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Recognition of *Candida albicans* by gingival fibroblasts: The role of TLR2, TLR4/CD14, and MyD88

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Abstract

Recent evidence indicates that nonprofessional immune cells such as epithelial cells, endothelial cells, and fibroblasts also contribute to innate immunity via secretion of cytokines. Fibroblasts are the principal type of cell found in the periodontal connective tissues and they are involved in the immune response during periodontal disease. The role of fibroblasts in the recognition of pathogens via Toll-like receptors (TLRs) has been established; however, few studies have been conducted concerning the involvement of innate immune receptors in the recognition of *Candida albicans* by gingival fibroblast. In the current study, we investigate the functional activity of TLR2, cluster of differentiation 14 (CD14), and myeloid differentiation primary response gene 88 (MyD88) molecules in the recognition of *C. albicans* by gingival fibroblast. First, we identified that gingival fibroblasts expressed TLR2, TLR3, and TLR4. Our results showed that TLR agonists had no effect on these receptors' expression by TLR2, MyD88, and CD14-deficient cells. Notably, *C. albicans* and a synthetic triacylated lipoprotein (Pam3CSK4) induced a remarkable increase of TLR3 expression on MyD88-deficient gingival fibroblasts. TLR4 expression levels were lower than TLR2 and TLR3 levels and remained unchanged after TLR agonist stimulation. Gingival fibroblasts presented morphological similarities; however, TLR2 deficiency on these cells leads to a lower proliferative response, whereas the deficiency on CD14 expression resulted in lower levels of type I collagen by these cells. In addition, the recognition of *C. albicans* by gingival fibroblasts had an effect on the secretion of cytokines and it was dependent on a specific recognition molecule. Specifically, tumor necrosis factor-α (TNF-α) production after the recognition of *C. albicans* was dependent on MyD88, CD14, and TLR2 molecules, whereas the production of interleukin-1β (IL-1β) and IL-13 was dependent on TLR2. These findings are the first to describe a role of gingival fibroblast in the recognition of *C. albicans* and the pathways involved in this

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Conflict of interest

The authors declare that they have no conflict of interest.

process. An understanding of these pathways may lead to alternative treatments for patients with periodontal disease.

Keywords

Candida albicans; Fibroblasts; Inflammation; Toll-like receptors

1. Introduction

Fibroblasts are the major mesenchymal cell type in connective tissue and deposit the collagen and elastic fibers of the extracellular matrix [1]. Fibroblasts exhibit considerable functional diversity [2]. Evidence suggests that fibroblasts are not a homogeneous population even within a single tissue, but there are different subsets of cells such as tissue macrophages and dendritic cells [3]. Recent evidence indicates that nonprofessional immune cells such as fibroblasts, epithelial cells, and endothelial cells also contribute to innate immunity via Toll-like receptor (TLR) activation and production of cytokines [4].

The immune system uses a variety of innate immune receptors to sense infectious agents. These receptors can be expressed on the cell surface or on intracellular compartments, or be segregated to blood and tissue fluids [5]. The best known innate immune receptors involved in the recognition of pathogens are pattern recognition receptors (PRRs) [6]. Among the PRRs, TLRs are the most characterized family [7]; however, other PRRs (e.g., integrins, C-type lectin receptors, nucleotide-binding oligomerization domain-like receptors, and inflammasomes) are equally important pathogens sensors [6]. Similar to immune cells, studies have suggested that fibroblasts might utilize TLRs for sense pathogens, resulting in the production of many mediators contributing to the control of the infection [8,9]. Gingival fibroblasts constitutively express TLR2, TLR3, and TLR4 [10], and also the adhesion molecules ICAM-1 (CD54) and CD44 [11,12]. Activation of gingival fibroblast with microbial products induced the production of proinflammatory cytokines [13,14]. These findings indicate that these sensors molecules in gingival fibroblasts are important to recognize different microorganisms and regulate the immune response via specific cytokine production.

Fibroblasts are the principal type of cell found in the periodontal connective tissues presenting a unique phenotype according to their origin, being not only involved in tissue healing, but also with immune response [15]. Periodontal tissues are frequently injured by bacterial species that exist in complexes in subgingival tissue [16]. These bacterial complexes are considered the main etiological factor of periodontal diseases. Recently, the relation of *Candida albicans* with periodontal disease has also been investigated [17], and the association of subgingival colonization of *C. albicans* with severity of chronic periodontitis was established [18]. In recent years, the role for TLRs to sense *C. albicans* and other fungal species had been investigated [18].

Thus, we hypothesized that these cells will have a particular response during the fungal infection, resulting in a modulation of the cytokines via the recognition of TLR2, CD14, and MyD88 molecules.

2. Material and methods

2.1. Mice

C57BL/6 mice [wild-type (WT)], TLR2 (TLR2-KO), CD14 (CD14-KO), and MyD88-deficient (MyD88-KO) mice (6–8 weeks old) were obtained from Ribeirão Preto Medical School, University of São Paulo. Each mouse was housed in an isolated cage, and food and water were provided ad libitum. All experimental procedures involving animals in this study were reviewed and approved by the Animal Research Ethics Committee of the Bauru School of Dentistry, University of São Paulo (CEEPA-Proc. No 039/2009).

2.2. Candida albicans and growth conditions

C. albicans (ATCC 10231) was grown overnight to generate yeast cells in Sabouraud dextrose broth (Difco Becton, Dickinson and Company, Le Pont de Claix, France) at 29 °C. Cells were harvested by centrifugation, washed twice with PBS, and re-suspended in culture medium (RPMI 1640). Yeast cell morphology was confirmed using the Gram method and a stereomicroscope (MS 23358; Wild Heerbrugg, Romanshorn, Switzerland). Cell counts and concentrations were determined using a Neubauer chamber (Propper Manufacturing, Long Island City, NY, USA) as described [19]. *C. albicans* yeasts were heat-killed for 30 min at 95 °C.

2.3. Murine gingival fibroblast

Fibroblasts were isolated from gingival tissue, as described [20]. Gingival tissue was removed under aseptic conditions and minced with scissors in DMEM containing 10% fetal calf serum. Gingival tissue from a single animal was placed in 15 ml medium in tissue falcon 50 mL centrifuge tubes (Corning, New York, NY, USA). Cells were allowed to grow out of the minced tissue, and when cells reached 70% confluence, they were passaged following trypsinization. Fibroblasts was cultivated in 75 cm^2 flasks (Corning, New York, NY, USA) and maintained at 37 °C, under 5% CO^2, changing the culture medium every 2 days. Cells were grown for 14 days (3–4 passages) before being used.

2.4. Culture of gingival fibroblasts

Cells were obtained by explants and cultivated in DMEM (Gibco™) supplemented with 15% fetal calf serum (Gibco™), 100 UI/L penicillin (Gibco™), 100 μg/L streptomycin (Gibco™), and 250 ng/L amphotericin B (Gibco™), and they were used between the third and fourth passages for all the analysis in the current study. The cells were cultured in six-well plates (5×10^5 fibroblasts/well) or in eight-well Lab-Tek (Hatfield, PA, USA) plates (2×10^5 fibroblasts/well) for immunofluorescence staining. After overnight attachment, they were stimulated by live or heat killed *C. albicans* 5:1(E:T) and 10:1(E:T), *Escherichia coli* lipopolysaccharide (LPS; 100 ng/mL) (InvivoGen, San Diego, CA, USA) or with a synthetic ligand of TLR2, PAMP3CSK4 [1 μg/mL] (InvivoGen). After 24, 48, and 96 h, supernatants were collected and stored at −20 °C until used to measure cytokine levels and the cells analyzed by flow cytometry.

2.5. Flow cytometry analysis

For immunostaining, APC-, PerCP-, PE-, and FITC conjugated Abs anti-CD282 (TLR2), CD283 (TLR3), CD284 (TLR4), and isotype-matched control monoclonal antibodies were used (BD Biosciences). The intracellular detection of TLR3 in fibroblasts was performed using Cytoflx/Cytoperm and Perm/Wash buffer from BD Biosciences according to the manufacturer's instructions. Fibroblasts were acquired according to forward and side scatter parameters. Data acquisition was performed using a FACS Callibur™ (BD Immunocytometry Systems, Franklin Lakes, NJ, USA) and the data were analyzed using CellQuest software (BD Biosciences).

2.6. Immunofluorescence

All immunofluorescence staining was performed in eight-well chamber slides (Lab-Tek), as described [21]. Fibroblasts were fixed using acetone at −20 °C for 10 min. Fixed cells were washed with phosphate buffered saline (PBS) 1× and stained overnight at 4 °C with anti-type I collagen (Millipore Corporation, Billerica, MA, USA), anti-actin α-smooth muscle (Sigma-Aldrich, St. Louis, MO, USA) in PBS containing 1% (wt/vol) BSA, followed by 1 h with Alexa 488-labeled anti–mouse immunoglobulin G (Vector Laboratories, Burlingame, CA, USA), then 10 min of staining with 4′6-diamidino 2 phenylindole (DAPI) (Vector Laboratories). The antibodies were diluted in 1:200 phosphate-buffered saline (PBS). Subsequently, the slides were analyzed by confocal laser scanning microscopy TCS-SPE with 63× magnification (Leica Microsystems, Koblenz, Wetzlar, Germany).

2.7. Proliferation assay

Gingival fibroblasts were first labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) following the manufacturer's instruction (Invitrogen, Carlsbad, CA), as described previously [20]. Fibroblasts (5×10^5/well) were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. After 48 h and 96 h culture, the cells were collected using trypsin (Sigma-Aldrich), and analyzed using flow cytometry. Fibroblast proliferation was characterized by sequential halving of CFSE fluorescence, generating equally spaced peaks on a logarithmic scale. Data was analyzed using CellQuest software (BD Biosciences).

2.8. Cytokine detection

Murine IL-13, IL-6, IL-1β, TGF-β and TNF-α levels were measured in the supernatants of cultured gingival fibroblasts using ELISA kits (BD Biosciences), according to the manufacturer's instructions.

2.9. Statistical analysis

Statistical differences were detected using ANOVA to compare multiple groups. Values are presented as mean ± SD or mean ± SEM. P values ≤.05 were considered statistically significant. Calculations were performed using the Prism 6.0 software program (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Role of TLR2, MyD88, and CD14 in the collagen and α-actin deposition in primary gingival fibroblasts

Primary fibroblast was established from the gingival biopsy of WT, TLR2, MyD88, and CD14-deficient mice. After the second culture passage, all cells were positive for CD44 and CD54 and negative for HLA-DR and CD68 by flow cytometry (data not shown). Fibroblasts usually produce some components as type I collagen or α-actin, among others, which are responsible for extracellular matrix and collagen deposition. Hence, we wanted to determine how the expression of type 1 collagen and α-actin in gingival fibroblasts is derived from WT, TLR2, MyD88, and CD14-deficient mice (Fig. 1). Constitutive α-actin expression was observed in all groups of gingival fibroblasts cells, indicating that the absence of these molecules had no effect on the physiology of fibroblasts (Fig. 1). We found a strong basal collagen deposition in WT, TLR2, and MyD88-deficient gingival fibroblasts; however, the collagen deposition was attenuated in CD14-deficient fibroblasts (Fig. 1).

Next, we determined the effects of TLR2, MyD88, and CD14 absence on basal gingival fibroblasts proliferation (Fig. 2A). We found that the percentage of fibroblasts able to proliferate in the presence of *E. coli* LPS, PAMP3CSK4, and *C. albicans* was not affected by the deficiency of MyD88 and CD14 when compared with WT fibroblasts; however, TLR2-deficient fibroblasts presented a significant decreased cell proliferation ($P < .05$). In relation to the expression of innate immune receptors, control gingival fibroblast express TLR2, TLR3, and TLR4, whereas a small percentage of TLR2 and CD14-deficient cells expressed these innate receptors. Expression of TLR2 was not detected on fibroblasts from TLR2, MyD88, and CD14-deficient mice (Fig. 2B). We next questioned the basal cytokine-producing ability of TLR2, MyD88, and CD14-deficient gingival fibroblasts. Consistent with previous results, these cells produced TGF-β, IL-1β, TNF-α, and IL-6 even without simulation. Unexpectedly, cultured TLR2-deficient cells failed to produce TGF-β and IL-1β, whereas MyD88-deficient cells failed to produce IL-6 and TNF-α (Fig. 2C).

3.2. TLR expression on gingival fibroblasts after C. albicans stimulation

Upregulation of TLR expression by fibroblasts in response to different stimuli was reported [22]. To assess whether *C. albicans* can mediate TLR regulation in murine gingival fibroblasts, we next evaluated the effect of *C. albicans* and TLRs agonists on TLR2, TLR3, and TLR4 expression by gingival fibroblast. *C. albicans*, LPS, and Pam3C promoted an increased expression of TLR2 on WT gingival fibroblasts (Fig. 3). However, TLR agonists had no effect on these receptors' expression by TLR2, MyD88, and CD14-deficient cells (Fig. 3). Notably, *C. albicans* and Pam3C induced a remarkable increase of TLR3 expression on MyD88-deficient gingival fibroblasts (Fig. 3). TLR4 expression levels were lower than TLR2 and TLR3 levels and remained unchanged after TLRs agonist stimulation. Collectively, our data demonstrated that TLRs are differentially expressed by gingival fibroblasts and that the synthesis of these receptors could be modulated by *C. albicans*.

3.3. C. albicans stimulated gingival fibroblast to produce flammatory in cytokines

Because *C. albicans* is sensed by innate immune receptors and can induce several cytokines [23–25], possible changes in cytokine production were studied to investigate the effects of innate immune receptors on the cytokine production by gingival fibroblasts after *C. albicans* challenge.

C. albicans, LPS, and Pam3C induced an increase of TNF-α production by WT and MyD88-deficient gingival fibroblasts (Fig. 4). TLR agonists had no significant effects on TNF-α production by TLR2 and CD14-deficient cells (Fig. 4).

Comparable proportional increase of IL-6 in LPS or PamP3C-stimulated wild type, TLR2, MyD88 and CD14-deficient gingival fibroblast was seen (Fig. 4). *C. albicans* stimulation decreased the production of this cytokine by CD14 and TLR2-deficient gingival fibroblasts (Fig. 4). In addition, *C. albicans* stimulation induced weak increase in the production of these inflammatory cytokines by TLR2 and MyD88-deficient gingival fibroblasts (Fig. 4).

C. albicans and PamP3C also could stimulate MyD88-deficient gingival fibroblast to produce IL-13 (Fig. 4). However, no or minimal increase in IL-13 production was detected after WT, TLR2, and CD14-deficient gingival fibroblast stimulation with TLR agonists (Fig. 4). We showed that TLR ligands, particularly LPS and heat-killed *C. albicans*, could negatively modulate TGF-β production by WT, TLR2, and CD14-deficient gingival fibroblast (Fig. 4). Nonetheless, TLRs agonist merely induced weak increases in the production of these inflammatory cytokines by MyD88-deficient gingival fibroblast (Fig. 4). PamP3C induced a weak increase of TGF-β production by WT and CD14-deficient gingival fibroblasts (Fig. 4). Our results report an increase of IL-1β secretion by TLR2-deficient gingival fibroblasts challenged with *C. albicans* and LPS. Conversely, *C. albicans* decreased IL-1β production by WT, MyD88, and CD14-deficient fibroblasts. PamP3C induced a weak increase on IL-1β production by gingival fibroblasts (Fig. 4).

4. Discussion

The involvement of fibroblasts in the development of the immune responses in different tissues has already been published [26–28]; however, any study has been conducted concerning the recognition of *C. albicans* by gingival fibroblasts. As evidence supporting the important role of PRRs in the recognition of *C. albicans* for the induction of an immune response accumulates [29] and considering the importance of obtaining more information about the role of fibroblasts during the inflammatory immune response in gingival tissue, the aim of this study was to investigate the involvement of innate immune receptors and the adaptor protein MyD88 in the recognition of *C. albicans* by gingival fibroblast.

The results obtained in the current study show that the absence of TLR2 did not influence basal collagen deposition by gingival fibroblasts. Recently, it was shown that absence of TLR2 decreased the synthesis of type I collagen [30]. These differences among the studies could be explained by the fact that fibroblasts from different anatomic locations differ markedly in their gene expression patterns and show site-specific variations in their transcriptional profiles that seem to be related to their location within the body [31]. In

addition, our results show absence of TLR2 decreased basal proliferation by gingival fibroblast. TLRs have been shown to bind endogenous molecules present in human tissue in the absence of infection [32]. TLR2 has been identified to activate cells in response to heat-shock protein 70 [33] and necrotic cells [32,34]. Recent reports have shown that HSC70 released from the damaged cells may stimulate human gingival fibroblasts (HGFs) [35,36]. Considering this information, our data suggest that DAMPs can directly activate gingival fibroblast via TLR-2 and regulated cell activation. Further work investigating the mechanism by which gingival fibroblasts can sense DAMPS in damaged tissues is required to determine the mechanism of action. We also speculate that TLR2 down-regulation in basal proliferation could be modulated by cytokines [37]. In fact, our results indicate that absence of TLR2 decreased basal TGF-β and IL-1β production by gingival fibroblasts. TGF-β_1 is a key mediator of fibroblast activation and has a major influence on cell proliferation [38]. Therefore, we can conclude that the production of TGF-β_1 resulting in fibroblast proliferation can be dependent on TLR2. Recognition of the *C. albicans* cell wall antigens involves TLR2 and the absence of TLR2 signals increases susceptibility to candidiasis [39]. Release of TGF-β down-regulates activated monocytes and macrophages, suppressing gamma interferon (IFN-γ)-induced production of nitric oxide, which would favor the dissemination and progression of *C. albicans* infection [40]. The current data collectively demonstrate that TGF-β is an important determinant of the host response to systemic candidiasis.

In our study, recognition of *C. albicans* by gingival fibroblasts involves TLR2 and fungus induced an increase in the percentage of $TLR2^+$ fibroblasts by control cells. TLR2 upregulation in gingival fibroblasts after *C. albicans* recognition could be modulated by cytokines [40]. Various stimuli have been shown to regulate expression of TLRs in fibroblasts, including pathogen structures and TLR ligands, such as LPS and proinflammatory cytokines, including IL-1β, TNF-α, and IFN-γ [41,42]. Indeed, our results showed that the stimulation with *C. albicans* increased TNF-α production. More important, our results demonstrated that TGF-β production by gingival fibroblasts is dependent on TLR2 signals, even after *C. albicans* stimulation or DAMPs. TLR2 has been shown to play an important role in the inflammatory reaction induced by fungi [43]. For these reasons, an increase in the expression of TLR2 after *C. albicans* could have relevant clinical consequences, leading to the amplification of the cellular response mediated by TLR2 to yeast antigens in periodontal injuries.

We have observed decreased basal collagen deposition by CD14-deficient gingival fibroblast. CD14 exists as a glycosylphosphatidylinositol-anchored protein on the cell surface [44], which lacks a cytoplasmic signaling domain, making it incapable of downstream signaling [45]. TLR-4/CD14/MD2 or TLR2/CD14 complexes are necessary for receptor function [45]. CD14 is a receptor for LPS combined with LPS-binding protein [46]. Although TLR-4/CD14 complex is related to LPS recognition, this receptor is also involved with the recognition of lipoteichoic acid, heat-shock proteins, and the fusion protein of respiratory syncytial virus [47,48]. In addition, CD14 played a significant role in the activation of nuclear factor-kappa B in response to necrotic cells in the presence or absence of TLR2. DAMPs can trigger innate immune responses and provides an important homeostatic mechanism by which fibroblasts can sense and mount wound-repair responses

in damaged tissues [49]. Considering that gingival fibroblasts express innate immune receptors, it is possible that DAMPs can directly activate them via TLR-4/CD14/MD2 or TLR2/CD14 complexes [49]. In addition, the reduction in collagen deposition may reflect on the decreased levels of TGF-β observed in the current study. Thus, these results suggest that signals mediated by innate immune receptors might contribute to the type I collagen production by murine gingival fibroblasts.

It has also been suggested that TLR4/CD14 is involved in the recognition of *C. albicans* [50,51] and TLR4-defective C3H/HeJ mice have been reported to be more susceptible to disseminated candidiasis [50]. Our results show that TLR4 expression by gingival fibroblasts was negatively modulated by recognition of *C. albicans*. Downregulation of the TLR4 could be related with the internalization of the receptor together with fungus, resulting in decreased expression of TLR4 on the cell membrane, as observed after *Aspergillus fumigatus* recognition by PBMC cells [52]. Also, because TLR activation could regulate the expression of TLRs itself, it might be reasonable to suspect whether the down-regulation of TLR4 was caused by signals mediated through the recognition of this yeast by other types of TLR. Indeed, our results showed that the expression of TLR4 by TLR2-deficient cells is upregulated by *C. albicans*, which could indicate that signals mediated by TLR2 influence TLR4 expression by gingival fibroblasts [53,54]. Another factor that would control the expression of TLR4 by gingival fibroblasts after *C. albicans* recognition is the production of cytokines. In the current study, we did not evaluate the effects of immune-modulating cytokines on TLR expression. However, our results showed that *C. albicans* decrease TNF production by CD14-deficient cells. TNF-α expression can be up-regulated by TLR4-triggered signaling transduction [55]. TLR4/MyD88 signal transduction pathway participates in enhanced expression of IL-1 and TNF-α in patients with temporomandibular disorders [55]. TNF-α inhibits synthesis of structural components such as type I collagen and induces production of stromal collagenases [56]. These cytokines released by fibroblasts after recognition of *C. albicans* are important to the host defense against candidiasis [57]. Recognition of *C. albicans* by gingival fibroblast could drive a balanced proinflammatory cytokine production that collaborate to Th17 and/or Th1 protective immune responses and killing fungal pathogen [58].

With regard to the specific signaling pathways activated by these fungi, responses to *C. albicans* have recently been reported to occur in a MyD88-independent manner. Although mice lacking the TLR signaling adapter protein MyD88 are susceptible to fungal infection [59–62], the precise role of individual TLR receptors in combatting *Candida* infections is less clear. We demonstrated that absence of MyD88 decreased the basal expression of TLR2 and TLR4, and IL-6 and TNF-α production and did not interfere with collagen deposition by gingival fibroblasts, reflecting that collagen deposition is independent from this adaptor molecule (MyD88) [63]; thus, in this case, another TLR signaling pathway should be required.

The current study for the first time demonstrates that *C. albicans* can induce TLR3 expression by gingival fibroblasts in a MyD88-dependent manner. One study has shown an increase of TLR3 by endothelial cells after the stimulus with *Candida albicans* via a MyD88-dependent pathway [64], but nothing about gingival fibroblasts has been reported.

Remarkably, IL-13, TNF-α, and IL-6 production by *C. albicans* was detected in MyD88 deficiency, indicating that MyD88-independent pathway may contribute to their expression in gingival fibroblasts. TLRs axis initiates inflammatory cascades via two distinct pathways, one MyD88-dependent and the other via Toll/IL-1 receptor domain-containing adaptor inducing IFN-beta (TRIF)-dependent pathways. MyD88 adaptor is involved in transmitting signals from TLR and interleukin 1 receptor (IL-1R) family members (with the exception of TLR3) [65]; TRIF is recruited to TLR3 and TLR4 and activates an alternative pathway that triggers the activation of nuclear factor-kappa B, MAPKs, and IRF3 [66]. These signaling cascades lead to the production of pro-inflammatory cytokines, type I IFNs, and chemokines. The precise mechanism of *C. albicans*-induced cytokines production by gingival fibroblasts in MyD88-independent pathway remains to be elucidated.

In summary, our findings demonstrate that gingival fibroblasts expressed TLR2, TLR3, TLR4, ICAM-I, and CD44 and recognized *C. albicans* producing TNF-α and IL-13. Moreover, the production of IL-13 after stimulation with heat-killed *C. albicans* was dependent on TLR2, whereas the production of TNF-α was dependent on TLR2, TLR4/CD14, and MyD88. Therefore, our results demonstrated preliminary new insights into the influence of innate immune receptors and the adaptor protein MyD88 in the recognition of *C. albicans* by gingival fibroblast.

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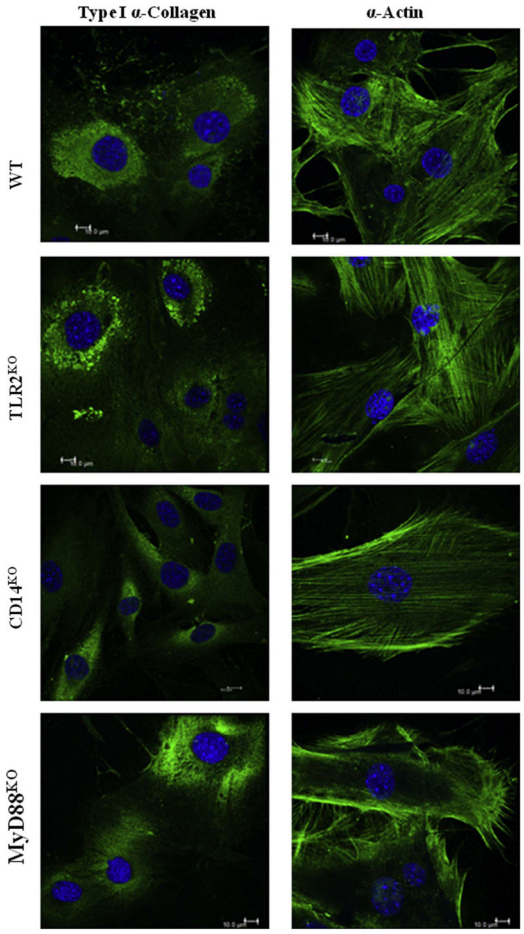

Fig. 1.

Representative immunofluorescence images of WT, TLR2, CD14, and MyD88-deficient fibroblasts. Localization collagen and α-actin in TLR2, MyD88, and CD14-deficient primary gingival fibroblasts. Primary fibroblasts were stained with anti-type I collagen (green; A, right panel) or anti-α-actin (green; B, left panel). Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI; blue). Scale bars = 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

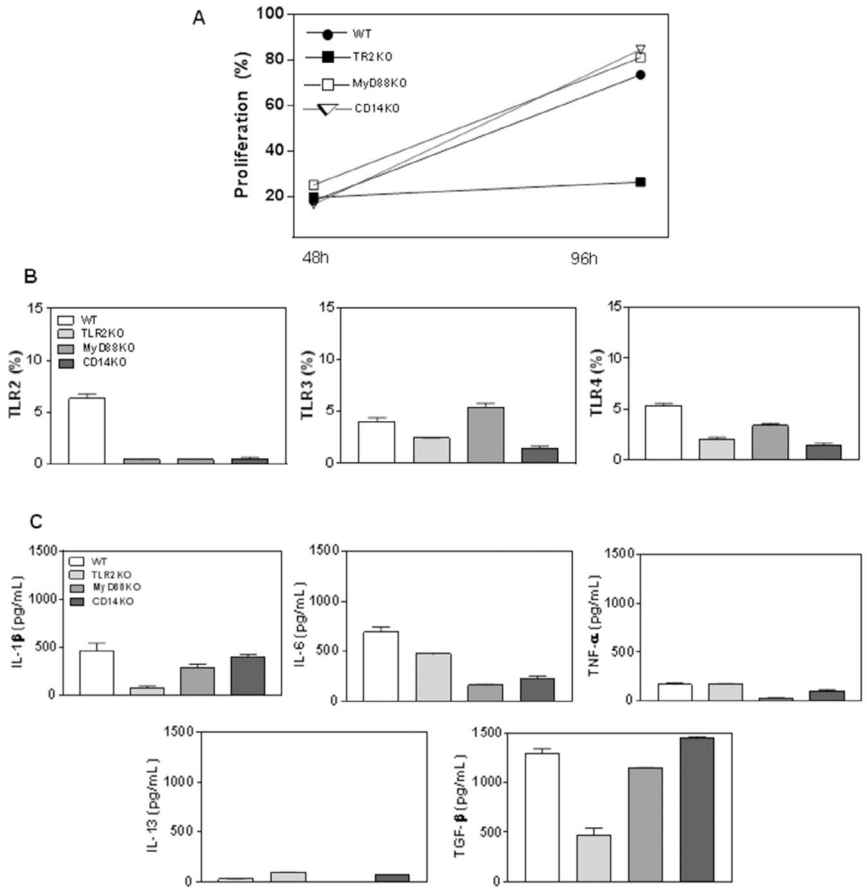

Fig. 2.

Characterization of TLR2, MyD88, and CD14-deficient gingival fibroblasts. (A) Primary fibroblast was established from the gingival biopsy of wild-type (WT), TLR2, MyD88, and CD14-deficient mice. After the second passage, cells were collected and marked with CFSE. After 48 and 96 h, the cells were collected and analyzed concerning the proliferative response by flow cytometry. (B) The expression of TLR2, TLR3, and TLR4 in gingival fibroblasts was analyzed by flow cytometry. (C) The primary fibroblast culture supernatants were collected for ELISA analyses of TNF-α, IL-6, IL-13, TGF-β and IL-1β. Data are representative of three independent experiments. The results were evaluated by ANOVA followed by Bonferroni test. *P < .05 vs. WT cells.

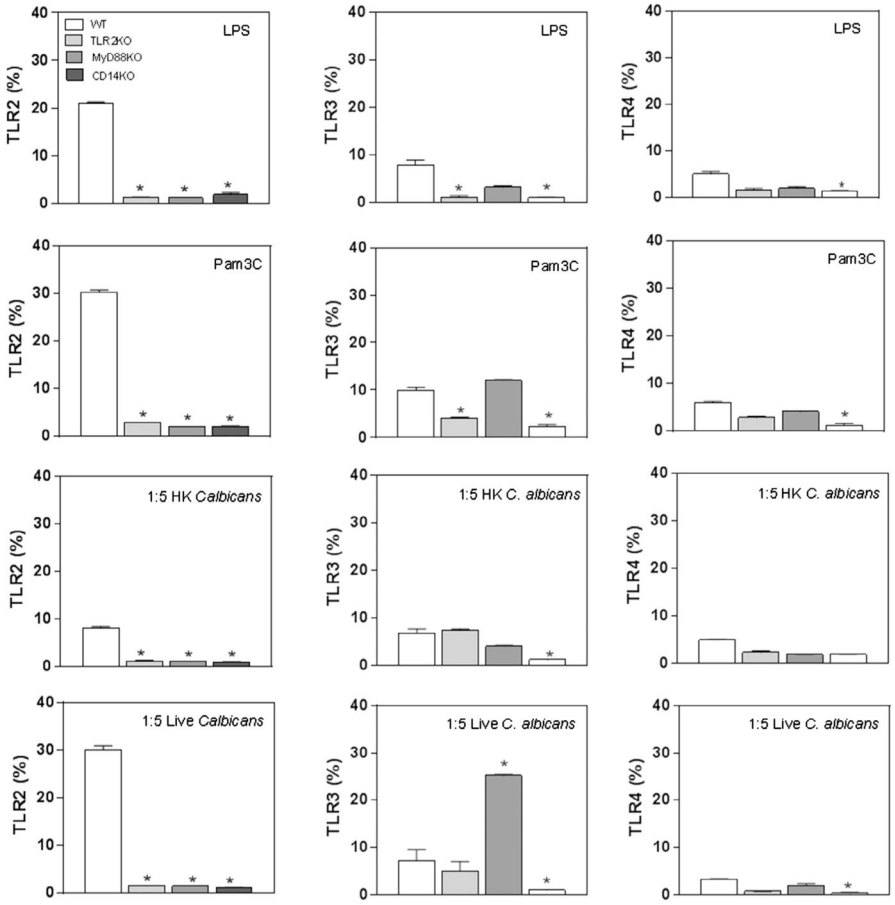

Fig. 3.

Innate immune receptors expression by TLR2, MyD88, and CD14-deficient gingival fibroblasts. The percentage of cells expressing TLR2, TLR3, and TLR4 was evaluated by flow cytometry. Data representative of three independent experiments. Results were evaluated by ANOVA test, followed by Bonferroni. *P < .05 vs. wild-type (WT) cells.

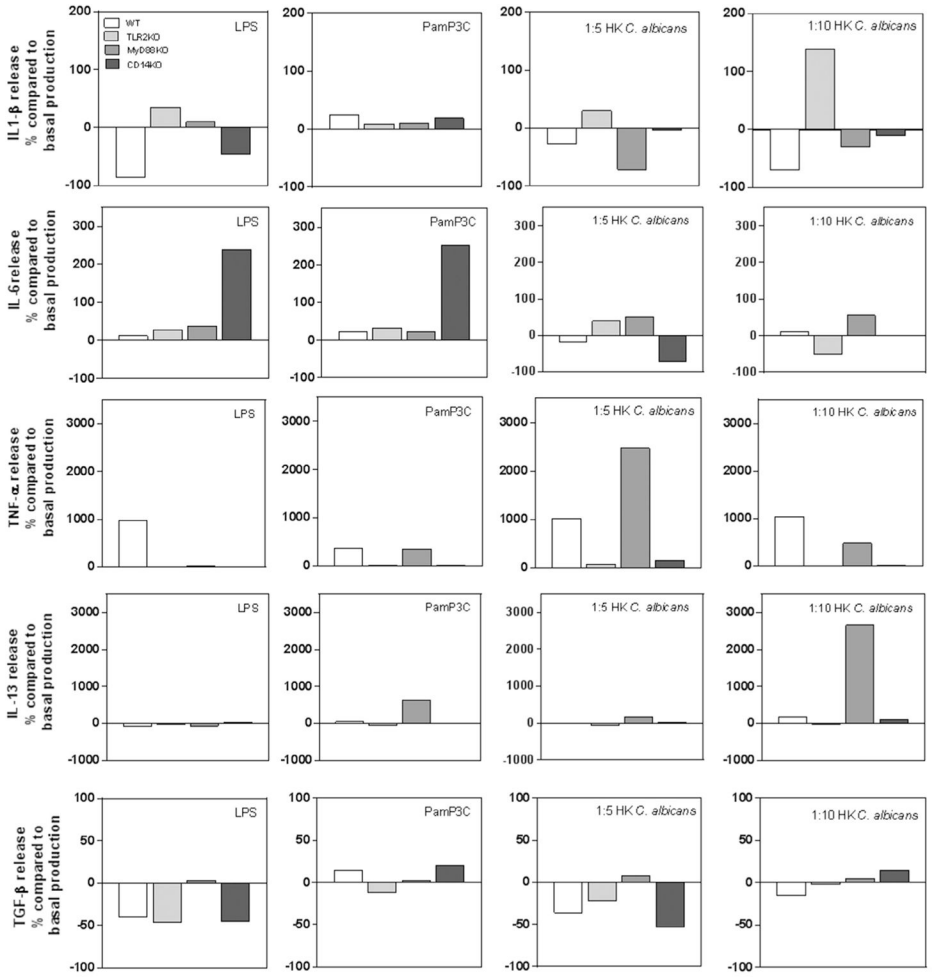

Fig. 4.

Candida albicans stimulated gingival fibroblast to produce inflammatory cytokines. The cell culture supernatants were collected for ELISA analyses of TNF-α, IL-6, IL-13, TGF-β and IL-1β. Data representative of three independent experiments. The results were evaluated by ANOVA followed by Bonferroni test. *P < .05 vs. wild-type (WT) cells.