Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Peripheral immune profile of periodontitis patients and reduced expression of CD11b in periodontitis

Mehmetcan Uytun
Muğla University
Deniz Genç
denizgenc@mu.edu.tr
Muğla University
Ali Batuhan Bayırlı
Muğla University
Emrah Türkmen
Istanbul Medipol University

Research Article

Keywords: CD11b, cell adhesion molecules, flow cytometry, natural killer T-cells, periodontitis

Posted Date: September 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10993186/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Oral Health on November 6th, 2026. See the published version at https://doi.org/10.1186/s12903-026-10401-7.

Abstract

Background

Periodontitis is an inflammatory disease caused by the complex interaction between plaque microorganisms and the host immune system that threatens oral health. Leukocyte recruitment plays important roles during the protective and pathological immune responses of many diseases, including periodontitis. CD14 and CD11b are adhesion molecules that promote adhesion of leukocytes to the vascular endothelium to defend against microorganisms. In this cross-sectional study, we aimed to determine the difference between the expression levels of CD14 and CD11b expressed on leukocytes and analyzed subclasses of lymphocytes in individuals with periodontitis compared to healthy individuals.

Methods

Fourteen periodontitis patients and fourteen healthy individuals were included in the study. Venous blood samples were collected from each individual. T lymphocyte subsets were analyzed by flow cytometry using peripheral blood mononuclear cells stained with anti-CD3, anti-CD4, and anti-CD8 antibodies. Natural killer (NK) cells and Natural killer T (NKT) cells were analyzed by flow cytometry using peripheral blood cells stained with anti-CD3, anti-CD16, and anti-CD56 antibodies. The monocyte adhesion molecule CD14 and the common leukocyte adhesion molecule CD11b were analyzed by flow cytometry.

Results

The proportions of NK and NKT cells were significantly higher in periodontitis patients than in healthy subjects ($p < 0.05$). CD11b expression was significantly lower on monocytes and T lymphocytes in periodontitis patients compared with healthy individuals ($p < 0.05$).

Conclusions

Periodontitis is associated with a distinct peripheral immune profile, characterized by reduced CD11b expression on monocytes and T lymphocytes and increased NK and NKT cell frequencies. This reduction in CD11b suggests a possible impairment of leukocyte adhesion-related immune mechanisms, which warrants further functional investigation.

Introduction

Periodontitis is a chronic inflammatory disease characterized by the progressive destruction of tooth-supporting tissues, including the alveolar bone, periodontal ligament, and gingiva [1]. Genetic

predisposition and environmental factors are known to play critical roles in the initiation and progression of periodontal tissue destruction [2]. The primary etiological determinant of the disease is the complex interaction between the local microbiota and the host, followed by the activation of inflammatory and immune responses [3]. Severe periodontitis has been reported to affect more than 10% of the global population, ranking it among the most prevalent chronic inflammatory diseases worldwide [4]. In addition, periodontal diseases have bidirectional associations with systemic conditions such as diabetes mellitus and cardiovascular diseases [5]. The chronic inflammatory nature of both periodontitis and systemic diseases may contribute to the excessive production of inflammatory cytokines by immune cells [6, 7].

Cytokines play a critical role in regulating the inflammatory response involved in the initiation and progression of periodontitis [8]. Interferon-gamma (IFN-γ), secreted mainly by T helper 1 (Th1) cells, can activate macrophages and enhance the production of various pro-inflammatory cytokines, thereby contributing to the amplification of periodontal inflammation [9]. Similarly, interleukin-17 (IL-17), produced by T helper 17 (Th17) cells, is considered an important mediator of periodontal tissue destruction, particularly through its role in promoting alveolar bone resorption [10].

The innate immune system, composed of effector leukocytes such as neutrophils, macrophages, and natural killer (NK) cells, represents the first line of defense against periodontal pathogens. These cells contribute to the control of the bacterial burden by mounting a rapid response against microorganisms; however, through the concomitant production of pro-inflammatory cytokines, they may also amplify the local inflammatory response and contribute to the persistence of tissue destruction [11, 12]. In addition, innate immune cells, including macrophages and dendritic cells, serve as a bridge between innate and adaptive immunity by presenting antigens to T and B lymphocytes and by shaping the direction and magnitude of adaptive immune responses through the cytokines they secrete [13, 14]. Accordingly, the pathogenesis of periodontitis is increasingly understood as a dynamic and mutually reinforcing network involving cytokine-mediated inflammatory processes and the activation of both innate and adaptive immune cells [15].

After maturation in the thymus, T cells enter the peripheral circulation and can be classified into distinct subpopulations, including alpha-beta (αβ) T cells, gamma delta (γδ) T cells, and natural killer T (NKT) cells, according to their T-cell receptor (TCR) expression profile and phenotypic characteristics [16]. γδ T cells are considered an important source of IL-17 production in several tissues, particularly in the gingiva [17]. NKT cells, which function as a bridge between innate and adaptive immunity, are distinguished from other immune cell subsets by their ability to rapidly secrete a broad range of cytokines shortly after activation [18, 19].

Cytokines are key mediators that regulate leukocyte recruitment to infected sites during inflammation. In addition, they play a critical role in immune cell adhesion to the endothelium and subsequent transmigration into tissues by inducing the expression of adhesion molecules on vascular endothelial cells [20, 21]. The absence or insufficient expression of the integrin glycoproteins CD11b and CD18,

which are essential for leukocyte adhesion, leads to the development of leukocyte adhesion deficiency (LAD). This disorder impairs the effective recruitment and transmigration of leukocytes to sites of infection and inflammation, thereby potentially resulting in severe periodontal destruction and marked alveolar bone loss [22]. Moreover, although CD14 does not belong to the integrin family, it has also been shown to play an important role in the adhesion of monocytes to the endothelium [23].

The aim of the present study was to comprehensively evaluate the peripheral immune response in patients with periodontitis and periodontally healthy individuals and to identify potential disease-associated immunological characteristics. To this end, the expression of adhesion molecules, including CD11b and CD14, lymphocyte subset distribution, and cytokine profiles were analyzed together to define a more integrated peripheral immune signature associated with periodontitis. Although these immunological variables have generally been investigated separately in the existing literature, to the best of our knowledge, no previous study has evaluated these parameters together using an integrated approach.

Materials and methods

Study Design and Participants

This study was conducted in accordance with the Declaration of Helsinki, as revised in 2013, and was approved by the Non-Interventional Clinical Research Ethics Committee of Istanbul Medipol University (approval no: 865; date: 17 July 2025). All participants were fully informed about the study protocol, and written informed consent was obtained prior to enrollment.

Participants were recruited from individuals who attended the Department of Periodontology, Faculty of Dentistry, Muğla Sıtkı Koçman University, between August 2025 and December 2025. Clinical and radiographic examinations were performed at the Faculty of Dentistry, Muğla Sıtkı Koçman University. A total of 28 participants, including 14 females and 14 males, who met the eligibility criteria were included in the study. The inclusion criteria were as follows: age between 18 and 65 years, absence of any systemic disease, no periodontal treatment within the previous 6 months, no medication use within the previous 6 months, and non-smoking status. The exclusion criteria were alcohol consumption, pregnancy or lactation, and the presence of active infection, such as an acute periodontal abscess. Eligible participants were allocated according to their periodontal status into either the periodontally healthy group or the generalized stage III/IV periodontitis group.

Study Groups

Participants were divided into two groups according to their periodontal status: the periodontitis group and the periodontally healthy group. Periodontal status was determined according to the 2017 classification system developed jointly by the European Federation of Periodontology and the American Academy of Periodontology [1, 24]. The eligibility criteria for each group were as follows.

Periodontitis group (P) (n = 14): Individuals diagnosed with generalized stage III or stage IV periodontitis were included in this group. The inclusion criteria for the periodontitis group were as follows: presence of ≥ 15 teeth, clinical attachment loss (CAL) of ≥ 5 mm and probing depth (PD) of ≥ 6 mm in more than 30% of teeth, and radiographic bone loss extending to the middle or apical third of the root.

Periodontally healthy group (H) (n = 14): Individuals diagnosed as periodontally healthy were included in this group. The inclusion criteria for the healthy group were as follows: presence of ≥ 15 teeth, CAL ≤ 1 mm and PD ≤ 3 mm at all teeth, absence of radiographic bone loss, and a bleeding on probing (BOP) score of < 10%

Clinical Evaluation

Clinical and radiographic examinations were performed to evaluate the periodontal status of the participants. Periodontal measurements were recorded at six sites per tooth using a Williams periodontal probe (Hu-Friedy, Chicago, IL, USA). As part of the clinical assessment, mean values for the plaque index (PI), gingival index (GI), PD and CAL were calculated. All periodontal measurements were performed by a single periodontist (M.U.). Prior to data collection, intra-examiner calibration was performed on 10 individuals diagnosed with stage III or stage IV periodontitis. Intra-examiner reliability was assessed using the intraclass correlation coefficient, yielding values of 0.92 for PD and 0.91 for CAL. These values indicated an acceptable level of measurement reliability.

Immunophenotyping

Ten-milliliter blood samples were collected on heparin, 8 mL of blood samples were used for intracellular cytokine analysis as described below, and 2 mL of blood samples were subjected to immunophenotyping analysis. The blood samples were incubated with anti-CD3, anti-CD4, anti-CD8 antibodies to identify T lymphocyte subsets, or stained with anti-CD3, anti-CD16, and anti-CD56 antibodies to identify NK and NKT cells, or stained with anti-CD3, anti-CD66b, anti-CD11b, and anti-CD14 to identify adhesion molecules on monocytes and neutrophils. T cells were also identified for γδ T cell and αβ T cell receptors by staining the cells with anti-γδT and anti-αβT receptor antibodies, respectively. All antibodies were obtained from BD Biosciences, USA. The incubation with antibodies was done in the dark in the room temperature. Flow cytometry (BD Accuri C6 Plus, USA) analysis was performed after washing stained cells with lysing buffer. To minimize potential measurement bias, all samples were identified by an anonymized code, and flow cytometric acquisition and gating were performed by a single investigator (D.G.) who was blinded to the periodontal status of the participants.

Cell Culture for Intracellular Cytokine Analysis

To analyze cytokine secreting CD4 + T lymphocytes, PBMC was isolated by ficoll density gradient solution. In brief, 8 mL of whole blood was transferred on 4 mL of ficoll solution (Sigma-Aldrich,

Germany) in a 15 mL falcon tube and centrifuged at 2000 rpm for 20 minutes. The buffy coat was collected and washed twice with RPMI 1640 medium (Sigma-Aldrich, Germany) supplemented with 10%fetal bovine serum and 1% Penicillin/Streptomicin (10.000 U/mL). The remaining cells were cultured with PMA/Ionomycin (0.5µg/mL) for 4 hours at 37°C in a humidified and 5%CO2 chamber. After the incubation period cells were washed twice with PBS, and subjected to intracellular cytokine analysis (BD Cytokine Kit). Cells were initially stained with cell surface antibodies (anti-CD3 / anti-CD4). A fixation and permeabilization solution was used to permeabilize cells for cytokine detection. Anti-IFNγ and anti-IL17 antibodies were used to stain CD3 + CD4+ T lymphocytes. Results were presented as percentage of the cytokine secreting cells.

Statistical Analysis

Demographic data and clinical periodontal parameters were analyzed using IBM SPSS Statistics version 21.0. The normality of continuous variables was assessed using the Shapiro–Wilk test. Normally distributed variables were compared between the periodontitis and periodontally healthy groups using the independent samples t-test, whereas non-normally distributed variables were analyzed using the Mann–Whitney U test. Categorical variables, including sex distribution, were analyzed using the Pearson chi-square test.

Flow cytometry data were analyzed using GraphPad Prism version 8.0 and are presented as mean ± standard deviation. Between-group comparisons (periodontitis vs periodontally healthy) of flow cytometric parameters were performed using the independent-samples t-test. A p value < 0.05 was considered statistically significant.

An a priori power analysis was performed using G*Power to determine the required total sample size for the two comparison groups, namely the periodontitis and periodontally healthy groups. Based on an effect size of 0.60, a statistical power of 0.80, and a Type I error rate of 0.05 [25], the required total sample size was calculated as 24 participants. Considering the possibility of sample loss, a total of 28 participants were included in the study.

Results

Demographic Parameters

A total of 28 individuals who met the eligibility criteria were included in the study, comprising 14 females and 14 males. No statistically significant difference was observed between the periodontitis and periodontally healthy groups in terms of age (p = 0.188). Similarly, sex distribution did not differ significantly between the study groups (p = 1.000) (Table 1).

Table 1
Demographics of the study groups

Age	**Periodontitis (*n* = 14)**	**Healthy (*n* = 14)**		**p-value**
Mean ± sd	34.00 ± 2.57	35.42 ± 3.00	t=-1.351	0.188
Mdn.(min.,max)	34 (30–39)	36 (30–40)		
Sex				
Female	7	7	χ^2<0.001	1
Male	7	7		

Values are presented as mean±standard deviation (sd), median (Mdn.), minimum (min.), maximum (max.),t: Independent samples t test, χ^2: Chi-squared test

Clinical Parameters

All clinical periodontal parameters, including PI, GI, PD, CAL, and BOP, were significantly higher in the periodontitis group than in the periodontally healthy group ($p < 0.001$) (Table 2).

Table 2
Group comparisons in terms of clinical parameters

PI	Healthy (n = 14)	Periodontitis (*n* = 14)		p-value
Mean ± sd	0.82 ± 0.26	2.23 ± 0.45	Z=-4.458	**< 0.001****
Mdn.(min.,max)	0.78 (0.56–1.62)	2.24 (1.58-3)		
GI				
Mean ± sd	0.78 ± 0.26	1.92 ± 0.27	t=-11.037	**< 0.001****
Mdn.(min.,max)	0.78 (0.19–1.25)	1.88 (1.36–2.45)		
CAL				
Mean ± sd	0.58 ± 0.21	5.00 ± 0.98	t=-16.35	**< 0.001****
Mdn.(min.,max)	0.65 (0.25–0.92)	4.52 (4.01–7.21)		
PD				
Mean ± sd	1.92 ± 0.25	4.37 ± 0.72	t=-11.90	**< 0.001****
Mdn.(min.,max)	1.95 (1.46–2.26)	4.12 (3.32–5.40)		
BOP				
Mean ± sd	0.08 ± 0.007	0.47 ± 0.08	t=-17.891	**< 0.001****
Mdn.(min.,max)	0.087 (0.08–0.1)	0.48 (0.35–0.59)		

Values are presented as mean±standard deviation (sd), median (Mdn.), minimum (min.), maximum (max.), Z: Mann Whitney U test, t: Independent samples t test

Ratio of T helper and CD8 + T Cells

The ratio of CD3 + CD4+ T helper lymphocytes was similar in periodontitis patients and healthy individuals, there was no significant difference between the groups ($p > 0.05$). The ratio of CD3 + CD8+ T cells were significantly low in periodontitis patients compared to healthy individuals ($p < 0.05$). CD3 + CD4+CD20 + cells were notably high in periodontitis patients compared to healthy subjects ($p < 0.05$), but there was no significant difference in CD3 + CD8+CD20 cells between two groups ($p > 0.05$) (Fig. 1A, Fig. 1B, and Fig. 1C).

Ratio of αβ and γδ T Cells

To further investigate T cell subset distribution in periodontitis, immunophenotypic analyses of αβ and γδ T cell populations were performed by flow cytometry. The percentage of αβ T cells was comparable between periodontitis patients (49.60 ± 5.38%) and healthy subjects (46.59 ± 5.25%), with no statistically significant difference detected between the groups ($p > 0.05$). In contrast, the proportion of γδ T cells was markedly elevated in periodontitis patients (9.27 ± 1.49%) compared with healthy individuals (2.47 ± 0.55%) ($p < 0.05$). These results indicate a selective expansion of γδ T cell populations in periodontitis, while the overall αβ T cell compartment remains relatively unchanged. Representative flow cytometric plots and quantitative analyses are shown in Fig. 1D and Fig. 1E.

Decreased expression of CD11b on Monocytes and Neutrophils

The expression of CD11b was significantly low in CD14 + cells in periodontitis patients (9.3 ± 2.6%) when compared with healthy individuals (79.3 ± 7.8%) ($p < 0.05$). As similar to monocytes, CD11b expression on CD66 + cells (neutrophils) were low in periodontitis patients (7.2 ± 1.8%) compared to healthy subjects (71.9 ± 5.4%) ($p < 0.05$). We further analyzed CD11b ratio in CD3- and CD3 + cells, and the results showed that CD11b ratio was significantly low in periodontitis patients both in CD3- cells (1.4 ± 0.8%) and CD3 + cells (2.2 ± 1.3%) compared to healthy individuals (15.7 ± 3.8%, 25.2 ± 5.4%, respectively ($p < 0.05$, $p < 0.05$) (Fig. 2).

Frequency of NK and NKT Cells

We analyzed CD3-CD56 + NK cell ratio and CD3 + CD56+ NKT cell ratio to determine the frequency of both cell types in periodontitis patients and compared those with healthy individuals. The ratio of CD3-CD56+ (NK) cells were significantly high in periodontitis patients (3.1 ± 0.4%) compared to healthy individuals (2.4 ± 0.3%) ($p < 0.05$). The ratio of CD3 + CD56+ (NKT) cells were significantly high in periodontitis patients (3.6 ± 1.1%) compared to healthy individuals (1.8 ± 0.7%) ($p < 0.05$). The two primary functions of human natural killer (NK) cells have traditionally been associated with distinct cell subsets. Thus, cytolytic activity is mostly restricted to the CD56-dim CD16 + subset, while cytokine production generally occurs in CD56-bright CD16+/− cells. Therefore, we further analyzed CD56dimCD16 + cells to determine the cytolytic NK cells, which may play a role in aggressive periodontitis. CD56dimCD16 + cells were significantly high in periodontitis patients (3.2 ± 0.7%) when compared with healthy subjects (1.1 ± 0.3%) ($p < 0.05$) (Fig. 3).

IFN-γ and IL-17 Secreting αβ and γδ T Cells

To further define the functional characteristics of T cell subsets in periodontitis, intracellular cytokine production was evaluated separately in αβ and γδ TCR-expressing T cells. Analysis of αβ T cells revealed that the proportion of IFN-γ–producing αβ T cells was significantly increased in mononuclear cells obtained from periodontitis patients (4.47 ± 0.91%) compared with healthy subjects (2.13 ± 0.56%) ($p <$

0.05). Conversely, the frequency of IL-17–producing αβ T cells was significantly reduced in periodontitis patients (0.16 ± 0.11%) relative to healthy controls (0.74 ± 0.12%) ($p < 0.05$).

Evaluation of γδ T cell subsets demonstrated that the percentage of IFN-γ–secreting γδ T cells was higher in periodontitis patients (1.02 ± 0.32%) than in healthy individuals (0.72 ± 0.41%); however, this difference did not reach statistical significance ($p > 0.05$). In contrast, IL-17–producing γδ T cells were markedly increased in periodontitis patients (5.26 ± 1.56%) compared with healthy controls (0.66 ± 0.35%) ($p < 0.05$). Collectively, these findings indicate that periodontitis is associated with distinct functional alterations in αβ and γδ T cell subsets, characterized by enhanced IFN-γ production in αβ T cells and pronounced IL-17 production within the γδ T cell compartment. Representative flow cytometric analyses and quantitative data are presented in Fig. 4A, Fig. 4B, and Fig. 4C.

IFN-γ and IL-17 Secreting CD4 + T Cells

To characterize CD4 + T helper cell subsets in periodontitis patients, intracellular IFN-γ and IL-17 expression was analyzed by flow cytometry following stimulation of peripheral blood mononuclear cells. Quantitative analysis demonstrated that the proportion of CD4 + IL-17 + T cells was significantly reduced in periodontitis patients (0.4 ± 0.3%) compared with healthy individuals (1.6 ± 0.7%) ($p < 0.05$). In contrast, the frequency of CD4 + IFN-γ + T cells did not significantly differ between the groups, although a slight increase was observed in periodontitis patients (9.3 ± 2.0%) compared with healthy controls (7.4 ± 1.8%) ($p > 0.05$). These findings suggest that periodontitis is associated with an altered CD4 + T cell cytokine profile characterized by reduced IL-17–producing helper T cells without significant changes in IFN-γ–producing CD4 + T cells. Representative flow cytometry analyses and quantitative data are presented in Fig. 4D, Fig. 4E.

Discussion

Periodontitis is a chronic inflammatory disease affecting teeth and their supporting tissues. Periodontitis is initiated by bacteria and can lead to tooth loss through inflammatory responses in the tissue. In recent years, it has been shown that the disease results from complex interactions between microbes and the immune response, and that these immune responses contribute to disease progression. In inflammatory responses, neutrophils and monocytes are the first cells to migrate into the tissue, and these cells are the initiators of lymphocyte-mediated immune responses. Adhesion molecules in mononuclear cells are effective in the migration of these cells into infectious tissue. In this study, we investigated the expression of adhesion molecules on phagocytes such as monocytes and neutrophils, and on T lymphocytes in patients with periodontitis compared with healthy individuals.

Adhesion molecules are crucial in the recruitment of mononuclear cells in the site of infection. CD11b, an integrin protein and complement 3 receptor (CR3), is expressed on the surface of many innate immune cells, including macrophages, granulocytes, NK cells, subsets of dendritic cells (DCs), and B cells [26]. CD11b, as a subunit of integrin αMβ2/Mac-1/CR3, is important for the adhesion, migration,

and phagocytosis of macrophages. CD11b deficiency has been reported to confer a higher susceptibility to bacteremia and sepsis in infections caused by pathogens [27]. In a case study in the literature, a deficiency of neutrophil integrin CD11b/CD18 was detected in the peripheral blood mononuclear cells of a patient with severe periodontitis. This patient was diagnosed with leukocyte adhesion deficiency (LAD-1) upon further immunological testing [28]. LAD-I-associated periodontitis is associated with an aggressive form of inflammatory bone loss due to a deficiency of neutrophil migration in periodontal infection [29]. Although studies conducted to date have separately demonstrated leukocyte integrin molecule expressions or LAD-associated periodontitis, there is no study yet that combines all immunological data. In the present study, we analyzed CD11b expression on monocytes, neutrophils, and CD3 + cells in peripheral blood mononuclear cells in periodontitis by comparing the results with healthy subjects. CD11b expression on monocytes and neutrophils was significantly lower in periodontitis patients compared with healthy individuals. Similarly, CD11b expression on CD3 + lymphocytes were notably lower in periodontitis patients compared with healthy individuals. These results suggest that aggressive periodontitis may be due to CD11b deficiency or leukocyte adhesion deficiency. In contrast, a study on a periodontitis mouse model showed that CD11b expression was observed in the periodontal tissues of mice with periodontitis, indicating that periodontitis causes inflammation in the periodontal tissues. The difference between our study and the study in the literature may be due to differences in experimental design, or it may be due to the lack of an experimental design for cells lacking CD11b expression in the study conducted in the experimental animal model. Therefore, further in vivo studies are needed to reveal whether CD11b deficiency or LAD-1 causes periodontitis.

We further analyzed αβ T cells and γδ T cells to determine the T cell receptor profile of periodontitis patients. γδ T lymphocytes, which mature in the thymus, constitute up to 5% of the total T lymphocyte population in adult humans. γδ T lymphocytes are distributed not only in the blood and lymphoid organs but also in various tissues such as the skin, lung and intestinal epithelium, and liver. Unlike αβ T cells, γδ T cells are MHC-unrestricted manner antigen presentation. The results of our study showed that there was a significant high frequency in γδ T lymphocytes in periodontitis patients' peripheral mononuclear cells compared to healthy subjects, while αβ T cell frequency was similar in both groups. In a study of periodontitis patients with chronic obstructive pulmonary disease (COPD), researchers concluded that the periodontitis-mediated γδ T immune mechanism promotes COPD progression. Previous work has shown that Porphyromonas gingivalis (P. gingivalis) promotes the activation of γδ T cells and M2 macrophages ex vivo [22]. This study is a pioneering study on the role of γδ T lymphocytes in periodontitis, but it should be questioned whether γδ T lymphocytes play a role in the progression of periodontitis or whether γδ T lymphocytes developing with COPD cause periodontitis. Another in vivo study demonstrated that γδ T cells play a pathological role during P. gingivalis infection, and their absence prevented alveolar bone loss. Further analysis in the same study revealed that γδ T cells are responsible for neutrophil and monocyte recruitment to the gingiva after exposure to P. gingivalis. It was suggested that γδ T cell ablation reduces osteoclastogenesis and dysregulates long-term immune responses in the gingiva [30]. The conclusion reached in the studies conducted to date is undoubtedly that gamma delta T lymphocytes play an important role in the progression of periodontitis.

There is currently increasing interest in researching the relationship between periodontal disease and autoimmunity. Periodontal disease-associated microorganisms comprise a diverse range of species that can inhabit the oral cavity and influence the onset or development of autoimmunity, characterized by the breakdown of tolerance mechanisms against self-antigens [31]. Chronic inflammation, which may occur after infection, can trigger autoimmunity through various mechanisms, including potentiation of autoimmunity by cytokines, epitope spreading, autoantigen complementation, overproduction of autoantigens, microbial translocation, molecular mimicry, superantigens, and activation or inhibition of autoimmunity-related receptors by microorganisms [32]. The presence of T cells expressing CD20 is well-established in autoimmunity and correlates with disease severity in autoimmune diseases. In addition, CD20 + T cells are found in the peripheral blood of healthy individuals at ~ 3–5% of the total CD3 T cell population, but increases in autoimmune patients [33]. In this study, we found that CD4 + T lymphocytes in periodontitis patients showed higher CD20 expression compared to healthy individuals. However, the CD20 ratio on CD8 + T lymphocytes in periodontitis patients was similar to that in healthy individuals. In addition, the ratio of CD4 + T cells was similar to that in healthy individuals, whereas the frequency of CD8 + T cells were significantly low in periodontitis patients compared to healthy individuals. We also analyzed the frequency of IFN-γ or IL-17 secreting CD4 + T cells to determine the peripheral cytokine profile of periodontitis patients. IL-17 secreting T lymphocytes have high abilities to stimulate acute and chronic inflammation that make these cells crucial player in development of autoimmune disorders [34]. Additionally, several studies have found that the type II IFN, IFN-γ, plays an essential role in the development and severity of systemic autoimmunity [35]. IL-6 is secreted by various cell types including macrophages, fibroblasts, adipocytes, and skeletal muscle cells. But the most abundant secretory cells for IL-6 are macrophages. IL-6 is a strong inducer of Th17 differentiation, it would be reasonable to hypothesize that IL-6 induces metabolic changes in T cells that trigger Th17 differentiation and function. Monocytes must migrate into tissue to differentiate phenotypically into macrophages. It is thought that decreased migration into tissue may be due to a lack of monocyte adhesion molecules, which in turn may be related to decreased IL-6 secretion due to a lack of macrophages in the tissue, leading to a decrease in Th17. We believe that in vivo experimental studies should be conducted regarding this assumption.

T lymphocytes consist of two populations, αβ T cells and γδ T cells, which can be distinguished by their expression of either the αβ T cell receptor (TCR) or γδ TCR. The antigen-binding heterodimer, composed of TCRαβ or TCRγδ, pairs with proteins of the CD3 complex to form the complete αβ or γδ TCR. Despite the similarities in the structure of TCRαβ and TCRγδ, these two receptors differ in important ways [36]. In the cellular immunity, γδ T cells differ from αβ T cells due to distinct properties that complexly shape their role in disease pathogenesis. αβ T cells, which constitute the predominant subset of CD3 + T cells, predominantly recognize peptide antigens presented by major histocompatibility complex (MHC) molecules, while γδ T cells recognize a broader array of antigens in an MHC-independent manner [37]. In addition, γδ T cells are important in mucosal immunity because they provide defense against various pathogens such as viruses, bacteria and fungi [38]. Th17 cells play a crucial role on periodontal inflammation. Cytokines secreted by Th17 cells are also major players in the pathogenesis of

periodontitis [39]. In the present study, we also analyzed αβ and γδ T cell subsets according to their cytokine profiles for the first time in periodontitis. Our results showed that IFN-γ secreting αβ T cells were notably high, while IL-17 secreting αβ T cells were significantly low in periodontitis patients' mononuclear cells compared to healthy individuals. In contrast, IL-17 secreting γδ T cells were significantly high in periodontitis patients' mononuclear cells when compared with healthy subjects. Studies have shown that IL-17 is a critical cytokine involved in host defense functions in the periodontium, including neutrophil recruitment, phagocytosis, and mucosal immunity. However, overexpression of IL-17 in periodontitis causes persistent chronic inflammation, local tissue destruction, and bone loss [40]. However, in terms of determining which cell subset is a source of IL-17 secretion, our results show that increased IL-17 secretion in periodontitis originates from gamma delta T lymphocytes rather than alpha beta T lymphocytes. These results suggest that there may be MHC-independent T lymphocyte responses, such as those in T lymphocytes with gamma delta TCR, rather than MHC-associated antigen presentation in antigen-presenting cells such as macrophages. These results actually suggest that patients with periodontitis are prone to autoimmunity, but the cause of autoimmunity may also be epitope spreading or molecular mimicry of bacterial microbiota.

Natural killer T (NKT) cells constitute a unique subset of T lymphocytes characterized by their surface antigens common in NK cells and T cells. Peripheral CD56dimCD16 + NK cells are cytotoxic NK cells acting mainly through the release of perforin and granzyme which activates apoptosis on target cells. As in many pathological conditions, changes in NK phenotype and function have been reported in periodontitis, primarily associated with impaired or increased toxicity [41]. We in this study observed that the ratio of CD3 + CD56+ NKT cells and CD3-CD56 + NK cells were significantly high in periodontitis patients compared to healthy subjects. We further analyzed CD56dimCD16 + cytotoxic NK cell subsets, and it was shown that cytotoxic NK cell ratio is notably high in periodontitis patients' mononuclear cells when compared with healthy individuals. Although NK cells play an important role in both the innate and adaptive immune response, their role in periodontal disease remains unclear. It is well known that increased number of these cells (CD56dim NK cells) which express KIR receptors, can affect osteoclast activity and trigger osteoclast apoptosis [42]. Therefore, increased number of CD56dimCD16 + cytotoxic NK cells may also be responsible for bone loss in periodontitis.

This study has certain limitations that should be considered when interpreting the findings. First, although the sample size was determined a priori by power analysis, the study was conducted in a relatively small cohort, and confirmation in larger populations would strengthen the generalizability of the results. Second, the cross-sectional design allows the identification of associations between periodontitis and the observed immune alterations but does not permit conclusions regarding causality or the temporal sequence of these changes. Third, the immune profile was characterized in peripheral blood and may therefore not fully reflect the local immune response within the periodontium. Gingival crevicular fluid (GCF) and periodontal tissue were not evaluated in the present study; future investigations incorporating local sampling, together with functional and tissue-level analyses in larger populations, may help to confirm the pathophysiological relevance and clinical applicability of these findings.

Conclusion

In conclusion, periodontitis is a complex, chronic multifactorial inflammatory disease, and the present study demonstrated a distinct peripheral immune profile in affected patients compared with periodontally healthy individuals. Although the frequency of CD4 + T helper cells was comparable between the groups, CD8 + T cells were reduced and CD4 + CD20+ T cells were increased in periodontitis patients. A marked reduction in CD11b expression was observed on monocytes, neutrophils, and CD3 + T cells, suggesting a possible impairment of leukocyte adhesion–related immune mechanisms that may, in turn, reduce the migration of phagocytes into the tissue. In addition, periodontitis patients exhibited increased frequencies of γδ T cells, NKT cells, and cytotoxic NK cell subsets. Cytokine profiling further indicated that IL-17 production was decrased in αβ and CD4 + T-cell subsets but markedly increased in γδ T cells, whereas IFN-γ production was increased in αβ T cells, suggesting that γδ T cells may represent an important peripheral source of IL-17 in periodontitis. Taken together, these findings characterize the peripheral immune alterations that accompany periodontitis and provide a basis for further studies on the immunopathogenesis of the disease.

Abbreviations

BOP
Bleeding on probing
CAL
Clinical attachment loss
CD
Cluster of differentiation
COPD
Chronic obstructive pulmonary disease
CR3
Complement receptor 3
DC
Dendritic cell
FSC
Forward scatter
GCF
Gingival crevicular fluid
GI
Gingival index
IFN-γ
Interferon-gamma
IL-6
Interleukin-6
IL-17

Interleukin-17

KIR

Killer immunoglobulin-like receptor

LAD

Leukocyte adhesion deficiency

Mac-1

Macrophage-1 antigen

MHC

Major histocompatibility complex

NK

Natural killer

NKT

Natural killer T

PBMC

Peripheral blood mononuclear cell

PBS

Phosphate-buffered saline

PD

Probing depth

PI

Plaque index

PMA

Phorbol 12-myristate 13-acetate

RPMI

Roswell Park Memorial Institute (culture medium)

SD

Standard deviation

SPSS

Statistical Package for the Social Sciences

SSC

Side scatter

TCR

T-cell receptor

Th1

T helper 1

Th17

T helper 17

Declarations

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Non-Interventional Clinical Research Ethics Committee of Istanbul Medipol University (approval/decision no: 865; date of decision: 17 July 2025). The study was conducted in accordance with the Declaration of Helsinki, as revised in 2013. All participants were informed about the study protocol and provided written informed consent prior to their inclusion in the study.

Funding

This research was conducted without any external funding.

Author Contribution

MU: Selection of patients, writing manuscriptDG: Study design, analyses, writing manuscript, supervisionABB: Selection of patients, writing manuscriptET: Selection of patients, writing manuscript

Acknowledgements

Not applicable.

Data Availability

All data supporting the findings of this study are available within the paper.

References

1. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol. 2018;89(Suppl 1):S173–82.
2. Bartold PM, Van Dyke TE, Periodontitis. A host-mediated disruption of microbial homeostasis. Unlearning learned concepts. Periodontol 2000. 2013;62(1):203–17.

3. Page RC, Schroeder HE. Pathogenesis of inflammatory periodontal disease. A summary of current work. Lab Invest. 1976;34(3):235–49.
4. Eke PI, Dye BA, Wei L, Slade GD, Thornton-Evans GO, Borgnakke WS, et al. Update on prevalence of periodontitis in adults in the United States: NHANES 2009 to 2012. J Periodontol. 2015;86(5):611–22.
5. Kuo LC, Polson AM, Kang T. Associations between periodontal diseases and systemic diseases: A review of the inter-relationships and interactions with diabetes, respiratory diseases, cardiovascular diseases and osteoporosis. Public Health. 2008;122(4):417–33.
6. Shinjo T, Nishimura F. The bidirectional association between diabetes and periodontitis, from basic to clinical. Jpn Dent Sci Rev. 2024;60:15–21.
7. Yamashita A, Soga Y, Iwamoto Y, Yoshizawa S, Iwata H, Kokeguchi S, et al. Macrophage-adipocyte interaction: Marked interleukin-6 production by lipopolysaccharide. Obes (Silver Spring). 2007;15(11):2549–52.
8. Preshaw PM, Taylor JJ. How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis? J Clin Periodontol. 2011;38(Suppl 11):60–84.
9. Takayanagi H, Ogasawara K, Hida S, Chiba T, Murata S, Sato K, et al. T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-γ. Nature. 2000;408(6812):600–5.
10. Takahashi K, Azuma T, Motohira H, Kinane DF, Kitetsu S. The potential role of interleukin-17 in the immunopathology of periodontal disease. J Clin Periodontol. 2005;32(4):369–74.
11. Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. Nat Immunol. 2015;16(4):343–53.
12. Nilsson BO. Mechanisms involved in regulation of periodontal ligament cell production of pro-inflammatory cytokines: Implications in periodontitis. J Periodontal Res. 2021;56(2):249–55.
13. Benakanakere M, Kinane DF. Innate cellular responses to the periodontal biofilm. Front Oral Biol. 2011;15:41–55.
14. Anderson KJ, Allen RL. Regulation of T-cell immunity by leucocyte immunoglobulin-like receptors: Innate immune receptors for self on antigen-presenting cells. Immunology. 2009;127(1):8–17.
15. Assuma R, Oates T, Cochran D, Amar S, Graves DT. IL-1 and TNF antagonists inhibit the inflammatory response and bone loss in experimental periodontitis. J Immunol. 1998;160(1):403–9.
16. Mousset CM, Hobo W, Woestenenk R, Preijers F, Dolstra H, van der Waart AB. Comprehensive phenotyping of T cells using flow cytometry. Cytometry A. 2019;95(6):647–54.
17. Wilharm A, Tabib Y, Nassar M, Reinhardt A, Mizraji G, Sandrock I, et al. Mutual interplay between IL-17-producing γδ T cells and microbiota orchestrates oral mucosal homeostasis. Proc Natl Acad Sci U S A. 2019;116(7):2652–61.

18. Matsuda JL, Naidenko OV, Gapin L, Nakayama T, Taniguchi M, Wang CR, et al. Tracking the response of natural killer T cells to a glycolipid antigen using CD1d tetramers. J Exp Med. 2000;192(5):741–54.
19. Van Kaer L, Parekh VV, Wu L. Invariant natural killer T cells: Bridging innate and adaptive immunity. Cell Tissue Res. 2011;343(1):43–55.
20. Kinane DF, Lappin DF, Culshaw S. The role of acquired host immunity in periodontal diseases. Periodontol 2000. 2024. https://doi.org/10.1111/prd.12562
21. Kinane DF, Adonogianaki E, Moughal N, Winstanley FP, Mooney J, Thornhill M. Immunocytochemical characterization of cellular infiltrate, related endothelial changes and determination of GCF acute-phase proteins during human experimental gingivitis. J Periodontal Res. 1991;26(3 Pt 2):286–8.
22. Hajishengallis G, Moutsopoulos NM. Role of bacteria in leukocyte adhesion deficiency-associated periodontitis. Microb Pathog. 2016;94:21–6.
23. Beekhuizen H, Blokland I, Corsèl-van Tilburg AJ, Koning F, van Furth R. CD14 contributes to the adherence of human monocytes to cytokine-stimulated endothelial cells. J Immunol. 1991;147(11):3761–7.
24. Chapple ILC, Mealey BL, Van Dyke TE, Bartold PM, Dommisch H, Eickholz P, et al. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol. 2018;89(Suppl 1):S74–84.
25. Serdar CC, Cihan M, Yücel D, Serdar MA. Sample size, power and effect size revisited: Simplified and practical approaches in pre-clinical, clinical and laboratory studies. Biochem Med (Zagreb). 2021;31(1):010502.
26. Abram CL, Lowell CA. The ins and outs of leukocyte integrin signaling. Annu Rev Immunol. 2009;27:339–62.
27. Sim H, Jeong D, Kim HI, Pak S, Thapa B, Kwon HJ, et al. CD11b deficiency exacerbates methicillin-resistant Staphylococcus aureus-induced sepsis by upregulating inflammatory responses of macrophages. Immune Netw. 2021;21(2):e13.
28. Dababneh R, Al-Wahadneh AM, Hamadneh S, Khouri A, Bissada NF. Periodontal manifestation of leukocyte adhesion deficiency type I. J Periodontol. 2008;79(4):764–8.
29. Xiong K, Ao K, Wei W, Dong J, Li J, Yang Y, et al. Periodontitis aggravates COPD through the activation of γδ T cell and M2 macrophage. mSystems. 2024;9(2):e00572–23.
30. Barel O, Aizenbud Y, Tabib Y, Jaber Y, Leibovich A, Horev Y, et al. γδ T cells differentially regulate bone loss in periodontitis models. J Dent Res. 2022;101(4):428–36.
31. Degasperi GR, Ossick MV, Pinheiro SL, Etchegaray A. Autoimmunity and periodontal disease: Arguing a possible correlation. Indian J Dent Res. 2020;31(4):615–20.
32. Suárez LJ, Garzón H, Arboleda S, Rodríguez A. Oral dysbiosis and autoimmunity: From local periodontal responses to an imbalanced systemic immunity. A review. Front Immunol. 2020;11:591255.

33. Vlaming M, Bilemjian V, Álvarez Freile J, Lourens HJ, van Rooij N, Huls G, et al. CD20 positive CD8 T cells are a unique and transcriptionally-distinct subset of T cells with distinct transmigration properties. Sci Rep. 2021;11(1):20499.
34. Tabarkiewicz J, Pogoda K, Karczmarczyk A, Pozarowski P, Giannopoulos K. The role of IL-17 and Th17 lymphocytes in autoimmune diseases. Arch Immunol Ther Exp (Warsz). 2015;63(6):435–49.
35. Pollard KM, Cauvi DM, Toomey CB, Morris KV, Kono DH. Interferon-γ and systemic autoimmunity. Discov Med. 2013;16(87):123–31.
36. Morath A, Schamel WW. αβ and γδ T cell receptors: Similar but different. J Leukoc Biol. 2020;107(6):1045–55.
37. Hu Y, Hu Q, Li Y, Lu L, Xiang Z, Yin Z, et al. γδ T cells: Origin and fate, subsets, diseases and immunotherapy. Signal Transduct Target Ther. 2023;8(1):434.
38. Kang I, Kim Y, Lee HK. Double-edged sword: γδ T cells in mucosal homeostasis and disease. Exp Mol Med. 2023;55(9):1895–904.
39. Huang N, Dong H, Luo Y, Shao B. Th17 cells in periodontitis and its regulation by A20. Front Immunol. 2021;12:742925.
40. Tang Z, Jin L, Yang Y. The dual role of IL-17 in periodontitis regulating immunity and bone homeostasis. Front Immunol. 2025;16:1578635.
41. Parisi L, Bassani B, Tremolati M, Gini E, Farronato G, Bruno A. Natural killer cells in the orchestration of chronic inflammatory diseases. J Immunol Res. 2017;2017:4218254.
42. Fathollahi A, Samimi LN, Akhlaghi M, Jamshidi A, Mahmoudi M, Farhadi E. The role of NK cells in rheumatoid arthritis. Inflamm Res. 2021;70(10):1063–73.

Figures

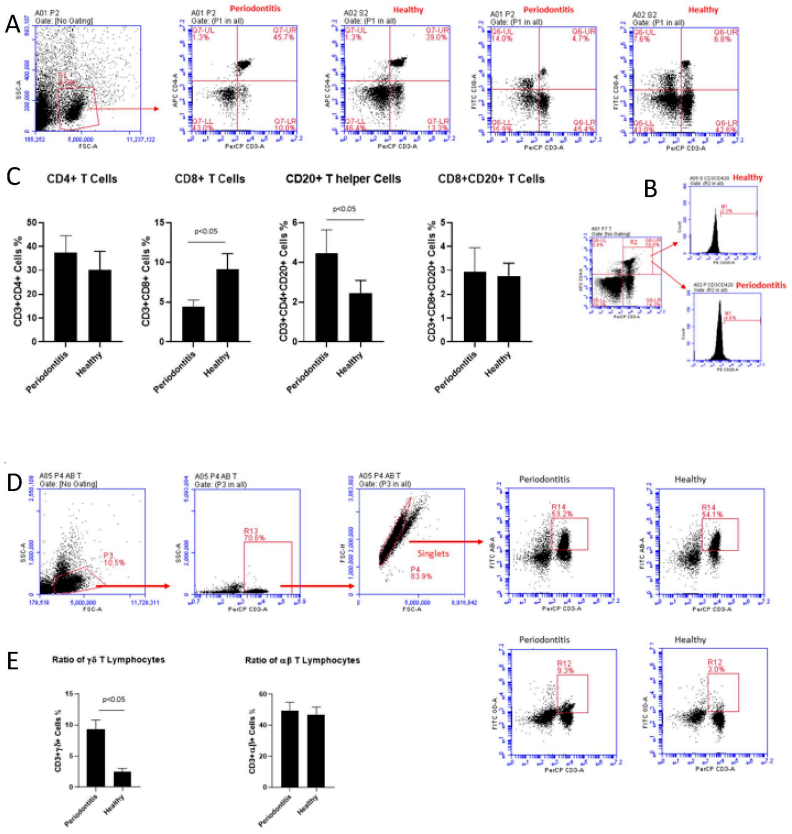


Figure 1

Flow cytometric analysis of peripheral blood T lymphocyte subsets in periodontitis patients and healthy controls. A) Total lymphocytes were gated from SSC-FSC dot plot. CD3+CD4+ or CD3+CD8+ double positive cells were analyzed. B) The histogram analysis of CD20+ T cell subsets are shown. The histogram analysis was performed for CD3+CD4+ or CD3+CD8+ cells. C) Statistical analysis of T cell subsets. The proportion of CD3+CD4+ T helper lymphocytes was comparable between periodontitis patients and healthy individuals, with no statistically significant difference observed ($p>0.05$). In contrast, the frequency of CD3+CD8+ cytotoxic T lymphocytes was significantly decreased in periodontitis patients compared with healthy controls ($p<0.05$). Furthermore, the percentage of CD3+CD4+CD20+ lymphocytes was significantly elevated in periodontitis patients ($p<0.05$), whereas no significant difference was detected in the proportion of CD3+CD8+CD20+ cells between the two groups ($p>0.05$). D) Immunophenotypic analysis of αβ and γδ T cell subsets in periodontitis patients and healthy controls. Representative flow cytometric plots and quantitative evaluation of αβ T cells. No statistically significant difference was observed in the proportion of αβ T cells between periodontitis patients and healthy individuals ($p>0.05$). E) Representative analysis and quantitative evaluation of γδ T cells. The percentage of γδ T cells was significantly increased in periodontitis patients compared with healthy controls ($p<0.05$). Data are presented as mean ± SD. *$p<0.05$ versus healthy controls.

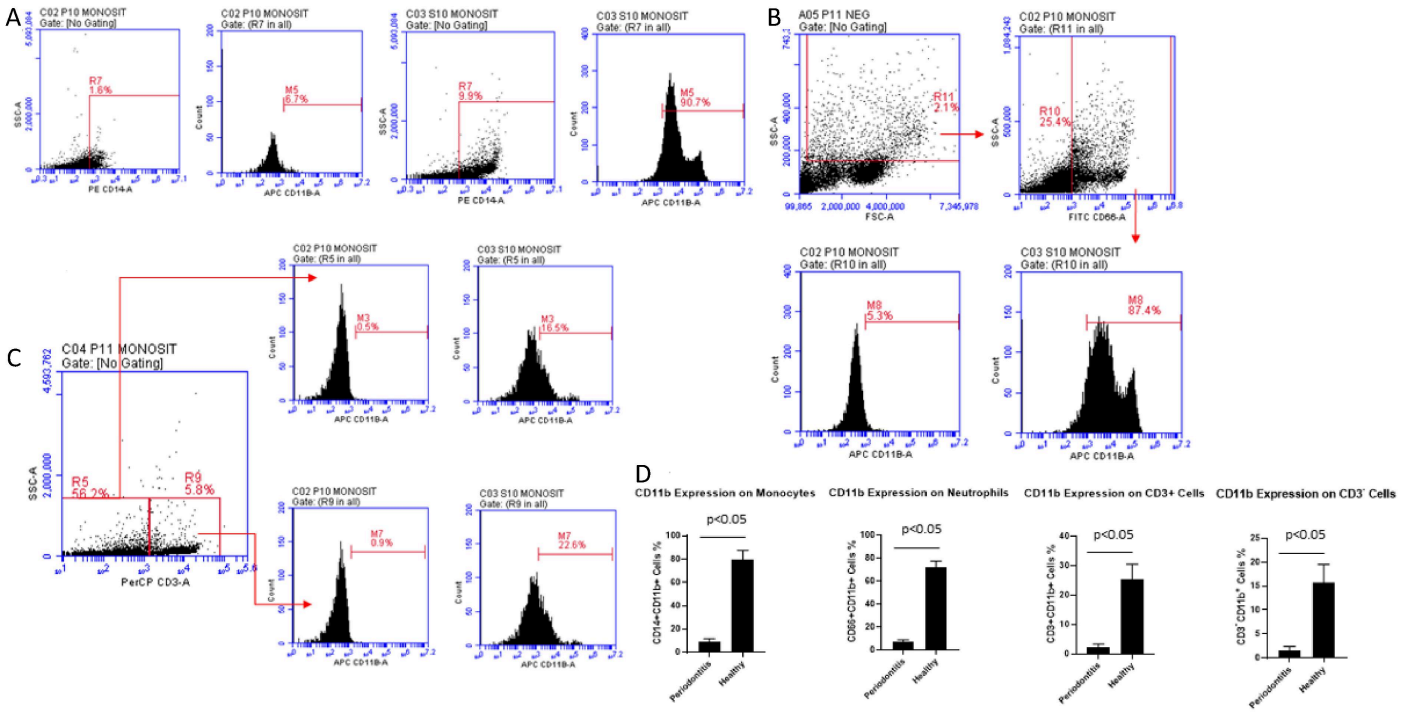


Figure 2

Flow cytometric analysis of CD11b expression on peripheral blood immune cell subsets in periodontitis patients and healthy controls. A) Representative analysis and quantitative evaluation of CD11b expression on CD14+ monocytes. The percentage of CD11b+CD14+ cells was significantly decreased in periodontitis patients compared with healthy individuals ($p<0.05$). B) Representative flow cytometric plots and quantitative analysis of CD11b expression on CD66+ neutrophils. CD11b expression on CD66+ cells was markedly reduced in periodontitis patients relative to healthy controls ($p<0.05$). C) Quantitative analysis of CD11b expression on CD3− and CD3+ lymphocytes. The proportion of CD11b+CD3− cells was significantly lower in periodontitis patients than in healthy individuals ($p<0.05$). D) Quantitative evaluation of CD11b expression on CD3+ lymphocytes. The percentage of CD11b+CD3+ cells was significantly reduced in periodontitis patients compared with healthy subjects ($p<0.05$). Data are presented as mean ± SD. *$p<0.05$ versus healthy controls.

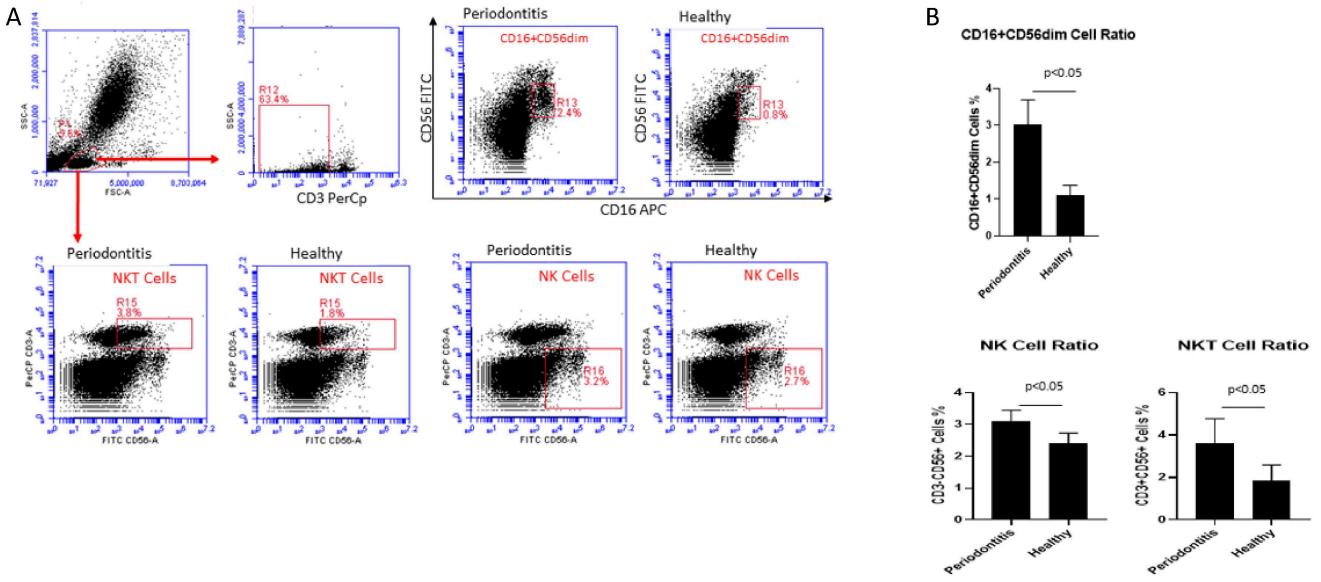


Figure 3

Flow cytometric analysis of NK and NKT cell subsets in periodontitis patients and healthy controls. A) Representative flow cytometric plots and quantitative analysis of CD3−CD56+ natural killer (NK) cells. The proportion of NK cells was significantly increased in periodontitis patients compared with healthy individuals ($p<0.05$). B) Representative analysis and quantitative evaluation of CD3+CD56+ natural killer T (NKT) cells. The percentage of NKT cells was significantly higher in periodontitis patients than in healthy controls ($p<0.05$). Quantitative analysis of $CD56^{dim}CD16^{+}$ cytolytic NK cell subsets. The frequency of $CD56^{dim}CD16^{+}$ cells was significantly elevated in periodontitis patients compared with healthy subjects. Data are presented as mean ± SD. *$p<0.05$ versus healthy controls.

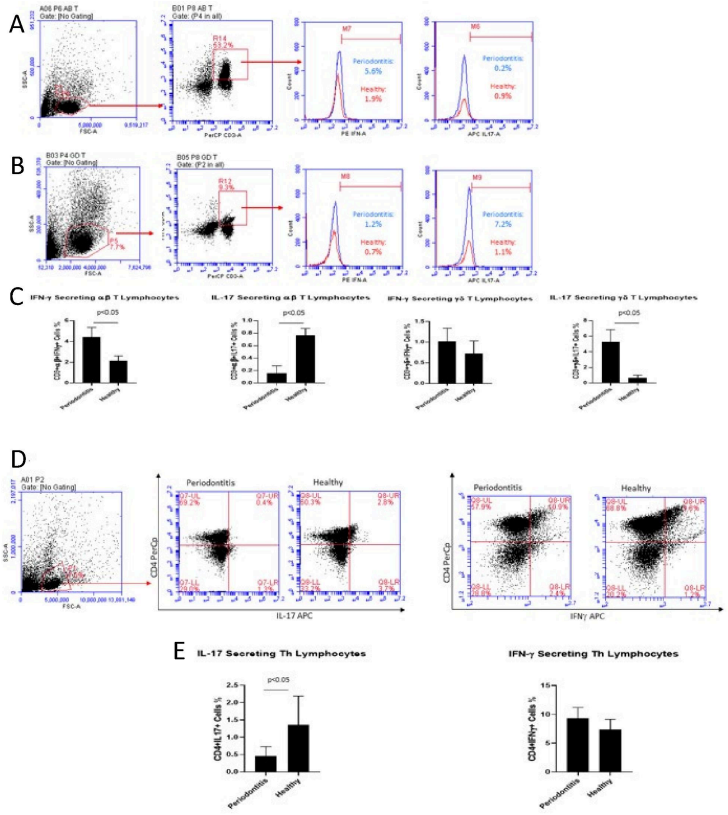


Figure 4

Flow cytometric analysis of cytokine-secreting αβ and γδ T cell subsets in periodontitis patients and healthy controls. A) Representative flow cytometric plots and quantitative analysis of IFN-γ–producing αβ T cells. The proportion of IFN-γ+ αβ T cells was significantly increased in periodontitis patients compared with healthy individuals ($p<0.05$). B) Representative flow cytometric plots and quantitative analysis of IFN-γ–producing γδ T cells. C) Quantitative evaluation of IL-17–producing αβ T cells. The percentage of IL-17+ αβ T cells was significantly decreased in periodontitis patients relative to healthy controls ($p<0.05$). The frequency of IFN-γ+ γδ T cells was higher in periodontitis patients than in healthy individuals; however, the difference did not reach statistical significance ($p>0.05$). Quantitative analysis of IL-17–producing γδ T cells. The proportion of IL-17+ γδ T cells was significantly elevated in periodontitis patients compared with healthy subjects ($p<0.05$). D) Flow cytometric analysis of cytokine-producing CD4+ T helper cell subsets in periodontitis patients and healthy controls. Representative flow cytometric plots and quantitative evaluation of CD4+IL-17+ T helper cells. The proportion of IL-17–secreting CD4+ T cells was significantly decreased in periodontitis patients compared with healthy individuals ($p<0.05$). E) Representative analysis and quantitative evaluation of CD4+IFN-γ+ T helper cells. No statistically significant difference was observed in the frequency of IFN-γ–producing CD4+ T cells between periodontitis patients and healthy controls ($p>0.05$). Data are presented as mean ± SD. *$p<0.05$ versus healthy controls.