AMERICAN SOCIETY FOR MICROBIOLOGY | Infection and Immunity®

HOST RESPONSE AND INFLAMMATION

Reduction of Articular and Systemic Inflammation by Kava-241 in a *Porphyromonas gingivalis*-Induced Arthritis Murine Model

Olivier Huck,[a,b] Jian You,[d] Xianxian Han,[d] Bin Cai,[c] James Panek,[c] Salomon Amar[d]

[a]INSERM (French National Institute of Health and Medical Research), UMR 1260, Regenerative Nanomedicine (RNM), FMTS, Strasbourg, France

[b]Université de Strasbourg, Faculté de Chirurgie Dentaire, Periodontology, Strasbourg, France

[c]Department of Chemistry, Boston University, Boston, Massachusetts, USA

[d]Department of Pharmacology, Department of Microbiology and Immunology, School of Medicine, New York Medical College, Valhalla, New York, USA

ABSTRACT Rheumatoid arthritis (RA) is an inflammatory disease that has been linked to several risk factors, including periodontitis. Identification of new anti-inflammatory compounds to treat arthritis is needed. We had previously demonstrated the beneficial effect of Kava-241, a kavain-derived compound, in the management of *Porphyromonas gingivalis*-induced periodontitis. The present study evaluated systemic and articular effects of Kava-241 in an infective arthritis murine model triggered by *P. gingivalis* bacterial inoculation and primed with a collagen antibody cocktail (CIA) to induce joint inflammation and tissular destruction. Clinical inflammation score and radiological analyses of the paws were performed continuously, while histological assessment was obtained at sacrifice. Mice exposed to *P. gingivalis* and a CIA cocktail and treated concomitantly with Kava-241 exhibited a reduced clinical inflammatory score and a decreased number of inflammatory cells and osteoclasts within joint. Kava-241 treatment also decreased significantly tumor necrosis factor alpha (TNF-α) in serum from mice injected with a Toll-like receptor 2 or 4 (TLR-2/4) ligand, *P. gingivalis*-lipopolysaccharide (LPS). Finally, bone marrow-derived macrophages infected with *P. gingivalis* and exposed to Kava-241 displayed reduced TLR-2/4, reduced mitogen-activated protein kinase (MAPK)-related signal elements, and reduced LPS-induced TNF-α factor (LITAF), all explaining the observed reduction of TNF-α secretion. Taken together, these results emphasized the novel properties of Kava-241 in the management of inflammatory conditions, especially TNF-α-related diseases such as infective RA.

KEYWORDS TNF, infection, inflammation, kavain, periodontitis

Rheumatoid arthritis (RA) is a systemic autoimmune disease that causes chronic inflammatory responses of the capsule around the joints and the development of fibrous tissue in the synovium. The development of the pathology leads to the destruction of articular cartilage, which is a consequence of sustained secretion of cytokines and cellular activation, especially of synovial macrophages (1). RA manifests by signs of inflammation, with the affected joints being swollen, painful, warm, and stiff. Clinically, RA is diagnosed based on the nine criteria listed according to the 2010 American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) classification (2). However, risk factors and the cellular and molecular mechanisms involved remain under investigation, with specific emphasis on the role of infectious pathogens. Interestingly, a role for periodontitis (PD) in aggravation of RA has been proposed, the two diseases sharing some common features (3).

PD is a chronic inflammatory disease of bacterial origin affecting tooth-supporting tissues. Progressive destruction of alveolar bone leads ultimately to tooth loss, with consequences on masticatory function, quality of life, and general health (4).

Received 17 May 2018 **Returned for modification** 4 June 2018 **Accepted** 11 June 2018

Accepted manuscript posted online 18 June 2018

Citation Huck O, You J, Han X, Cai B, Panek J, Amar S. 2018. Reduction of articular and systemic inflammation by Kava-241 in a *Porphyromonas gingivalis*-induced arthritis murine model. Infect Immun 86:e00356-18. https://doi.org/10.1128/IAI.00356-18.

Editor Marvin Whiteley, Georgia Institute of Technology School of Biological Sciences

Address correspondence to Salomon Amar, salomon_amar@nymc.edu.

Several studies described the association between PD and systemic chronic diseases such as cardiovascular diseases, diabetes, and RA (5–8). Recently, in a nationwide, population-based study in Taiwan, the risk of RA was higher in PD-affected patients (hazard ratio, 1.89; confidence interval [CI], 1.56 to 2.29) (5). Interestingly, some interventional trials concluded that nonsurgical treatment of PD led to improvements of markers of activity in RA patients, emphasizing the plausibility of the association between the two diseases (6).

In this context, special attention has been placed on the role of infectious agents, given that antibiotics had a beneficial effect in the management of certain cases of RA (7) and strategies to alter microbiome or induced immune activation are suggested for the treatment of RA (8). More specifically, *Porphyromonas gingivalis*, a purported periodontal pathogen, was proposed to play a key role in RA. *P. gingivalis*, a Gram-negative anaerobic bacterium, is frequently found in PD lesions and is associated with a dysbiotic flora (9). This bacterium is able to spread at a distance, and its DNA has been detected in synovial fluid from RA patients (10). Nevertheless, it is the only prokaryote expressing a peptidyl arginine deiminase (PAD) and able to induce protein citrullination (11). In patients with RA, a correlation between the presence of *P. gingivalis* and anti-citrullinated protein antibodies has already been observed (12). Its detrimental effect was also confirmed *in vivo* in several mouse models, in which *P. gingivalis* infection exacerbated collagen antibody (AB)-induced arthritis through different mechanisms, including modification of the gut microbiota and the associated autoimmune response (13, 14), neutrophil activation (15), or induction of bone destruction (16).

Control of inflammation is a key element of RA treatment, and several drugs targeting cytokines such as tumor necrosis factor alpha (TNF-α) or interleukin-6 (IL-6) are currently being used. However, side effects such as parenteral delivery and immune suppression and the high cost of this treatment warrant alternatives (17). Kavain, a compound extracted from the *Piper methysticum* plant, has been credited for its antiarthritic and anti-inflammatory properties (18, 19). Due to its potential toxicity, optimized compounds have been developed with interesting results regarding prevention or management of inflammation (20). Recently, we demonstrated that Kava-241, a synthetic kava analog, reduced TNF-α secretion in *Escherichia coli* lipopolysaccharide (LPS)-stimulated RAW cells but also prevented alveolar bone loss and inflammation associated with *P. gingivalis*-induced PD in a collagen antibody-primed mouse model (21).

Therefore, the aim of this study was to evaluate the potential anti-inflammatory effect of Kava-241 on arthritis development and joint inflammation in a *P. gingivalis*-stimulated collagen antibody-induced arthritis (CAIA) mouse model and determine its effect on molecular pathways upstream of TNF-α secretion.

RESULTS

***P. gingivalis* injection triggered AB-induced arthritis.** Following AB and *P. gingivalis* injections, the clinical arthritis score of mice was evaluated daily during 17 days. AB injections alone did not induce inflammatory clinical signs of the paws during the whole follow-up (Fig. 1). Interestingly, clinical signs of inflammation were detected in the AB + *P. gingivalis* group from day 11, reaching a plateau from days 14 to 17. These data show that *P. gingivalis* is an effective trigger exacerbating arthritis in AB-primed mice.

Kava-241 reduced joint swelling. To evaluate the potential therapeutic effect of Kava-241 on infective arthritis, AB + *P. gingivalis*-injected mice were treated concomitantly with the compound delivered intraperitoneally (40 mg/kg of body weight). This therapeutic intervention reduced the intensity of the clinical inflammation and delayed its detection (Fig. 1 and 2A), highlighting the anti-inflammatory properties of Kava-241. Radiographic findings were consistent with early-phase arthritis, represented by extensive soft tissue edema and joint space widening (Fig. 2B). This extensive tissue edema displayed classic radiographic images consistent with sausage-like fingers. The radio-

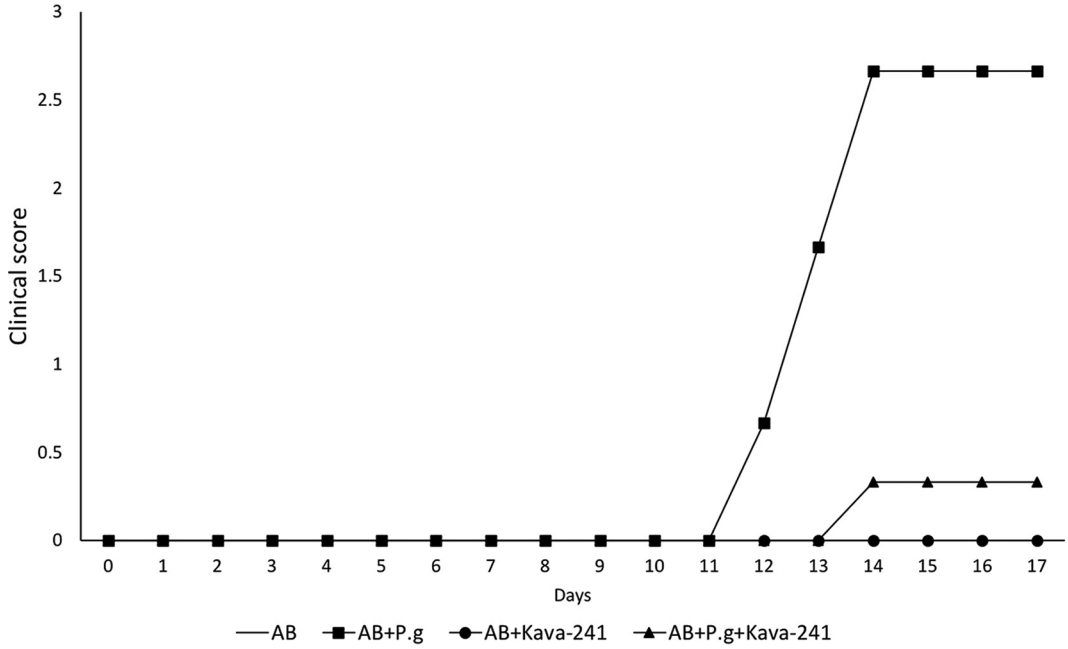

FIG 1 Clinical score of arthritis. Scores were evaluated daily in all 4 groups (AB injected; AB + *P. gingivalis* [P.g] injected; AB + Kava-241; AB + *P. gingivalis* + Kava-241) from day 1 to sacrifice (day 17).

graphic images exhibited joint space widening typical of joint effusion and/or synovial hypertrophy.

Kava-241 reduced inflammatory cells recruitment and osteoclast activation. Histological analysis was performed at the joint site to evaluate signs of inflammation, cartilage and bone destruction, and bone repair (Fig. 3 and 4). The AB + *P. gingivalis*-injected group was associated with the highest index score, reflecting intense infiltrate inflammatory cells, predominantly neutrophils, macrophages, and lymphocytes. Furthermore, signs of edema and synovial hyperplasia were clearly observed. The *P. gingivalis*-only-injected group displayed very mild inflammatory infiltrate and no edema or hyperplasia, while the AB-injected group had virtually no inflammation ($P < 0.05$). Regarding bone and cartilage destruction, the AB + *P. gingivalis* group exhibited a higher index score (high osteoclastic activity and cartilage destruction) than did the *P. gingivalis*-only-injected group ($P < 0.05$) (Fig. 4). No bone or cartilage destruction was observed in the AB-injected group. Interestingly, Kava-241 treatment reduced by about 30% the inflammatory infiltrate, by 60% the cartilage destruction, and by 35% the bone destruction induced by AB + *P. gingivalis* injection. Consistent with these results, Kava-241 treatment promoted bone repair ($P < 0.05$) (Fig. 3 and 4).

Kava-241 reduced systemic inflammation. To evaluate the effect of Kava-241 on systemic inflammation, mice were injected with *P. gingivalis*-LPS, and TNF-α secretion was measured in Kava-241-treated and untreated mice (Fig. 5). After 3 h, TNF-α concentration was significantly reduced (56%, $P < 0.05$) in the Kava-241-treated group. Similar results were observed at 6 h, demonstrating the ability of this compound to prevent systemic inflammation.

Kava-241 reduced TNF-α through decrease of TLR-4, TLR-2, MAPKs, and LITAF. To determine the molecular effects of Kava-241 on TNF-α secretion and upstream signal elements, Toll-like receptor 4 (TLR-4), TLR-2, mitogen-activated protein kinases (MAPKs), and LPS-induced TNF-α factor (LITAF) were evaluated in *P. gingivalis*-infected bone marrow macrophages (BMMs). As expected, *P. gingivalis* increased the TNF-α secretion (Fig. 6)-related cascade including TLR-2 and phosphorylated forms of MAPKs expression, including extracellular signal-regulated kinase (ERK), Jun N-terminal protein kinase (JNK), and AKT (Fig. 7). Kava-241 treatment of *P. gingivalis*-infected BMMs

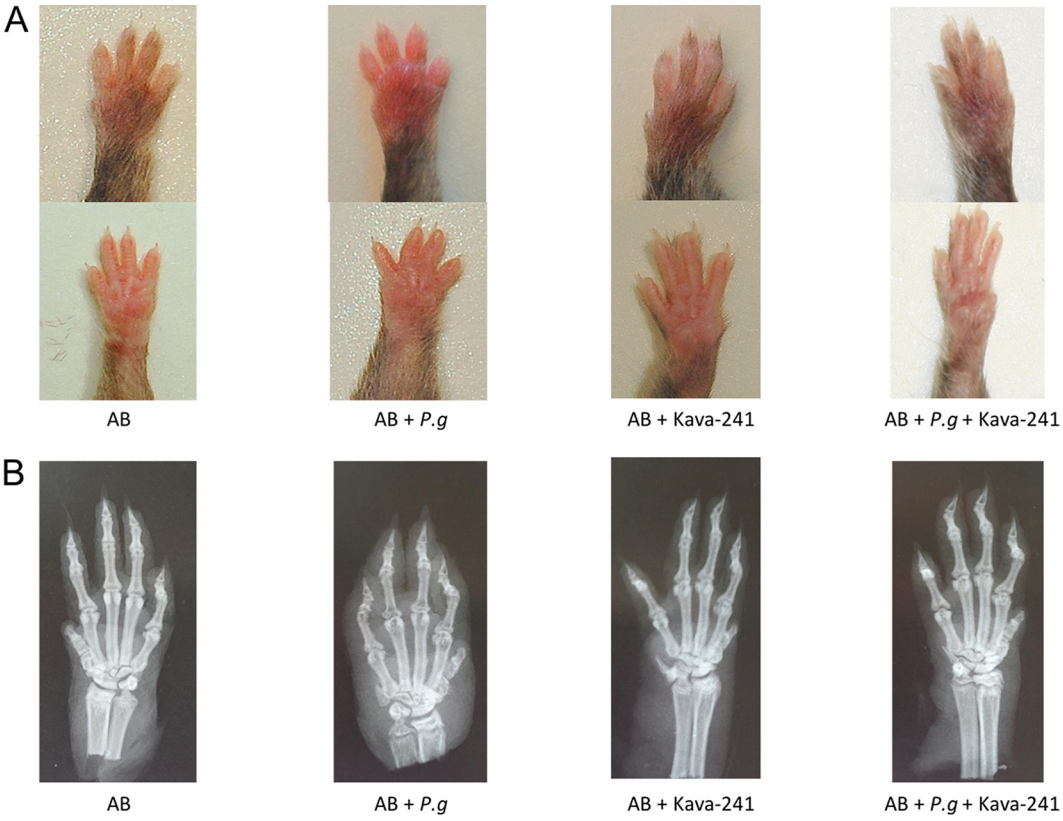

FIG 2 Clinical examination of articular inflammation at forepaws and joints. (A) Paw inflammation. Paws (dorsal/palm) were examined daily in all groups (AB injected; AB + *P. gingivalis* injected; AB + Kava-241; AB + *P. gingivalis* + Kava-241). Pictures were taken on the day of sacrifice. Note the swollen paws in the AB + *P. gingivalis* group and the significant reduction of swelling in the AB + *P. gingivalis* + Kava-241 group. (B) X-ray evaluation of infective arthritis. Radiographic findings were consistent with early-phase arthritis represented by extensive soft tissue edema and joint space widening. Radiographic images in the AB + *P. gingivalis* group show sausage-like fingers with joint space widening typical of joint effusion and/or synovial hypertrophy. Note the disappearance of the sausage-like fingers and lack of widening of joint space in the AB + *P. gingivalis* + Kava-241 group.

reduced TNF-α secretion in a dose-dependent manner (40% decrease for 20 μg/ml, 70% for 100 μg/ml, 90% for 200 μg/ml) (Fig. 6A). Treatment with Kava-241 modulated significantly TLR-2/4-associated signal pathway elements at multiple levels of the cascade. Kava-241 reduced TLR-4, TLR-2, and phosphorylated MAPK expression in *P. gingivalis*-infected BMMs (Fig. 7). Interestingly, Kava-241 decreased significantly all the tested phosphorylated proteins, emphasizing its potential to inhibit kinases activation.

Interestingly, LITAF expression was reduced by Kava-241 treatment in *P. gingivalis*-infected BMMs, possibly resulting in reduced TNF-α expression, hence explaining the reduced articular and systemic inflammation.

DISCUSSION

In this study, we demonstrated the potential therapeutic properties of Kava-241, a kava-derived compound, in the context of infective arthritis and systemic inflammation management. Here, we showed that Kava-241 significantly reduced TNF-α secretion through modulation of the TLR-4/TLR-2-associated cascade and decreased significantly phosphorylation of MAPKs such as ERK1/2, JNK, AKT, and p38.

Periodontal pathogens, especially *P. gingivalis*, have been associated with arthritis exacerbation through increase of the inflammatory and autoimmune responses. In mouse models, this detrimental effect of periodontal pathogens on arthritis has been described as more-severe symptoms of arthritis in animals primed by collagen antibodies and infected by one or several periodontal pathogens (16). The methods used to infect animals with periodontal pathogens and to induce infective arthritis seem to

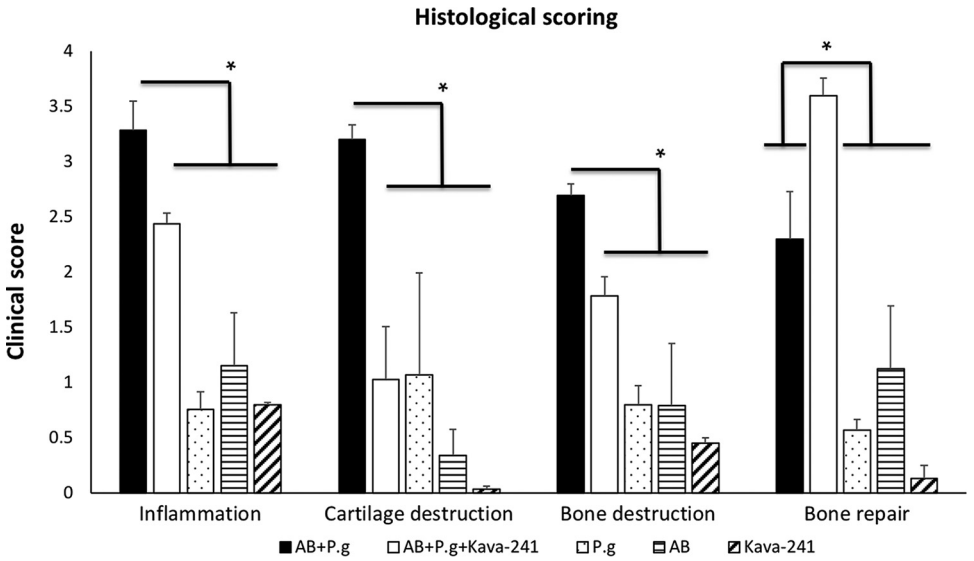

FIG 3 Histological analysis of arthritis. Levels of inflammation, cartilage destruction, and bone destruction and repair were evaluated on histological sections of phalangeal joint in each group (AB injected; AB + *P. gingivalis* injected; AB + Kava-241; AB + *P. gingivalis* + Kava-241) (7 mice/group); *, $P < 0.05$. Note that Kava-241 treatment reduced by about 30% the inflammatory infiltrate, by 60% the cartilage destruction, and by 35% the bone destruction induced by AB + *P. gingivalis* injection. Furthermore, Kava-241 treatment promoted bone repair ($P < 0.05$).

influence the time needed to be able to observe clinical signs (15, 16, 22). In this study, we decided to evaluate the effect of an acute infection with *P. gingivalis* through repeated intraperitoneal injections of *P. gingivalis* strain 381. Such a procedure amplified significantly the arthritic effect of AB injection, inducing significant swelling of the

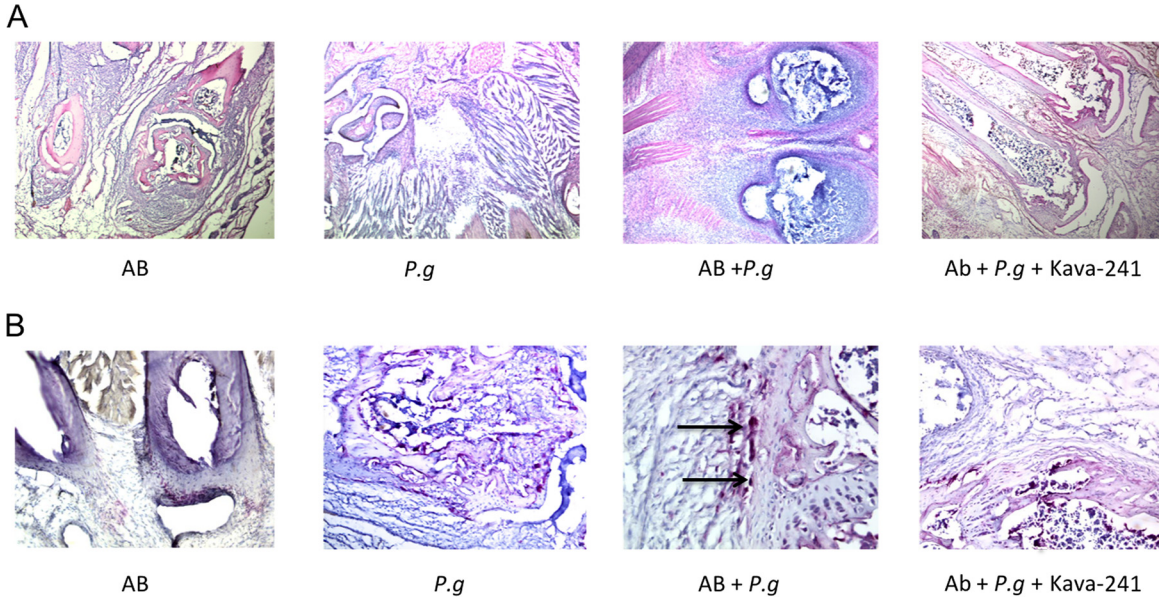

FIG 4 (A) Histological views. H&E staining for inflammation. (B) TRAP staining (arrows show TRAP-stained multinuclear cells). Histological sections performed at the joint site are representative of each group (AB injected; AB + *P. gingivalis* injected; AB + Kava-241; AB + *P. gingivalis* + Kava-241) (7 mice/group). Note the absence of inflammation and TRAP-stained multinuclear cells in AB-treated animals or *P. gingivalis*-only-treated animals. Note that the AB + *P. gingivalis*-injected group was associated with an intense infiltrate of inflammatory cells, predominantly neutrophils, macrophages, and lymphocytes. Furthermore, signs of edema and synovial hyperplasia were clearly observed. The *P. gingivalis*-only-injected group displayed very mild inflammatory infiltrate and no edema or hyperplasia, while the AB-injected group had virtually no inflammation ($P < 0.05$). Regarding bone and cartilage destruction, the AB + *P. gingivalis* group exhibited a higher osteoclastic activity and cartilage destruction than did the *P. gingivalis*-only-injected group ($P < 0.05$). No bone or cartilage destruction was observed in the AB-injected group (magnification, ×100).

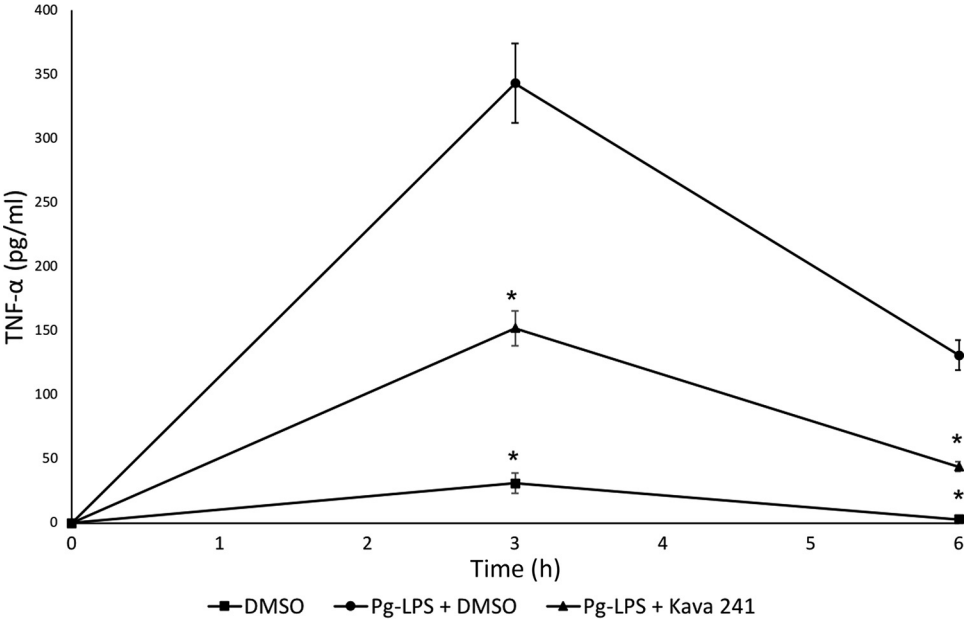

FIG 5 TNF-α concentration in *P. gingivalis*-LPS-stimulated mice. *P. gingivalis*-LPS (50 μg/100 μl) was injected intraperitoneally. Thirty minutes later, mice were treated with DMSO (control; 25 μl/600 μl) or Kava-241 (40 mg/kg/600 μl). Sera were taken at 3 and 6 h, and the TNF-α concentration was evaluated with ELISA. Results are presented as means $\pm$ standard deviations (SD). *, $P < 0.05$.

paws, with a peak reaching a plateau after 13 days and histological modifications at joint sites confirming the contributive pathogenic role of *P. gingivalis* in RA. *P. gingivalis* has been detected in synovial fluid (23) and in synovial tissue (24). *P. gingivalis* is an invasive bacterium, and its presence within joint contributes to increased inflammatory cell infiltration and tissular destruction through exacerbation of the autoimmune response (16). The observed destruction was corroborated with radiographic findings; however, it should be stated that these signs could not be considered specific and can be seen in different types of arthritis during the early phase. In animals subjected to bacterial injections, these findings may represent septic arthritis (which may be confirmed by culture of synovial fluid or the identification of live bacteria and polymorphonuclear leukocytes in the joint space). Alternatively, these findings may correspond

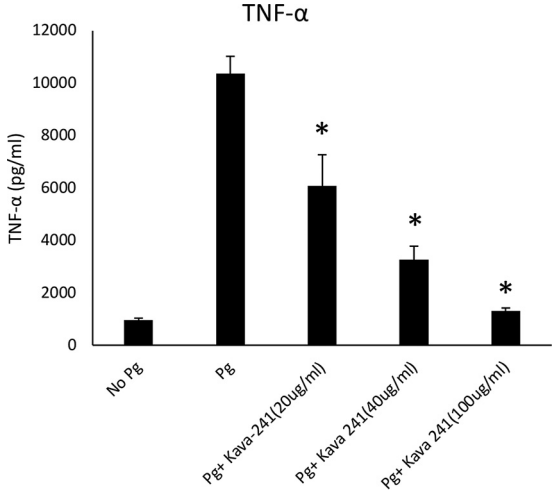

FIG 6 TNF-α secretion in BMMs after *P. gingivalis* infection and Kava-241 treatment. *, $P < 0.05$. Dose response of Kava-241 treatment shows significant reduction of TNF-α secretion in BMMs after *P. gingivalis* infection.

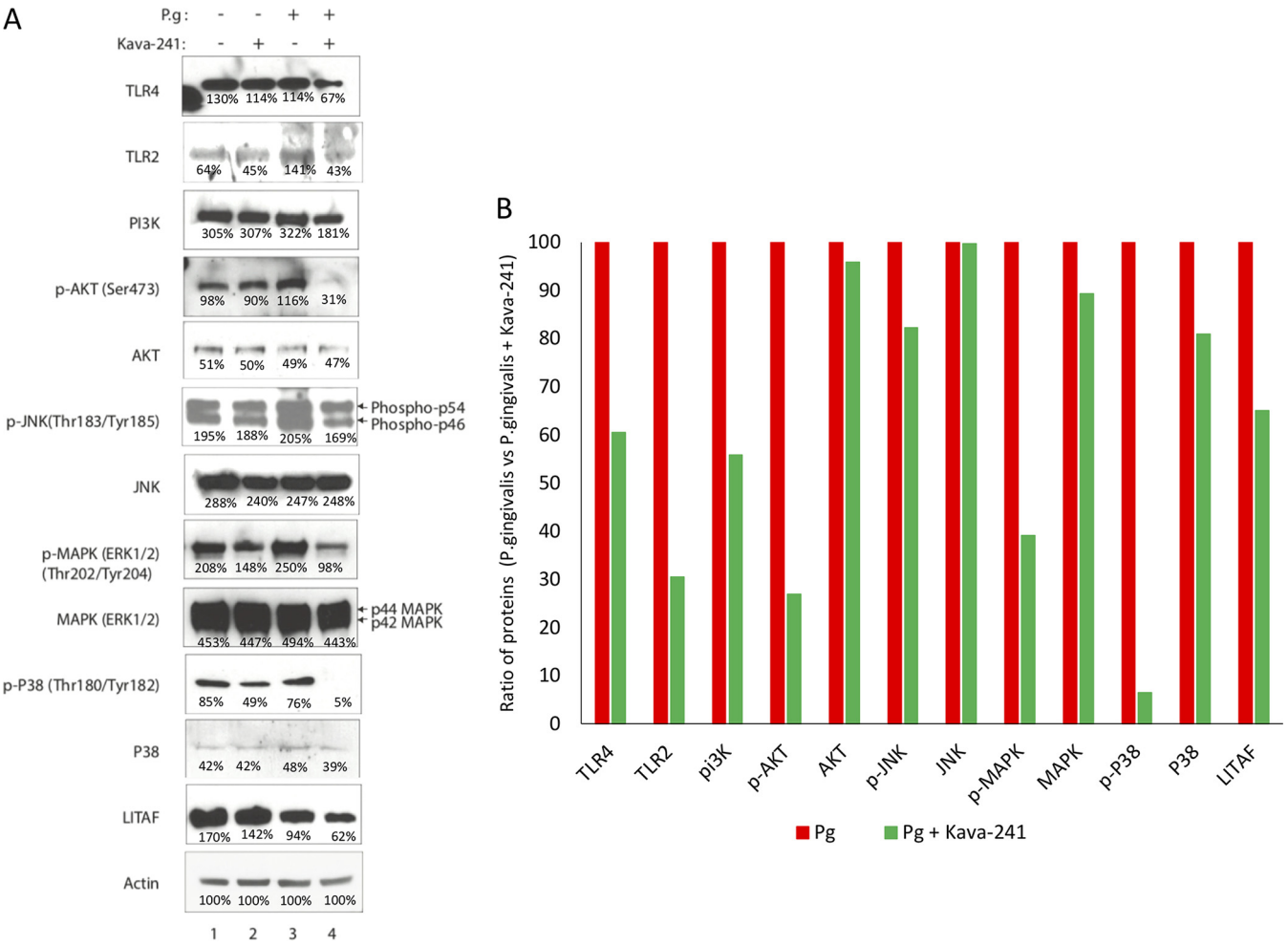

FIG 7 Modulation of MAPK-related proteins in *P. gingivalis*-infected BMMs by Kava-241. (A) Western blots of TLR4/2, MAPKs and LITAF. Significant reduction of all the proteins tested after Kava-241 treatment in *P. gingivalis*-infected cells can be observed. Percentages represent the ratios of protein versus actin in each sample. (B) Protein expression in *P. gingivalis*-infected and *P. gingivalis* + Kava-241-treated cells.

to reactive or immune-mediated arthritis as determined by a longer follow-up of the disease course and response to immune-modulation therapy.

It should be emphasized that arthritis in CIA mouse models is strain dependent. The rationale for using strain 381 in this study was based on the facts that it was recovered in clinical samples from patients suffering from both diseases (25) and that previous studies have demonstrated that strain 381 expresses common outer membrane proteins (26); in addition, our laboratory has significant experience using this strain. This may not be the only viable approach, as it was demonstrated that *P. gingivalis* strain W83, a strain lacking fimbriae, displayed arthritogenic properties even before immunization while strain 2561, a strain having several types of fimbriae on its membrane, did not. This strain-specific effect could be explained by differences in cell adhesion properties stemming from environmental factors or in induction of protein citrullination (27).

Several underlying mechanisms contributing to the association between PD and RA have been identified, highlighting a role for bacterial recognition and activation of inflammatory pathways leading to cytokine secretion. *P. gingivalis* is able to directly activate several cell types, including macrophages, fibroblasts, or chondrocytes, contributing to the sustained inflammation and cartilage destruction (21, 28, 29). For instance, modulation of TLR-2 and -4 mediates the inflammatory response to *P. gingivalis* expression (30), leading to an increase of TNF-α, IL-1, IL-6, or IL-17 at the RA

lesion site (31). An increase of cytokine levels, especially TNF-α, is correlated to activation of matrix metalloproteinases (MMPs), which play a critical role in cartilage destruction in RA (32). Nevertheless, *P. gingivalis*-associated PD induces a chronic systemic inflammation that is considered a contributing factor of RA (33). Systemic TNF-α increase, which could be induced by acute or chronic infectious and inflammatory diseases such as PD, has been proposed as one of the mechanisms explaining the PD-RA association (3). For instance, it has been observed in RA patients that PD was associated with high levels of TNF-α (34); therefore, its reduction during RA management is aimed.

Kavain has been demonstrated to be an effective anti-inflammatory property as evidenced by reduction of TNF-α (35, 36), a cytokine implicated in both PD and RA and targeted in RA management (37). Recently, we demonstrated the promising anti-inflammatory effect of Kava-241 that was associated with significant reduction of the inflammation, inflammatory cell recruitment, and alveolar bone loss induced by *P. gingivalis* at the periodontal level (21). Here, we demonstrate that Kava-241 strongly decreased the development of infective arthritis while reducing paw swelling, joint inflammation, and bone and cartilage destruction and promoted bone repair. Interestingly, at the systemic level, Kava-241 was able to reduce the secretion of TNF-α induced by *P. gingivalis*-LPS, illustrating its promising systemic anti-inflammatory properties.

To investigate the anti-inflammatory effect of Kava-241, we focused on the pathway associated with TNF-α secretion as alluded to by the parent molecule kavain in LPS-stimulated macrophages (35). Macrophages are key cells in the inflammatory process associated with RA (38). Here, treatment with Kava-241 reduced significantly TNF-α secretion induced by *P. gingivalis* infection. Interestingly, this effect was associated with reduced expression of TLRs and to a decrease of phosphorylated ERK, JNK, MAPK, AKT, and p38. These data confirm those described for the flavokawain A, the predominant kava chalcone for which reduction of cytokine secretion was associated with the modulation of the same pathway (39). TLR-2 and TLR-4 are highly expressed in RA synovial tissue-lining macrophages. They play a central role in Gram-negative infections and have a key role in the recognition of pathogen-associated molecular patterns (PAMPs) such as LPS but also of endogenous molecules released during necrosis or cellular stress (40). The inhibition of TLR-2/4 and targeting of their downstream pathways are currently being considered as an innovative therapeutic strategy for RA (41). The evidence of decreased TLR-2 and TLR-4 expression caused by Kava-241 could be considered a novelty, as this specific effect was not described previously.

The cytokine secretion induced by bacterial by-products such as LPS in macrophages is regulated mainly by MAPKs such as JNK, p38, and ERK (42). Here, Kava-241 reduced significantly phosphorylated protein levels induced by the infection (Fig. 8). Phosphorylation is associated with kinase activation. Therefore, its inhibition leads to blockade of the kinase cascade contributing to NF-κB activation and TNF-α secretion. The regulation of JNK phosphorylation is of importance, as JNK is upregulated in RA and is a potential mechanism of excessive extracellular matrix degradation and its deficiency protected mice in an animal model of arthritis (43).

p38 is expressed in the inflamed joints of patients with RA, and its activation is observed in animal models of the disease, in macrophages and fibroblasts of the synovial lining layer, and at sites of bone erosion. It contributes to the overexpression of proinflammatory cytokines, chemokines, MMPs, and enzymes such as COX-2 in the inflamed synovium. Phosphorylated p38 is also detected in the vascular endothelial cells of the blood vessels infiltrating the synovium, where it regulates the expression of genes encoding cell adhesion molecules (44). It has been considered a potential target in the treatment of RA; however, several clinical trials were ineffective due to the high complexity of its regulation. Here, Kava-241 reduced significantly the phosphorylation of p38, emphasizing its potential use as a therapeutic compound in the treatment of RA.

ERK is a well-described mitogen-activated protein kinase (MAPK) expressed in synovial tissues and synovial fibroblasts during RA (45) and is implicated in the TNF-α-mediated IL-8 secretion (46). Due to its upstream regulation role on NF-κB and

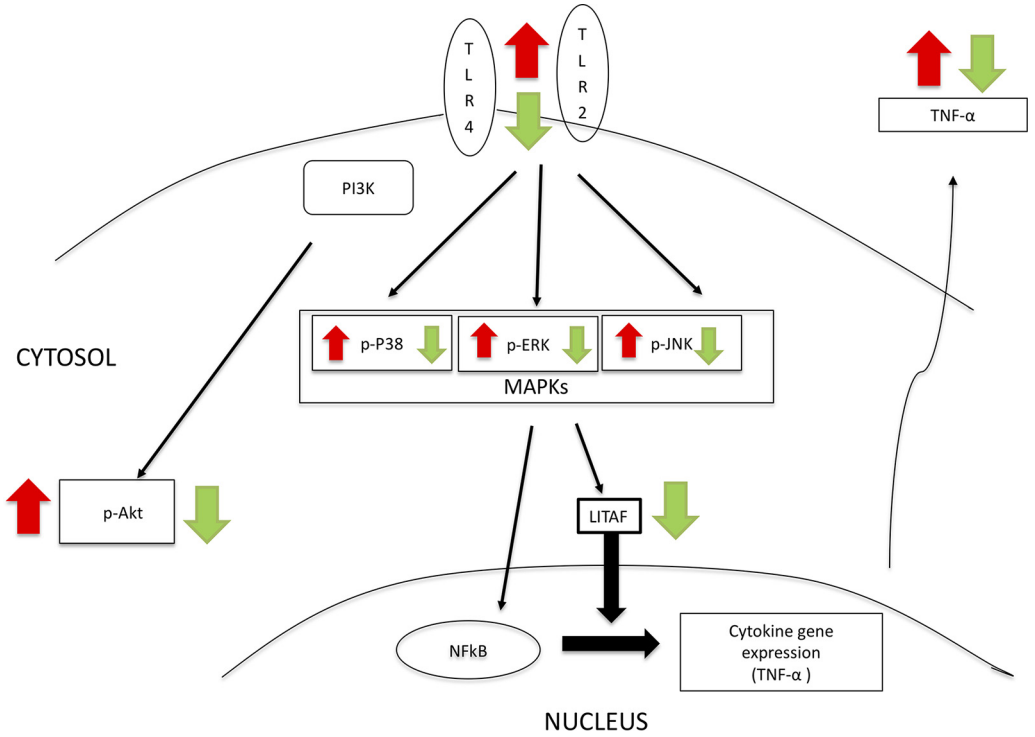

FIG 8 Schematic representation of Kava-241-induced effects on MAPKs and LITAF involved in TNF-α secretion. Red arrows represent the effects induced by *P. gingivalis* infection at the protein level. Green arrows represent the effects associated with Kava-241 treatment in BMMs infected by *P. gingivalis* at the protein level. *P. gingivalis* infection increased significantly TNF-α secretion after activation of TLRs and p-MAPKs. Conversely, Kava-241 counteracted these effects by blocking TNF-α secretion.

TNF-α release, decrease of phosphorylated ERK is a recurrent target for new pharmaceutical drugs or compounds (47).

Interestingly, the Kava-241 anti-inflammatory effect was associated with a reduced expression of LITAF and phosphorylated ERK. LITAF is a key regulator of TNF-α expression induced by LPS, and the LITAF pathway is activated after TLR-2/4-mediated signal-inducing phosphorylation of LITAF by kinases such as p38 (35). Phosphorylated LITAF binds to STAT6 before translocation to the nucleus, where it activates proinflammatory gene expression, including TNF-α (48), and has already been identified as a potential therapeutic target in inflammatory diseases (49). LITAF was found to be associated with arthritis development in a similar mouse model involving AB and LPS, and its genetic ablation was associated with improvement of the LPS arthritis (50). The present data highlight the similarity of signal transduction changes between native kavain and Kava-241. Indeed, we previously reported that native kavain reduced cytokine secretion through the modulation of the ERK2-LITAF pathway (35). Therefore, the reduction of LITAF by Kava-241 unveils some novel anti-inflammatory properties of this compound and may open potential therapeutic targets, since LITAF was already associated with several inflammatory diseases (48, 50–52) and targeted by some drugs, such as minocycline (49).

Nevertheless, the activation of the phosphatidylinositol 3-kinase (PI3K)/AKT pathway is also of importance in the development of RA, contributing to the expression of a variety of proinflammatory, proliferative, and antiapoptotic molecules leading to sustained inflammation but also synovial hyperplasia (53). Here, *P. gingivalis* infection increased the AKT level. This effect was totally counteracted by Kava-241 treatment. Targeting AKT has already been proposed to inhibit the inflammatory response in RA, emphasizing the interesting properties of Kava-241 (54).

Control of inflammation is one of the main goals of RA treatments, and drugs targeting TNF-α are under development, as current treatment may be related to severe

side effects. Kava-241 may be a good candidate to treat inflammatory diseases, as it displays a reduced cytotoxicity and strong anti-inflammatory properties. Therefore, future studies should focus on optimization of the mode of delivery to determine precisely its pharmacokinetic properties *in vivo* to allow its evaluation at the human clinical level.

MATERIALS AND METHODS

Bacterial culture. *P. gingivalis* strain 381 (ATCC, Manassas, VA, USA) was cultured in Schaedler anaerobe broth (Oxoid Ltd., Basingstoke, Hampshire, England), supplemented with hemin (5 μg/ml; Sigma-Aldrich, St. Louis, MO), menadione (1 μg/ml; Sigma-Aldrich, St. Louis, MO), and sodium bicarbonate (420 μg/ml; Sigma-Aldrich, St. Louis, MO) in an anaerobic chamber with 85% N_2, 10% H_2, and 5% CO_2 at 37°C.

Mice. Twenty-eight 6-week-old, pathogen-free DBA1/BO male mice (Taconic Farm, Rensselaer, NY) were used in this study. Mice were fed sterile food and distilled water *ad libitum*. All procedures were approved by the local ethical committee. Mice were randomly assigned to one of the following experimental groups: (i) an AB-injected group, (ii) an AB + *P. gingivalis*-injected group, (iii) an AB-injected and Kava-241-treated group, and (iv) an AB + *P. gingivalis*-injected and Kava-241-treated group.

Induction of arthritis and scoring. Arthritis was induced by two consecutive injections of ArthritoMab (AB) antibody cocktail (CIA-MAB-2C; MD Bioproducts, Oakdale, MN, USA). A 7-mg dose was injected at baseline, and a second injection (of 4 mg) was done at day 5. For *P. gingivalis*-injected groups, 3 intraperitoneal injections of 5×10^8 bacteria/100 μl were performed at days 2, 8, and 11. Mice were sacrificed after 17 days.

Forepaws were evaluated to score arthritis using a visual qualitative assessment scoring as follows: 0, no paw swelling; 1, mild swelling; 2, moderate swelling; 3, severe swelling (50). Inflammation was also evaluated with X-ray examination.

Injection of Kava-241 compound. Kava-241 is a compound derived from kavain and has been synthesized as described previously (21). In Kava-241-treated groups, 7 intraperitoneal injections (40 mg/kg) were performed. The first injection was performed 3 days before the first AB injection and at days 1, 3, 7, 8, 9, and 10.

***P. gingivalis*-LPS injection.** A 50-μg/100 μl dose of ultrapure *P. gingivalis*-LPS (Invivogen, CA, USA) was injected intraperitoneally. After 30 min, 600 μl of Kava-241 (40 mg/kg) in phosphate-buffered saline (PBS) was injected. As a control, the same volume of dimethyl sulfoxide (DMSO) as that used to dissolve Kava-241 powder was used. Serum was collected at 0, 3, and 6 h.

Tissue preparation. After 17 days of experimentation, mice were euthanized by CO_2 inhalation. Phalangeal joint and intact surrounding tissues were fixed with 4% freshly prepared paraformaldehyde (Sigma-Aldrich, St. Louis, MO) in PBS (pH 7.2) for 24 h at 4°C. Following fixation, specimens were consecutively washed with 5%, 10%, and 15% glycerol (American Bioanalytical, Natick, MA) in PBS, each for 15 min at 4°C, and decalcified in an EDTA tetrasodium salt dihydrate solution (Sigma-Aldrich, St. Louis, MO) for 14 days at 4°C. Samples were then immersed in 30% sucrose (Sigma-Aldrich, St. Louis, MO) in PBS until embedding. The tissue block was embedded with a Histoprep compound (Fisher Scientific, Hanover Park, IL) for cryostat sectioning. Serial mesiodistal sections (5 μm) parallel to the long axis of the phalangeal joint were made and stained with hematoxylin (HE; Fisher Scientific, Hanover Park, IL) and eosin (Acros Organics, Morris Plains, NJ).

TRAP staining. Osteoclasts were detected by TRAP staining. Five-micrometer-thick histological slides were exposed to the TRAP solution, containing N,N-dimethylformamide (EM Science), 3.7 mM fast red violet LB di-azonium salt (Sigma), 6.4 mM tartaric acid (Sigma), and 0.4% $MgCl_2$ in 0.2 M sodium acetate buffer (pH 5.0) for 10 min at 37°C. The slides were then washed for 30 min before being counterstained with HE for 5 s. Osteoclasts were identified as being positively stained for TRAP and possessing a ruffled border with an underlying lacuna in contact with bone.

Histological scoring. Samples were scored for inflammation, bone destruction, bone formation/repair, and cartilage destruction. To score inflammation, a scale of 0 to 4 was used, with 0 corresponding to no signs of inflammation, 1 to mild infiltration of inflammatory cells, 2 to mild inflammation with small hyperplasia in the synovial lining layer, 3 to synovial edema, hyperplasia, and more pronounced inflammation, and 4 to severe synovial hyperplasia and cellular infiltration. Bone destruction was scored using a scale of 0 to 4, with 0 corresponding to no signs of bone destruction, 1 to osteoclast activation, 2 to presence of some osteoclasts lacunas, 3 to presence of many osteoclasts lacunas and signs of bone resorption, and 4 to severe bone resorption and erosion. Bone formation/repair was evaluated as follows: 0 for intact bone with some activated mature chrondrocytes in the nonarticular surface, 1 when alkaline phosphatase activity was detected in the phalangeal area, 2 for osteoblast activity in the trabecular epiphyseal area, 3 for strong osteoblast activity in all trabecular bone, 3.5 for osteoblast activity in cortical bone, and 4 for strong osteoblast activity in cortical bone. Finally, cartilage destruction was evaluated on a scale of 0 to 4 according to the intensity of cartilage staining.

Mouse macrophage isolation and infection. Bone marrow macrophages (BMMs) were harvested as previously described (55) and cultured during 7 days in RPMI medium supplemented with L929 and 10,000 U/liter penicillin, 100 mg/liter streptomycin, and 10% fetal bovine serum in a humidified atmosphere (5% CO_2) at 37°C. According to the experimental design, cells were infected by *P. gingivalis* (multiplicity of infection [MOI], 20) and/or treated with 50 to 200 μg ml^{-1} of synthesized Kava-241.

Western blotting. Cells were harvested and washed twice with PBS. Proteins from the whole cells were purified from each experimental sample. The Bio-Rad protein assay system (Bio-Rad) was used to

determine the protein concentration of cell lysates. Cell lysates (20 μg total proteins per lane) were applied to SDS polyacrylamide gels, and proteins were detected by Western blotting. The following antibodies (all from Cell Signaling) were directed against p-AKT (Ser473) (number 9271), p-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (number 9101), p-p38 MAPK (Thr180/Tyr182) (number 9211), p-SAPK/JNK (Thr183/Tyr185) (81E11) (number 4668), β-actin (D6A8) rabbit monoclonal antibody (MAb) number 8457, anti-rabbit IgG, horseradish peroxidase (HRP)-linked antibody (number 7074). Anti-TLR-4 antibody (ab13556) was obtained from Abcam, MA, USA. Anti-LITAF (sc-66945), PI3-kinase (sc-423), and TLR2 (sc-21759) were obtained from Santa Cruz, TX, USA. Anti-AKT (number 44609), MAPK, p38 (AHO 1202), and JNK (number 44690) antibodies were purchased from Fisher Scientific, USA. Signals on immunoblots were quantified using ImageJ according to the manufacturer's instructions.

TNF-α ELISA. The supernatants from infected cells and mouse serum were subjected to an enzyme-linked immunosorbent assay (ELISA) for the determination of TNF-α concentration with an Invitrogen kit (KMC3011; Thermo Fisher, Dublin, OH, USA). ELISA immunoreactivity was quantified using a microplate reader (Bio-Rad, Hercules, CA, USA).

Statistical analysis. All experiments were performed at least in triplicate (biological and technical replicates). Data were analyzed for statistical significance with XLStat (Addinsoft, New York, NY, USA). *P* values were calculated with the Mann-Whitney *t* test or analysis of variance (ANOVA) one-way *t* test for multiple comparisons.

ACKNOWLEDGMENT

This study was supported by NIH/NIDCR grant R01 DE 014079.

REFERENCES

1. Udalova IA, Mantovani A, Feldmann M. 2016. Macrophage heterogeneity in the context of rheumatoid arthritis. Nat Rev Rheumatol 12:472–485. https://doi.org/10.1038/nrrheum.2016.91.
2. Felson DT, Smolen JS, Wells G, Zhang B, van Tuyl LHD, Funovits J, Aletaha D, Allaart CF, Bathon J, Bombardieri S, Brooks P, Brown A, Matucci-Cerinic M, Choi H, Combe B, de Wit M, Dougados M, Emery P, Furst D, Gomez-Reino J, Hawker G, Keystone E, Khanna D, Kirwan J, Kvien TK, Landewé R, Listing J, Michaud K, Martin-Mola E, Montie P, Pincus T, Richards P, Siegel JN, Simon LS, Sokka T, Strand V, Tugwell P, Tyndall A, van der Heijde D, Verstappen S, White B, Wolfe F, Zink A, Boers M. 2011. American College of Rheumatology/European League against Rheumatism provisional definition of remission in rheumatoid arthritis for clinical trials. Ann Rheum Dis 70:404–413. https://doi.org/10.1136/ard.2011.149765.
3. Culshaw S, McInnes IB, Liew FY. 2011. What can the periodontal community learn from the pathophysiology of rheumatoid arthritis? J Clin Periodontol 38:106–113. https://doi.org/10.1111/j.1600-051X.2010.01669.x.
4. Papapanou PN, Susin C. 2017. Periodontitis epidemiology: is periodontitis under-recognized, over-diagnosed, or both? Periodontol 2000 75:45–51. https://doi.org/10.1111/prd.12200.
5. Chou Y-Y, Lai K-L, Chen D-Y, Lin C-H, Chen H-H. 2015. Rheumatoid arthritis risk associated with periodontitis exposure: a nationwide, population-based cohort study. PLoS One 10:e0139693. https://doi.org/10.1371/journal.pone.0139693.
6. Kaur S, Bright R, Proudman SM, Bartold PM. 2014. Does periodontal treatment influence clinical and biochemical measures for rheumatoid arthritis? A systematic review and meta-analysis. Semin Arthritis Rheum 44:113–122. https://doi.org/10.1016/j.semarthrit.2014.04.009.
7. Sandhya P, Danda D, Sharma D, Scaria V. 2016. Does the buck stop with the bugs?: an overview of microbial dysbiosis in rheumatoid arthritis. Int J Rheum Dis 19:8–20. https://doi.org/10.1111/1756-185X.12728.
8. Rosenbaum JT, Asquith MJ. 2016. The microbiome: a revolution in treatment for rheumatic diseases? Curr Rheumatol Rep 18:62. https://doi.org/10.1007/s11926-016-0614-8.
9. Deng Z-L, Szafrański SP, Jarek M, Bhuju S, Wagner-Döbler I. 2017. Dysbiosis in chronic periodontitis: key microbial players and interactions with the human host. Sci Rep 7:3703. https://doi.org/10.1038/s41598-017-03804-8.
10. Reichert S, Haffner M, Keyßer G, Schäfer C, Stein JM, Schaller H-G, Wienke A, Strauss H, Heide S, Schulz S. 2013. Detection of oral bacterial DNA in synovial fluid. J Clin Periodontol 40:591–598. https://doi.org/10.1111/jcpe.12102.
11. Gabarrini G, de Smit M, Westra J, Brouwer E, Vissink A, Zhou K, Rossen JWA, Stobernack T, van Dijl JM, van Winkelhoff AJ. 2015. The peptidylarginine deiminase gene is a conserved feature of Porphyromonas gingivalis. Sci Rep 5:13936. https://doi.org/10.1038/srep13936.
12. Lappin DF, Apatzidou D, Quirke A-M, Oliver-Bell J, Butcher JP, Kinane DF, Riggio MP, Venables P, McInnes IB, Culshaw S. 2013. Influence of periodontal disease, Porphyromonas gingivalis and cigarette smoking on systemic anti-citrullinated peptide antibody titres. J Clin Periodontol 40:907–915. https://doi.org/10.1111/jcpe.12138.
13. Yamakawa M, Ouhara K, Kajiya M, Munenaga S, Kittaka M, Yamasaki S, Takeda K, Takeshita K, Mizuno N, Fujita T, Sugiyama E, Kurihara H. 2016. Porphyromonas gingivalis infection exacerbates the onset of rheumatoid arthritis in SKG mice. Clin Exp Immunol 186:177–189. https://doi.org/10.1111/cei.12847.
14. Sato K, Takahashi N, Kato T, Matsuda Y, Yokoji M, Yamada M, Nakajima T, Kondo N, Endo N, Yamamoto R, Noiri Y, Ohno H, Yamazaki K. 2017. Aggravation of collagen-induced arthritis by orally administered Porphyromonas gingivalis through modulation of the gut microbiota and gut immune system. Sci Rep 7:6955. https://doi.org/10.1038/s41598-017-07196-7.
15. de Aquino SG, Talbot J, Sônego F, Turato WM, Grespan R, Ávila-Campos MJ, Cunha FQ, Cirelli JA. 2017. The aggravation of arthritis by periodontitis is dependent of IL-17 receptor A activation. J Clin Periodontol 44:881–891. https://doi.org/10.1111/jcpe.12743.
16. Chukkapalli S, Rivera-Kweh M, Gehlot P, Velsko I, Bhattacharyya I, Calise SJ, Satoh M, Chan EKL, Holoshitz J, Kesavalu L. 2016. Periodontal bacterial colonization in synovial tissues exacerbates collagen-induced arthritis in B10.RIII mice. Arthritis Res Ther 18:161. https://doi.org/10.1186/s13075-016-1056-4.
17. Burmester GR, Pope JE. 2017. Novel treatment strategies in rheumatoid arthritis. Lancet 389:2338–2348. https://doi.org/10.1016/S0140-6736(17)31491-5.
18. Shimoda LMN, Park C, Stokes AJ, Gomes HH, Turner H. 2012. Pacific island 'Awa (Kava) extracts, but not isolated kavalactones, promote proinflammatory responses in model mast cells. Phytother Res 26:1934–1941. https://doi.org/10.1002/ptr.4652.
19. Teschke R, Lebot V. 2011. Proposal for a kava quality standardization code. Food Chem Toxicol 49:2503–2516. https://doi.org/10.1016/j.fct.2011.06.075.
20. Pollastri MP, Whitty A, Merrill JC, Tang X, Ashton TD, Amar S. 2009. Identification and characterization of kava-derived compounds mediating TNF-alpha suppression. Chem Biol Drug Des 74:121–128. https://doi.org/10.1111/j.1747-0285.2009.00838.x.
21. Alshammari A, Patel J, Al-Hashemi J, Cai B, Panek J, Huck O, Amar S. 2017. Kava-241 reduced periodontal destruction in a collagen antibody primed *Porphyromonas gingivalis* model of periodontitis. J Clin Periodontol 44:1123–1132. https://doi.org/10.1111/jcpe.12784.
22. Marchesan JT, Gerow EA, Schaff R, Taut AD, Shin S-Y, Sugai J, Brand D, Burberry A, Jorns J, Lundy SK, Nuñez G, Fox DA, Giannobile WV. 2013. Porphyromonas gingivalis oral infection exacerbates the development

and severity of collagen-induced arthritis. Arthritis Res Ther 15:R186. https://doi.org/10.1186/ar4376.
23. Martinez-Martinez RE, Abud-Mendoza C, Patiño-Marin N, Rizo-Rodríguez JC, Little JW, Loyola-Rodríguez JP. 2009. Detection of periodontal bacterial DNA in serum and synovial fluid in refractory rheumatoid arthritis patients. J Clin Periodontol 36:1004–1010. https://doi.org/10.1111/j.1600-051X.2009.01496.x.
24. Totaro MC, Cattani P, Ria F, Tolusso B, Gremese E, Fedele AL, D'Onghia S, Marchetti S, Di Sante G, Canestri S, Ferraccioli G. 2013. Porphyromonas gingivalis and the pathogenesis of rheumatoid arthritis: analysis of various compartments including the synovial tissue. Arthritis Res Ther 15:R66. https://doi.org/10.1186/ar4243.
25. Mikuls TR, Thiele GM, Deane KD, Payne JB, O'Dell JR, Yu F, Sayles H, Weisman MH, Gregersen PK, Buckner JH, Keating RM, Derber LA, Robinson WH, Holers VM, Norris JM. 2012. Porphyromonas gingivalis and disease-related autoantibodies in individuals at increased risk of rheumatoid arthritis. Arthritis Rheum 64:3522–3530. https://doi.org/10.1002/art.34595.
26. Curtis MA, Slaney JM, Carman RJ, Johnson NW. 1991. Identification of the major surface protein antigens of Porphyromonas gingivalis using IgG antibody reactivity of periodontal case-control serum. Oral Microbiol Immunol 6:321–326. https://doi.org/10.1111/j.1399-302X.1991.tb00502.x.
27. Jung H, Jung SM, Rim YA, Park N, Nam Y, Lee J, Park S-H, Ju JH. 2017. Arthritic role of Porphyromonas gingivalis in collagen-induced arthritis mice. PLoS One 12:e0188698. https://doi.org/10.1371/journal.pone.0188698.
28. Huck O, Al-Hashemi J, Poidevin L, Poch O, Davideau J-L, Tenenbaum H, Amar S. 2017. Identification and characterization of miRNA differentially expressed in macrophages exposed to *Porphyromonas gingivalis* infection. Infect Immun 85:e00771-16. https://doi.org/10.1128/IAI.00771-16.
29. Röhner E, Detert J, Kolar P, Hocke A, N'Guessan P, Matziolis G, Kanitz V, Bernimoulin JP, Kielbassa A, Burmester GR, Buttgereit F, Pischon N. 2010. Induced apoptosis of chondrocytes by Porphyromonas gingivalis as a possible pathway for cartilage loss in rheumatoid arthritis. Calcif Tissue Int 87:333–340. https://doi.org/10.1007/s00223-010-9389-5.
30. Makkawi H, Hoch S, Burns E, Hosur K, Hajishengallis G, Kirschning CJ, Nussbaum G. 2017. Porphyromonas gingivalis stimulates TLR2-PI3K signaling to escape immune clearance and induce bone resorption independently of MyD88. Front Cell Infect Microbiol 7:359. https://doi.org/10.3389/fcimb.2017.00359.
31. Hao L, Zhu G, Lu Y, Wang M, Jules J, Zhou X, Chen W. 2015. Deficiency of cathepsin K prevents inflammation and bone erosion in rheumatoid arthritis and periodontitis and reveals its shared osteoimmune role. FEBS Lett 589:1331–1339. https://doi.org/10.1016/j.febslet.2015.04.008.
32. Malemud CJ. 2017. Matrix metalloproteinases and synovial joint pathology. Prog Mol Biol Transl Sci 148:305–325. https://doi.org/10.1016/bs.pmbts.2017.03.003.
33. Arimatsu K, Yamada H, Miyazawa H, Minagawa T, Nakajima M, Ryder MI, Gotoh K, Motooka D, Nakamura S, Iida T, Yamazaki K. 2014. Oral pathobiont induces systemic inflammation and metabolic changes associated with alteration of gut microbiota. Sci Rep 4:4828. https://doi.org/10.1038/srep04828.
34. Nilsson M, Kopp S. 2008. Gingivitis and periodontitis are related to repeated high levels of circulating tumor necrosis factor-alpha in patients with rheumatoid arthritis. J Periodontol 79:1689–1696. https://doi.org/10.1902/jop.2008.070599.
35. Tang X, Amar S. 2016. Kavain inhibition of LPS-induced TNF-α via ERK/LITAF. Toxicol Res 5:188–196. https://doi.org/10.1039/C5TX00164A.
36. Yuan H, Gupte R, Zelkha S, Amar S. 2011. Receptor activator of nuclear factor kappa B ligand antagonists inhibit tissue inflammation and bone loss in experimental periodontitis. J Clin Periodontol 38:1029–1036. https://doi.org/10.1111/j.1600-051X.2011.01780.x.
37. Bek S, Bojesen AB, Nielsen JV, Sode J, Bank S, Vogel U, Andersen V. 2017. Systematic review and meta-analysis: pharmacogenetics of anti-TNF treatment response in rheumatoid arthritis. Pharmacogenomics J 17:403–411. https://doi.org/10.1038/tpj.2017.26.
38. Wang Y, Han C-C, Cui D, Li Y, Ma Y, Wei W. 2017. Is macrophage polarization important in rheumatoid arthritis? Int Immunopharmacol 50:345–352. https://doi.org/10.1016/j.intimp.2017.07.019.
39. Kwon D-J, Ju SM, Youn GS, Choi SY, Park J. 2013. Suppression of iNOS and COX-2 expression by flavokawain A via blockade of NF-κB and AP-1 activation in RAW 264.7 macrophages. Food Chem Toxicol 58:479–486. https://doi.org/10.1016/j.fct.2013.05.031.
40. Molteni M, Gemma S, Rossetti C. 2016. The role of Toll-like receptor 4 in infectious and noninfectious inflammation. Mediators Inflamm 2016:6978936–6978939. https://doi.org/10.1155/2016/6978936.
41. Elshabrawy HA, Essani AE, Szekanecz Z, Fox DA, Shahrara S. 2017. TLRs, future potential therapeutic targets for RA. Autoimmun Rev 16:103–113. https://doi.org/10.1016/j.autrev.2016.12.003.
42. Harikrishnan H, Jantan I, Haque MA, Kumolosasi E. 2018. Anti-inflammatory effects of hypophyllanthin and niranthin through down-regulation of NF-κB/MAPKs/PI3K-Akt signaling pathways. Inflammation 10:787–712. 41:984–995. https://doi.org/10.1007/s10753-018-0752-4.
43. Lee S-I, Boyle DL, Berdeja A, Firestein GS. 2012. Regulation of inflammatory arthritis by the upstream kinase mitogen activated protein kinase kinase 7 in the c-Jun N-terminal kinase pathway. Arthritis Res Ther 14:R38. https://doi.org/10.1186/ar3750.
44. Clark AR, Dean JL. 2012. The p38 MAPK pathway in rheumatoid arthritis: a sideways look. Open Rheumatol J 6:209–219. https://doi.org/10.2174/1874312901206010209.
45. Schett G, Tohidast-Akrad M, Smolen JS, Schmid BJ, Steiner CW, Bitzan P, Zenz P, Redlich K, Xu Q, Steiner G. 2000. Activation, differential localization, and regulation of the stress-activated protein kinases, extracellular signal-regulated kinase, c-JUN N-terminal kinase, and p38 mitogen-activated protein kinase, in synovial tissue and cells in rheumatoid arthritis. Arthritis Rheum 43:2501–2512. https://doi.org/10.1002/1529-0131(200011)43:11<2501::AID-ANR18>3.0.CO;2-K.
46. Namba S, Nakano R, Kitanaka T, Kitanaka N, Nakayama T, Sugiya H. 2017. ERK2 and JNK1 contribute to TNF-α-induced IL-8 expression in synovial fibroblasts. PLoS One 12:e0182923. https://doi.org/10.1371/journal.pone.0182923.
47. Li X, Peng F, Xie C, Wu W, Han X, Chen L. 2013. (E)-3-(3,4-Dimethoxyphenyl)-1-(5-hydroxy-2,2-dimethyl-2H-chromen-6-yl)prop-2-en-1-one ameliorates the collagen-arthritis via blocking ERK/JNK and NF-κB signaling pathway. Int Immunopharmacol 17:1125–1133. https://doi.org/10.1016/j.intimp.2013.10.001.
48. Zou J, Guo P, Lv N, Huang D. 2015. Lipopolysaccharide-induced tumor necrosis factor-α factor enhances inflammation and is associated with cancer. Mol Med Rep 12:6399–6404. https://doi.org/10.3892/mmr.2015.4243.
49. Pang T, Wang J, Benicky J, Saavedra JM. 2012. Minocycline ameliorates LPS-induced inflammation in human monocytes by novel mechanisms including LOX-1, Nur77 and LITAF inhibition. Biochim Biophys Acta 1820:503–510. https://doi.org/10.1016/j.bbagen.2012.01.011.
50. Merrill JC, You J, Constable C, Leeman SE, Amar S. 2011. Whole-body deletion of LPS-induced TNF-α factor (LITAF) markedly improves experimental endotoxic shock and inflammatory arthritis. Proc Natl Acad Sci U S A 108:21247–21252. https://doi.org/10.1073/pnas.1111492108.
51. Bushell KN, Leeman SE, Gillespie E, Gower AC, Reed KL, Stucchi AF, Becker JM, Amar S. 2011. LITAF mediation of increased TNF-α secretion from inflamed colonic lamina propria macrophages. PLoS One 6:e25849. https://doi.org/10.1371/journal.pone.0025849.
52. Chang WJ, Niu XP, Hou RX, Li JQ, Liu RF, Wang Q, Wang CF, Li XH, Yin GH, Zhang KM. 2015. LITAF, HHEX, and DUSP1 expression in mesenchymal stem cells from patients with psoriasis. Genet Mol Res 14:15793–15801. https://doi.org/10.4238/2015.December.1.31.
53. Singh K, Colmegna I, He X, Weyand CM, Goronzy JJ. 2008. Synoviocyte stimulation by the LFA-1-intercellular adhesion molecule-2-Ezrin-Akt pathway in rheumatoid arthritis. J Immunol 180:1971–1978. https://doi.org/10.4049/jimmunol.180.3.1971.
54. Li S, Chen J-W, Xie X, Tian J, Deng C, Wang J, Gan H-N, Li F. 2017. Autophagy inhibitor regulates apoptosis and proliferation of synovial fibroblasts through the inhibition of PI3K/AKT pathway in collagen-induced arthritis rat model. Am J Transl Res 9:2065–2076.
55. Zhang X, Goncalves R, Mosser DM. 2008. The isolation and characterization of murine macrophages. Curr Protoc Immunol Chapter 14:Unit 14.1. https://doi.org/10.1002/0471142735.im1401s83.