (−1,082, −819, −592) was significantly different between vascular disease (VD) patients with CP and VD patients without CP, indicating protection against CP (39).

In the present study, we observed a predominance of the ATA haplotype in the CP group and the GCC haplotype in the HC group. This finding was statistically significant in a multiple logistic regression model adjusted for age and ethnicity (p=0.046). Furthermore, individuals with the risk haplotypes ATA, ACT and ACC exhibited a 3.5-fold increase in CP risk (p=2.4E−15; OR=3.459) (Table 3).

The genotypes IL-10-1082GG, IL-10-1082GA and IL-10-1082AA correspond to high, intermediate and low producers of the cytokine IL-10, respectively. These genotypes are solely responsible for phenotype, independent of nucleotide variation at positions -819 and -592. Therefore, ATA/ATA, ACC/ATA, ATA/ACA, ACA/ACC and ACC/ACC were classified as low IL-10 production phenotypes; GCC/ACC, GCC/GTA, GTA/GTC, ACC/GCA, ACA/GCC and GTA/ACC were classified as intermediate IL-10 production phenotypes; and GCA/GCC, GTA/GTA, GTA/GCC and GCC/GGC were classified as high IL-10 production phenotypes (40). Low IL-10 production causes an excess of inflammatory cytokines in the periodontal lesions in the chronic form of this disease. This inflammation is a putative risk indicator of the progression of periodontal disease (38). However, the haplotype profiles for high IL-10 production conferred significant protection against CP; therefore, individuals who are high producers of IL10 are most likely protected against CP due to the anti-inflammatory cytokines that negatively regulate the immune response against periodontopathogenic bacteria (7).

We showed that the low IL-10 production phenotype (ATA/ATA, ACC/ATA, ATA/ACA, ACA/ACC and ACC/ACC genotypes) significantly increases the risk of developing CP (p=0.0.4; OR=2.7.7). In contrast, the high IL-10 phenotype (GCA/GCC, GTA/GTA, GTA/GCC and GCC/GGC genotypes) protects against CP (p=0.0.3; OR=0.2.8). This result supports previous studies that demonstrate that the high production of IL-10 promotes the anti-inflammatory immune response.
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which is important for protection against the bacteria that cause periodontitis.

6. ACKNOWLEDGMENTS

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7. REFERENCES


13. H. Sasaki, Y. Okamatsu, T. Kawai, R. Kent, M. Taubman, P. Stashenko: The interleukin-10 knockout mouse is highly susceptible to Porphyromonas gingivalis-induced alveolar
DOI: 10.1111/j.1600-0765.2004.00760.x

DOI: 10.1111/j.1600-0757.2010.00340.x

DOI: 10.1128/CVI.00334-13


DOI: 10.1002/1529-0131(199906)42:6<1101::AID-ANR6>3.0.CO;2-Y

DOI: 10.1038/sj.gene.6363657

DOI: 10.1186/cc8182

DOI: 10.1016/j.cytogfr.2008.11.006

DOI: 10.1016/j.ejca.2006.11.003

DOI: 10.1371/journal.pone.0040007

DOI: 10.1371/journal.pone.0017063

DOI: 10.1086/427888

DOI: 10.1002/humu.21159


DOI: 10.1086/319501

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