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18beta-Glycyrrhetic Acid Inhibits Periodontitis Via Glucocorticoid-Independent NF- κ B Inactivation In IL-10 Deficient Mice

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Abstract

Background and objective—18beta-glycyrrhetic acid (GA) is a natural anti-inflammatory compound derived from licorice root extract (*Glycyrrhiza glabra*). The effect of GA on experimental periodontitis and its mechanism of action were determined in the present study.

Methods—Periodontitis was induced by oral infection with *Porphyromonas gingivalis* W83 in IL-10 deficient mice. The effect of GA, which was delivered by subcutaneous injections in either prophylactic or therapeutic regimens, on alveolar bone loss and gingival gene expressions was determined on day 42 after initial infection. The effect of GA on LPS-stimulated macrophages, T cell proliferation, and osteoclastogenesis was also examined *in vitro*.

Results—GA administered either prophylactically or therapeutically dramatically reduced infection-induced bone loss in IL-10 deficient mice, which are highly disease-susceptible. Although GA has been reported to exert its anti-inflammatory activity via down-regulation of 11-beta hydroxysteroid dehydrogenase-2 (HSD2), which converts active glucocorticoids (GC) to their inactive forms, GA did not reduce HSD2 gene expression in gingival tissue. Rather, under GC-free conditions, GA potently inhibited LPS-stimulated proinflammatory cytokine production and RANKL-stimulated osteoclastogenesis, both of which are NF- κ B-dependent. GA furthermore suppressed LPS- and RANKL-stimulated phosphorylation of NF- κ B p105 *in vitro*.

Conclusion—These findings indicate that GA inhibits periodontitis by inactivation of NF- κ B in an IL-10 and GC-independent fashion.

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Keywords

18beta-glycyrrhetic acid; periodontal disease; NF- κ B; IL-10 deficient mouse

Introduction

Periodontitis is a chronic inflammatory bone destructive disease that is induced by a complex of oral pathogens, including *Porphyromonas gingivalis* (1). In addition, host immune responses play an important role in the induction and disease progression (2,3). Thus, regulation of the host immune and inflammatory response is a possible therapeutic strategy in ameliorating this disease.

18beta-glycyrrhetic acid (GA) is an active component of licorice root extract (*Glycyrrhiza glabra*). In traditional medicine, beneficial therapeutic effects of GA have been reported in various diseases including inflammation, ulcers, and cancer (4). Several mechanisms of the GA-mediated anti-inflammatory effect have been proposed, including modulation of glucocorticoid (GC) metabolism, alteration of IL-10 production, and down-regulation of nuclear factor kappa B (NF- κ B)(5–7). In experimental arthritis, GA reduced inflammatory bone destruction, which correlated with an inhibition of 11-beta-hydroxysteroid dehydrogenase-2 (HSD2) that converts active corticosteroids to their inactive forms (8). This finding suggests that increased local active GC is a possible mechanism of GA-mediated inhibition of inflammation. However, despite these correlative data, the precise mechanism(s) of action of GA remains unclear.

In the present study, we investigated possible therapeutic effects of GA on infection-stimulated alveolar bone loss in a well-established IL-10-deficient mouse model of periodontitis (9). We have previously reported that IL-10 deficient mice are highly susceptible to *P. gingivalis*-induced periodontal disease, consistent with their hyperinflammatory response phenotype (10). The hypothesis tested was that GA inhibits periodontitis via local increases in active GCs that is mediated by a down-regulation of HSD2. We here demonstrate that GA inhibits periodontal bone loss in this model, but that this likely involves modulation of the pro-inflammatory transcription factor NF- κ B rather than inhibition of HSD2 and local GC levels.

Materials and methods

Reagents and treatments

Reagents including 18beta-glycyrrhetic acid (MW470.70, purity=97%, insoluble in water and ethanol, and soluble in dimethyl sulfoxide (DMSO)), olive oil (highly refined, low acidity), and DMSO were purchased from Sigma-Aldrich (St. Louis, MO). Mice in GA treated groups were given an olive oil suspension of GA (30mg/kg weight/time) following two treatment regimens. For prophylaxis, GA was delivered by subcutaneous (s, c.) injection on days 0, 2, 4, 13, 20, 27, and 34 relative to infection with *Porphyromonas gingivalis* W83. To test therapeutic effects, GA was injected s.c. on days 9, 11, 13, 20, 27, and 34 after infection. Controls received injections of olive oil alone following the prophylactic schedule. For *in vitro* studies, 10mM GA in DMSO was diluted to 10 μ M or 1 μ M in culture medium. DMSO alone served as a control.

Mice

IL-10 deficient (IL-10^{-/-}) mice (B6.129P2-Il10^{tm1Cgn}/J) on a C57BL/6J background were purchased from the Jackson Laboratory (Bar Harbor, ME). Animals were maintained under

specific pathogen-free conditions in the Forsyth Institute animal facility as described (10). The use of animals was approved by the Forsyth Institutional Animal Care and Use Committee.

Bacterial culture and infection regimen

P. gingivalis W83 (*Pg*) was grown, harvested, and resuspended at 10^{10} cells/ml in PBS containing 2% carboxymethyl cellulose (CMC; Sigma-Aldrich) as described (9). Prior to oral infection, 6 to 7 week old mice received antibiotic treatment (sulfatrim suspension, 10 ml/100 ml of drinking water) to reduce endogenous oral flora (9). Animals were then orally inoculated with 10^9 *Pg* six times beginning on day 0 at 2 day intervals. Negative controls received the same volume of CMC without bacteria. Colonization of mice by *Pg* was determined by RT-PCR as described below.

Sample preparation

Animals were killed on day 42 after the initial oral infection. Mandibles were isolated, hemisected, and defleshed for bone loss measurements as described (9). Gingival tissues were isolated for RNA extraction, and were disrupted by FastPrep-24 using Matrix A (both MP Biomedicals, Solon, OH) and TRIzol reagent (Invitrogen, Carlsbad, CA).

Bone loss measurements

Alveolar bone loss was determined on digital images of mandibular molar teeth and alveolar bone morphometrically as described (9). Results were expressed in mm^2 .

Quantitative RT-PCR (qPCR) and RT-PCR

Gene expression of HSD1, HSD2, IL-12p40, and $\beta 7$ -integrin in gingival tissue was assessed by qPCR as described (10). Predesigned primers for GAPDH (catalog number QT00309099, Qiagen, Valencia, CA), HSD1 (QT00107303), HSD2 (QT00252609), $\beta 7$ -integrin (QT00105091), and RANKL (QT00147385) were used with the QuantiFast SYBR Green PCR Kit (Qiagen) following the manufacturer's instructions. The GAPDH gene was used as an internal control. All reactions were carried out in triplicate. The level of gene expression was determined by comparison with standard curves generated with known copy number samples.

To confirm the colonization of *Pg*, total RNA samples, which contain both host and bacterial RNA, was subjected to RT-PCR to detect the *Pg* 16S rRNA gene as described previously (10).

Macrophage cultures

RPMI1640 (Sigma-Aldrich) supplemented with 2mM L-glutamine (Invitrogen) and 10% charcoal/dextran-treated FBS (to remove glucocorticoids; HyClone, Waltham, MA) was used for all cell culture experiments. Resident peritoneal macrophages isolated from IL-10^{-/-} mice (11) were plated at 10^5 cells/well in 96-well plates ($n = 4$), and were stimulated with *E. coli* LPS (Sigma- Aldrich, 2 $\mu\text{g}/\text{ml}$) for 24 h at 37°C in an atmosphere of 5% CO₂/95% air. After the incubation, culture supernatants were subjected to ELISA for cytokines. Adherent macrophages were subjected to protein extraction for Western blot.

T cell proliferation assays

Splenic T cells isolated from IL-10^{-/-} mice (10) were plated at 10^5 cells/well in 96-well plates coated with anti-mCD3/mCD28 antibodies (both BD Biosciences, San Jose, CA), and stimulated with concanavalin A (Con A, Sigma- Aldrich, 1 $\mu\text{g}/\text{ml}$) in the presence/absence of

GA for 7 days. The proliferation of T cells was determined using the CellTiter 96 Aqueous assay (Promega, Madison, WI).

Osteoclast differentiation

Osteoclastogenesis was induced in RAW264.7 cells by recombinant mouse RANKL (Receptor Activator for NF- κ B Ligand; PeproTech, Rocky Hill, NJ), and TRAP-positive polykaryons with >3 nuclei were enumerated as osteoclast-like cells (12). The cells were also subjected to protein extraction for Western blot.

ELISA

IL-1 β , IL-6, IL-12, and IFN γ were quantified by ELISA (R&D Systems, Minneapolis, MN) following the manufacturer's instructions. Cytokine concentrations were expressed as pg/ml.

Protein extraction and Western blot

Cellular protein was extracted using Tris-Glycine SDS Sample Buffer (Invitrogen). After SDS-PAGE of protein samples (10 μ g), NF- κ B2 p100/p52, phosphorylated NF- κ B2 p100, and phosphorylated NF- κ B p105 was detected using specific antibodies (Cell Signaling Technologies, Danvers, MA). Mouse GAPDH served as a control (antibody from Ambion, Austin, TX).

Statistics

The effect of GA treatment was evaluated statistically by one-way ANOVA with the Bonferroni multiple comparison test.

Results

Effect of GA on *Pg*-induced alveolar bone loss

We have previously characterized IL-10 deficient mice as a hyperinflammatory model of periodontitis, secondary to an increase in the expression of IL-12 and the induction of strong Th1 responses. IL-10 $^{-/-}$ mice were infected orally with *Pg* as described in Methods. Infection was confirmed by the detection of *Pg* in all infected, but not in non-infected, animals on day 42 as determined by RT-PCR. As shown in Figure 1, oral infection of IL-10 $^{-/-}$ with *Pg* induced significant alveolar bone loss after 42 days compared to uninfected controls. Strikingly, GA (30mg/kg) administered either prophylactically (days 0–32) or therapeutically (days 9–34) completely inhibited *Pg*-induced bone loss, resulting in bone levels that were indistinguishable from uninfected controls.

Effect of GA on gingival gene expression

It has been suggested that GA acts by inhibiting the expression of HSD2 which degrades glucocorticoids. We therefore evaluated gingival gene expression of HSD1 and HSD2 by qPCR on day 42. GA did not modulate the expression of HSD1. Surprisingly GA also failed to reduce HSD2 gene expression in gingival tissue (Figure 2), and instead up-regulated HSD2 gene expression in the treated animals, although this difference was not statistically significant ($p=0.07$). We also examined the effect of GA on gingival gene expression of IL-12p40, β 7-integrin, and RANKL, because these genes are crucial in the induction of Th1 immune responses as well as for osteoclastogenesis (10,13,14). However no significant effect of GA on these genes was observed on day 42 (data not shown).

Suppressive effect of GA on cell functions in IL-10^{-/-} mice

Since macrophages and T cells play key roles in the induction of periodontitis in IL-10^{-/-} mice (10), we determined if GA inhibits proinflammatory cytokine production by *E. coli* LPS-stimulated IL-10^{-/-} macrophages *in vitro*. As shown in Figure 3A, GA significantly suppressed the production of IL-1 β and IL-12p70 by macrophages in a dose-dependent fashion, whereas there was no effect on IL-6, indicating a lack of toxicity of this compound.

Next, we examined the effect of GA on T cell proliferation and IFN γ production. GA modestly up-regulated T cell proliferation compared to positive controls, again indicating that it is not toxic. GA also failed to inhibit IFN γ production by T cells (Figure 3B).

Finally we determined the effect of GA on RANKL-stimulated osteoclastogenesis. As shown in Figure 3C, GA profoundly inhibited the formation of TRAP⁺ osteoclast-like polykaryons in a RAW264.7 cell model. Essentially no TRAP⁺ cells were observed in GA-treated cultures.

GA suppresses phosphorylation of NF- κ B

To further explore the mechanism of GA action, we determined the effects of GA on the activation of NF- κ B *in vitro*. LPS-induced phosphorylation of NF- κ B p105 in IL-10^{-/-} macrophages was significantly down-regulated by 1 μ M of GA (Figure 4A). In addition, GA inhibited RANKL-induced phosphorylation of NF- κ B p105 (Figure 4B). There was no effect of GA on the phosphorylation of NF- κ B p100/p52 or NF- κ B p100 (data not shown). These data suggest that inhibition of NF- κ B phosphorylation may underlie the protective anti-inflammatory effect of GA on *Pg*-induced periodontitis in IL-10^{-/-} mice.

Discussion

GA has been utilized as an anti-inflammatory agent for more than two thousand years (4), and in recent studies inhibited joint destruction in experimental adjuvant-induced autoimmune arthritis (8). The protective effect of GA on arthritis has been attributed to local increases in GC levels, via inhibition of HSD2 which mediates GC inactivation.

IL-10^{-/-} mice have a hyperinflammatory phenotype and are highly susceptible to *Pg*-induced alveolar bone destruction (9). Oral infection of *Pg* induces 5 times more alveolar bone loss in IL-10^{-/-} than in wild type C57BL/6J mice that are resistant to periodontitis (10). Hence, we chose IL-10^{-/-} mice as a stringent model to test the therapeutic potential and mechanism of action of GA. As we previously reported, the susceptibility of IL-10^{-/-} mice to *Pg*-induced periodontitis is dependant on a hyperinflammatory state established via IL-12-mediated polarized Th1 immune responses (10).

In the present studies we demonstrate that GA completely inhibits *Pg*-induced bone loss when delivered in either prophylactic or therapeutic regimens, suggesting a mode of action of GA that is exerted at the time of, as well as following periodontal disease initiation. GA also has therapeutic impact in wild-type animals, since it significantly suppressed ligature- and *Pg* infection-induced alveolar bone loss in wild-type Lewis rats (H.S., unpublished observations). The observation that GA inhibits bone loss in both IL-10^{-/-} and wild type animals clearly indicates that its mode of action is IL-10 independent.

GA has been reported to mediate anti-inflammatory effects in target tissues by increasing active GCs via down-regulation of HSDs (15). In contrast to autoimmune arthritis (8), we found that GA failed to down-regulate gingival expression of HSDs, indicating that the HSD-GC axis appears not to be a key mechanism in GA-mediated suppression of alveolar bone loss.

In contrast GA significantly suppressed a number of NF- κ B-dependent events including the production of IL-1 β and IL-12p70 by LPS-stimulated macrophages (16,17). *Pg* LPS was not employed in this study due to its weak and inconsistent effects in this assay system (H.S., unpublished observations). However IL-6, IFN γ and T cell proliferation, all of which are less NF- κ B-dependent (18–21), were unaffected. Consistent with NF- κ B as a primary point of regulation (22), GA completely inhibited RANKL-stimulated osteoclastogenesis and LPS-induced phosphorylation of NF- κ B p105 (NF- κ B1) but not NF- κ B2 p100/p52 or NF- κ B2 p100, under GC-free conditions *in vitro*. Taken together, these data strongly indicate that GA-mediated blockade of infection-stimulated alveolar bone loss is dependant on its ability to inhibit the activation of NF- κ B, and does not involve modulation of GCs or IL-10.

NF κ –B1 represents the canonical NF- κ B pathway whereas NF- κ B2 is involved in the non-canonical pathway. The canonical pathway is activated by various stimuli such as cytokines and microbial components. NF- κ B1 was also up-regulated in bone marrow cells derived from tail-suspended mice, a model of microgravity in which animals rapidly become osteoporotic (23). Although Rel-A complexes (Rel-A/c-Rel/p50) will be released from the I κ B α inhibitor in the canonical pathway (24), Rel-A was reported as a key molecule in inhibition of osteolytic osteomyelitis (25). NF- κ B p50/p50 homodimers mediate reactive arthritis, which is an infection-induced inflammation that also involves urethra and eyes (26). Of interest, in a sepsis arthritis model, systemic inhibition of NF- κ B2 using antisense oligonucleotides failed to suppress bone loss (27). In contrast, non-canonical pathway activation closely relates to autoimmunity, and is not activated by most of the classical NF- κ B inducers such as proinflammatory cytokines (28). In addition to rheumatoid arthritis, NF- κ B2 appears to be important in induction of juvenile idiopathic arthritis and psoriatic arthritis (29). These findings suggest that NF- κ B1, but not NF- κ B2, may play an important role in infection-stimulated bone loss.

The apparent lack of effect of GA on GC metabolism in periodontitis vs arthritis models may reflect inherent differences in the pathways activated by infection vs an autoimmune disease. HSDs are key regulators of GC pre-receptor metabolism (15), by interconverting active and inactive GCs. GA preferentially inhibits HSD2, which converts active GCs to their inactive form, but does not affect HSD1, which converts inactive GCs to their active form (5). HSD2 was elevated in autoimmune arthritis (30), and was also significantly up-regulated in synovial macrophages of rheumatoid arthritis patients compared to normal synovium (31). These correlations are consistent with a key role of HSD2 and local GCs in the protective effect of GA in autoimmune arthritis, which is a sterile inflammatory condition. However, direct evidence for the role of this mechanism is still lacking, and as noted earlier, other mechanisms of action have been reported (6,7). On the other hand, pro-inflammatory TNF α and IL-1 β significantly down-regulate HSD2 in placental trophoblasts (32), suggesting a GA-independent up-regulatory pathway of active GCs. In contrast, we demonstrate that GA can exert its inhibitory effects via an HSD- and GC-independent mechanism, indicating the complexity of the GA-HSD2-GC axis. Thus, further studies are essential to precisely examine the effect of GA on the NF- κ B pathways and HSD-GC axis using NF- κ B1^{-/-} and HSD2^{-/-} mice (33,34).

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References

1. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. *Periodontol* 2000. 1994; 5:78–111. [PubMed: 9673164]
2. Teng YT. Protective and destructive immunity in the periodontium: Part 1--innate and humoral immunity and the periodontium. *J Dent Res*. 2006; 85:198–208. [PubMed: 16498065]
3. Teng YT. Protective and destructive immunity in the periodontium: Part 2--T-cell-mediated immunity in the periodontium. *J Dent Res*. 2006; 85:209–219. [PubMed: 16498066]
4. Shibata S. A drug over the millennia: pharmacognosy, chemistry, and pharmacology of licorice. *Yakugaku Zasshi*. 2000; 120:849–862. [PubMed: 11082698]
5. Classen-Houben D, Schuster D, Da Cunha T, et al. Selective inhibition of 11beta-hydroxysteroid dehydrogenase 1 by 18alpha-glycyrrhetic acid but not 18beta-glycyrrhetic acid. *J Steroid Biochem Mol Biol*. 2009; 113:248–252. [PubMed: 19429429]
6. Abe M, Akbar F, Hasebe A, Horiike N, Onji M. Glycyrrhizin enhances interleukin-10 production by liver dendritic cells in mice with hepatitis. *J Gastroenterol*. 2003; 38:962–967. [PubMed: 14614603]
7. Kang OH, Kim JA, Choi YA, et al. Inhibition of interleukin-8 production in the human colonic epithelial cell line HT-29 by 18 beta-glycyrrhetic acid. *Int J Mol Med*. 2005; 15:981–985. [PubMed: 15870903]
8. Ferrer E. Emerging therapeutic strategies for chronic inflammatory diseases. *Drug News Perspect*. 2006; 19:353–358. [PubMed: 16971972]
9. Sasaki H, Okamatsu Y, Kawai T, Kent R, Taubman M, Stashenko P. The interleukin-10 knockout mouse is highly susceptible to *Porphyromonas gingivalis*-induced alveolar bone loss. *J Periodontal Res*. 2004; 39:432–441. [PubMed: 15491348]
10. Sasaki H, Suzuki N, Kent R Jr, Kawashima N, Takeda J, Stashenko P. T cell response mediated by myeloid cell-derived IL-12 is responsible for *Porphyromonas gingivalis*-induced periodontitis in IL-10-deficient mice. *J Immunol*. 2008; 180:6193–6198. [PubMed: 18424741]
11. Sasaki H, Hou L, Belani A, et al. IL-10, but not IL-4, suppresses infection-stimulated bone resorption in vivo. *J Immunol*. 2000; 165:3626–3630. [PubMed: 11034365]
12. Okamatsu Y, Kim D, Battaglini R, Sasaki H, Spate U, Stashenko P. MIP-1 gamma promotes receptor-activator-of-NF-kappa-B-ligand-induced osteoclast formation and survival. *J Immunol*. 2004; 173:2084–2090. [PubMed: 15265944]
13. Jorgensen C, Travaglio-Encinoza A, Bologna C, D'Angeac AD, Reme T, Sany J. Human mucosyl lymphocyte marker expression in synovial fluid lymphocytes of patient with rheumatoid arthritis. *J Rheumatol*. 1994; 21:1602–1607. [PubMed: 7799335]
14. Kawai T, Matsuyama T, Hosokawa Y, et al. B and T lymphocytes are the primary sources of RANKL in the bone resorptive lesion of periodontal disease. *Am J Pathol*. 2006; 169:987–998. [PubMed: 16936272]
15. Tomlinson JW, Stewart PM. Cortisol metabolism and the role of 11beta-hydroxysteroid dehydrogenase. *Best Pract Res Clin Endocrinol Metab*. 2001; 15:61–78. [PubMed: 11469811]
16. Wang P, Wu P, Siegel MI, Egan RW, Billah MM. Interleukin (IL)-10 inhibits nuclear factor kappa B (NF kappa B) activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. *J Biol Chem*. 1995; 270:9558–9563. [PubMed: 7721885]
17. Blackwell TS, Christman JW. The role of nuclear factor-kappa B in cytokine gene regulation. *Am J Respir Cell Mol Biol*. 1997; 17:3–9. [PubMed: 9224203]
18. Lee SY, Reichlin A, Santana A, Sokol KA, Nussenzweig MC, Choi Y. TRAF2 is essential for JNK but not NF-kappaB activation and regulates lymphocyte proliferation and survival. *Immunity*. 1997; 7:703–713. [PubMed: 9390693]
19. Hawiger J. Innate immunity and inflammation: a transcriptional paradigm. *Immunol Res*. 2001; 23:99–109. [PubMed: 11444396]
20. Wang XC, Jobin C, Allen JB, Roberts WL, Jaffe GJ. Suppression of NF-kappaB-dependent proinflammatory gene expression in human RPE cells by a proteasome inhibitor. *Invest Ophthalmol Vis Sci*. 1999; 40:477–486. [PubMed: 9950608]

21. Rogler G, Gelbmann CM, Vogl D, et al. Differential activation of cytokine secretion in primary human colonic fibroblast/myofibroblast cultures. *Scand J Gastroenterol.* 2001; 36:389–398. [PubMed: 11336164]
22. Iotsova V, Caamano J, Loy J, Yang Y, Lewin A, Bravo R. Osteopetrosis in mice lacking NF-kappaB1 and NF-kappaB2. *Nat Med.* 1997; 3:1285–1289. [PubMed: 9359707]
23. Ishijima M, Ezura Y, Tsuji K, et al. Osteopontin is associated with nuclear factor kappaB gene expression during tail-suspension-induced bone loss. *Exp Cell Res.* 2006; 312:3075–3083. [PubMed: 16889770]
24. Gilmore TD. Introduction to NF-kappaB: players, pathways, perspectives. *Oncogene.* 2006; 25:6680–6684. [PubMed: 17072321]
25. Yip KH, Zheng MH, Feng HT, Steer JH, Joyce DA, Xu J. Sesquiterpene lactone parthenolide blocks lipopolysaccharide-induced osteolysis through the suppression of NF-kappaB activity. *J Bone Miner Res.* 2004; 19:1905–1916. [PubMed: 15476591]
26. Collantes E, Valle Blazquez M, Mazorra V, Macho A, Aranda E, Munoz E. Nuclear factor-kappa B activity in T cells from patients with rheumatic diseases: a preliminary report. *Ann Rheum Dis.* 1998; 57:738–741. [PubMed: 10070274]
27. Gjertsson I, Hultgren OH, Collins LV, Pettersson S, Tarkowski A. Impact of transcription factors AP-1 and NF-kappaB on the outcome of experimental *Staphylococcus aureus* arthritis and sepsis. *Microbes Infect.* 2001; 3:527–534. [PubMed: 11418326]
28. Xiao G, Rabson AB, Young W, Qing G, Qu Z. Alternative pathways of NF-kappaB activation: a double-edged sword in health and disease. *Cytokine Growth Factor Rev.* 2006; 17:281–293. [PubMed: 16793322]
29. Roman-Blas JA, Jimenez SA. Targeting NF-kappaB: a promising molecular therapy in inflammatory arthritis. *Int Rev Immunol.* 2008; 27:351–374. [PubMed: 18853343]
30. Hardy R, Rabbitt EH, Filer A, et al. Local and systemic glucocorticoid metabolism in inflammatory arthritis. *Ann Rheum Dis.* 2008; 67:1204–1210. [PubMed: 18420938]
31. Haas CS, Creighton CJ, Pi X, et al. Identification of genes modulated in rheumatoid arthritis using complementary DNA microarray analysis of lymphoblastoid B cell lines from disease-discordant monozygotic twins. *Arthritis Rheum.* 2006; 54:2047–2060. [PubMed: 16804865]
32. Chisaka H, Johnstone JF, Premyslova M, Manduch Z, Challis JR. Effect of pro-inflammatory cytokines on expression and activity of 11beta-hydroxysteroid dehydrogenase type 2 in cultured human term placental trophoblast and human choriocarcinoma JEG-3 cells. *J Soc Gynecol Investig.* 2005; 12:303–309.
33. Lamhamedi-Cherradi SE, Zheng S, Hilliard BA, et al. Transcriptional regulation of type I diabetes by NF-kappa B. *J Immunol.* 2003; 171:4886–4892. [PubMed: 14568969]
34. Kotelevtsev Y, Brown RW, Fleming S, et al. Hypertension in mice lacking 11beta-hydroxysteroid dehydrogenase type 2. *J Clin Invest.* 1999; 103:683–689. [PubMed: 10074485]

Figure 1. GA inhibits the induction and progression of infection-induced alveolar bone loss in IL-10 deficient mice

A. Representative images of defleshed hemi-mandibles. Negative: non-infected, vehicle alone, n=9; Positive: *P. gingivalis* infected, vehicle alone, n=8; Prophylactic: *P. gingivalis* infected, GA 30mg/kg, day 0 to 34, n=9, Therapeutic: *P. gingivalis* infected, GA 30mg/kg, day 9 to 34, n=8. Area enclosed by black line: area of exposed cementum. B. Effect of GA on alveolar bone loss. Groups are as described above. Vertical bars: SD, * p<0.05 by ANOVA/Bonferroni test.

Figure 2. GA does not inhibit gingival gene expression of HSD1 or HSD2

Gingival gene expression of HSDs was determined by qPCR on day 42. Change-fold values are based on positive control. Closed columns: positive control (infected, no treatment), Shaded columns: negative control (no infection, no treatment), Open columns: (infected, treated with prophylactic regimen). Vertical bars: SD. No significant differences were detected by ANOVA/Bonferroni test.

Figure 3. GA suppresses proinflammatory cytokine production and osteoclastogenesis *in vitro*
 A. The effect of GA on cytokine production by LPS-stimulated IL-10^{-/-} macrophages. Negative: no LPS, no GA; Positive: LPS, no GA; GA: LPS, GA 10μM or 1μM, n=4/group. Vertical bars: SD, * p<0.05 by ANOVA/Bonferroni test. B. The effect of GA on T cell proliferation and IFNγ production. Negative: no ConA, no GA; Positive: Con A, no GA; GA: Con A, GA 10μM or 1μM, n=4/group, Vertical bars: SD, * p<0.05 by ANOVA/Bonferroni test. C. The effect of GA on RANKL-stimulated osteoclastogenesis by RAW264.7 cells. Negative: no RANKL, no GA; Positive: RANKL, no GA; GA: RANKL, GA 10μM or 1μM. n=4/group. Vertical bars: SD, * p<0.0005 by ANOVA/Bonferroni test.

Figure 4. GA inactivates the phosphorylation of NF- κ B p105

A. Inactivation of NF- κ B in LPS-stimulated IL-10^{-/-} macrophages. Lane 1: Negative (vehicle alone), Lane 2: Positive (LPS, vehicle), Lane 3: GA (LPS, 1 μ M GA). B. Inactivation of NF- κ B in RANKL-stimulated RAW264.7 cells. Lane 1: Negative (vehicle alone), Lane 2: Positive (RANKL, vehicle), Lane 3: GA (RANKL, 1 μ M GA).