In Vivo Up-Regulation of Kinin B₁ Receptors after Treatment with Porphyromonas gingivalis Lipopolysaccharide in Rat Paw

Fabiana N. Dornelles, Diógenes S. Santos, Thomas E. Van Dyke, João B. Calixto, Eraldo L. Batista, Jr., and Maria M. Campos

Programa de Pós-Graduação em Biologia Celular e Molecular, Pontificia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil (F.N.D.); Centro de Pesquisas em Biologia Molecular e Funcional, Instituto de Pesquisas Biomédicas, Pontificia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil (D.S.S., E.L.B.); Department of Periodontology and Oral Biology, Goldman School of Dental Medicine, Boston University, Boston, Massachusetts (T.E.V.D.); Department of Pharmacology, Centro de Biological Sciences, Universidade Federal de Santa Catarina, Florianópolis, Brazil (J.B.C.); and School of Dentistry (E.L.B., M.M.C.) and Institute of Toxicology (M.M.C.), Pontificia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil

Received May 3, 2009; accepted June 24, 2009

ABSTRACT

It has been demonstrated that kinin B₁ receptors are highly up-regulated under several stressful stimuli, such as infection. However, there is no evidence indicating whether Porphyromonas gingivalis lipopolysaccharide (Pg-LPS) might lead to B₁ receptor up-regulation. In this study, we demonstrate that Pg-LPS injection into the rat paw resulted in a marked functional up-regulation of B₁ receptors (as measured by an increase of B₁ receptor-induced edema), which was preceded by a rapid rise in B₁ receptor mRNA expression. The local administration of Pg-LPS also resulted in a prominent production of the proinflammatory cytokine tumor necrosis factor α (TNF-α), followed by an increase of neutrophil influx; both events were observed at periods before B₁ receptor induction. The functional and molecular Pg-LPS-elicited B₁ receptor up-regulation was significantly reduced by the glucocorticoid dexamethasone (0.5 mg/kg s.c.), and to a lesser extent by the chimeric anti-TNF-α antibody infliximab (1 mg/kg s.c.). Of high relevance, we show for the first time that a single administration of the proresolution lipid mediator (5S,12R,18R)-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid (resolvin E1; 300 ng/rat i.p.) was able to markedly down-regulate Pg-LPS-driven B₁ receptor expression, probably by inhibiting TNF-α production and neutrophil migration. Collectively, the present findings clearly suggest that Pg-LPS is able to induce the up-regulation of B₁ receptors through mechanisms involving TNF-α release and neutrophil influx, which are largely sensitive to resolvin E1. It is tempting to suggest that kinin B₁ receptors might well represent a pivotal pathway for the inflammatory responses evoked by P. gingivalis and its virulence factors.

Kinins are a group of biologically active peptides involved in several physiological and pathological conditions, such as vasodilatation, increased vascular permeability, and cellular migration (Calixto et al., 2004). The actions of kinins are mediated by the activation of two different G-protein-coupled receptors, named B₁ and B₂ (Marceau et al., 1998; Calixto et al., 2004). In general, B₂ receptors are expressed in a constitutive manner, mediating most of the physiological actions evoked by kinins, and exhibiting higher affinity for bradykinin (BK) and kallidin (Calixto et al., 2004). In contrast, B₁ receptors show high affinity for the active metabolites des-Arg⁹-BK and des-Arg¹⁰-kallidin. They are usually absent under normal conditions, but can be up-regulated after tissue injury and during inflammatory and infectious diseases (Marceau et al., 1998; Calixto et al., 2004).

Periodontal disease is a chronic infection that causes gingival inflammation and destruction of the supporting structure of the teeth, leading to bone resorption and tooth loss (Nishida et al., 2001; Carayol et al., 2006; Ohno et al., 2008). Specific groups of bacteria have been associated with periodontal destruction; among them, Porphyromonas gingivalis is a Gram-negative strain that harbors well known virulence factors with pathogenic potential. The host immune response

ABBREVIATIONS: AUC, area under the time-response curve; BK, bradykinin; MPO, myeloperoxidase; LPS, lipopolysaccharide; Pg-LPS, Porphyromonas gingivalis LPS; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; TLR, Toll-like receptor; TNF-α, tumor necrosis factor α; ELISA, enzyme-linked immunosorbent assay; resolvin E1, RvE1, (5S,12R,18R)-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid.
to bacterial products, such as lipopolysaccharide (LPS), is a key to the establishment and progression of periodontal tissue destruction (Carayol et al., 2006). Toll-like receptors (TLRs) are pattern recognition receptors that distinguish microbial structures, which generate cytokine-based responses involved in the induction of adaptive immunity. LPS from Gram-negative enterobacteria commonly signals through TLR4; however, *P. gingivalis* LPS (Pg-LPS) seems to signal mainly via TLR2 (Burns et al., 2006; Zhou and Amar, 2007; Hajishengallis et al., 2008). TLR2 activation by Pg-LPS triggers the downstream stimulation of a myriad of second messengers and transcription factors, leading to the release of proinflammatory cytokines, such as TNF-α and interleukin-1β (Muthukuru et al., 2005; Kikkert et al., 2007). Few studies have demonstrated a possible connection between periodontal pathogens and kinin production in a process involving B2 receptor activation (Imamura et al., 2004; Hu et al., 2006; Brechter et al., 2008); nevertheless, there is no available evidence showing how Pg-LPS might lead to kinin B2 receptor modulation.

The rat paw edema is a very well characterized model for studying the *in vivo* up-regulation of B1 receptors after several stimuli, including the local administration of LPS from *Escherichia coli* (Campos et al., 2006). The present study was aimed at investigating whether the local treatment with the periodontal pathogen Pg-LPS might induce functional and molecular up-regulation of the kinin B1 receptors by use of the rat paw edema experimental paradigm.

Resolvin E1, recently identified as an omega-3 eicosapentaenoic acid derivative lipid mediator generated during the resolution phase of inflammation, has proved to be a potent inhibitor of neutrophil migration and cytokine production *in vivo* (Serhan et al., 2000, 2002). In our study, we have also evaluated how resolvin E1 can modulate kinin B1 receptors up-regulation after Pg-LPS local administration.

**Materials and Methods**

**Drugs and Chemical Reagents.** The following drugs and reagents were used: LPS from *P. gingivalis* (InvivoGen, San Diego, CA); dexamethasone, EDTA, hexadecyltrimethyl ammonium bromide, tetramethylbenzidine, phenylmethylsulfonyl fluoride, benzamethonium chloride, aprotinin A (Sigma-Aldrich, St. Louis, MO); infliximab (Remicade, Centocor, Horsham, PA); resolvin E1 (Cayman Chemical, Ann Arbor, MI); des-Arg9-BK (H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-OH; Bachem Biosciences, King of Prussia, PA); NaPO4, hydrogen peroxide, NaCl, and Tween 20 (all from Merck, Haar, Germany). Most drugs were prepared and stocked in saline solution (NaCl 0.9%) or phosphate-buffered saline (PBS), except resolvin E1 that was provided in absolute ethanol. The final concentration of ethanol never exceeded 0.1% and did not display any effect per se.

**Animals.** In this study, nonfasted male Wistar rats (6–8 per group, 140–180 g) obtained from the Central Biotery of Universidade Federal de Pelotas (Brazil) were used. The animals were housed in groups of five and maintained in a temperature (22 ± 2°C) and humidity-controlled room (60–80%) with a 12/12 h light/dark cycle (lights on at 7:00 AM), and food and water were available ad libitum. Rats were adapted at the laboratory for a period of 1 h before experimental procedures. Tests were performed between 8:00 AM and 6:00 PM. Each animal was used only once, and was immediately euthanized at the end of the experimental period by isoflurane inhalation. The reported experiments were conducted in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigations of experiments in conscious animals (Zimmermann, 1983) and were preapproved by the Institutional Ethics Committee (Comité de Ética para o Uso de Animais Pontificia Universidade Catolica do Rio Grande do Sul).

**B1 Receptor-Mediated Rat Paw Edema.** This series of experiments was accomplished according to the method described by Passos et al. (2004), with minor modifications. In brief, the animals received a 0.1 ml intradermal (i.d.) injection in one hind paw (right paw) of PBS (composition: 137 mM NaCl, 2.7 mM KCl, and 10 mM phosphate buffer) containing the selective kinin B1 receptor agonist methyl-[9-2H]des-Arg9-BK (100 nmol/paw). The contralateral paw (left paw) received 0.1 ml of PBS and was used as the control. Edema was measured with a plethysmometer (Ugo Basile, Comerio, Italy), at several time points (10, 20, 30, 60, and 120 min) after injection of des-Arg9-BK. The edema is expressed in milliliters as the difference between the right and left paws.

In most experiments, animals were locally pretreated with Pg-LPS (3 μg/paw diluted in PBS; 1–72 h beforehand) at the same site of des-Arg9-BK injection. Control animals received the same volume of PBS solution (0.1 ml). The dose of Pg-LPS was selected on the basis of pilot experiments and did not evoke any significant alteration of paw volume per se. In all experiments, the intradermal injections were performed under slight anesthesia with isoflurane (1 ml/ml). Technical information on Pg-LPS used in the present study indicates that at low concentrations (<1 μg/ml), it induces TLR2 activation, and at higher concentrations (>10 μg/ml), it induces marked TLR2 and weaker TLR4 stimulation (Darveau et al., 2004).

**Mechanisms Responsible for Functional B1 Up-Regulation in Rats Pretreated with Pg-LPS.** To determine some of the possible mechanisms underlying the up-regulation of des-Arg9-BK-induced paw edema after Pg-LPS local administration, separate groups of animals were pretreated systemically with the anti-inflammatory steroid dexamethasone (0.5 mg/kg s.c., 2 h before Pg-LPS administration), the chimeric monoclonal anti-TNF-α antibody infliximab (1 mg/kg s.c., 15 min before Pg-LPS), or the proresolubilization lipid mediator resolvin E1 (RvE1, 300 ng/rat i.p., 30 min before Pg-LPS). Control animals received the vehicle at the same schedules of treatment. In these experimental sets, edema caused by des-Arg9-BK (100 nmol/paw) was evaluated 24 h after Pg-LPS (3 μg/paw) treatment, as Pg-LPS-induced functional up-regulation of B1 receptors peaked between 6 and 36 h.

The doses of inhibitors were selected based on previous studies and pilot experiments. These doses were demonstrated to be effective in different in vivo models of inflammation (Passos et al., 2004; Schwab et al., 2007; Seadi Pereira et al., 2009).

**Expression of B1 Receptor mRNA in the Rat Paw.** The expression of B1 receptor mRNA was measured by real-time PCR, following the methodology described by Batista et al. (2005). Rats were treated with Pg-LPS (3 μg/paw) and were euthanized at different intervals of time (1–5 h). PBS-treated paws were used as control. After euthanasia, the subcutaneous tissue of the paws was removed in RNase-free conditions and transferred to tubes containing RNA stabilization reagent (RNA later; Ambion, Austin, TX). Immediately thereafter, the tissues were processed according to the protocol of a RNA purification commercial kit (RNasy, Qiagen, Valencia, CA). Tissues were initially frozen in liquid nitrogen and ground with a mortar and pestle. RNA samples were then homogenized, and total RNA was isolated. All RNA samples were subjected to on-column DNase I (Sigma-Aldrich) treatment to remove trace amounts of genomic DNA. RNA concentrations and purity were determined spectrophotometrically at 260 and 260:280 nm, respectively. Samples presenting 260:280 ratios of 1.8 or higher were included in the analysis. RNA quality was assessed through formaldehyde denaturing 1.2% agarose gels stained with SybrGold (Molecular Probes, Eugene, OR) to check for the presence of clear 18S and 28S bands, and no smearing. For cDNA synthesis, 200 ng of total RNA was primed with random hexamers and reverse transcribed with use of an AML-V Reverse Transcriptase Kit (TaqMan Reverse Transcription Kit; Applied Biosystems, Foster City, CA). Quantification of B1 re-
ceptor mRNA was performed through fluorescence-based real-time PCR. To this end, approximately 100 ng of cDNA were amplified in duplicates by use of TaqMan-based chemistry with specific primers and FAM-labeled probes for rat kinin B1 receptor (Assays-on-Demand, Applied Biosystems), and β-actin as an endogenous control for normalization (Endogenous Controls, Applied Biosystems). The reaction plate was run in duplicate for every condition. Amplifications were performed in a thermal cycler (ABI 7500, Applied Biosystems) for 50 cycles; the fluorescence was collected at each amplification cycle and the data analyzed by the 2^ΔΔCt method for expression relative quantification. Before indicating this method, validation of the assays and efficiency of amplification of rat β-actin and kinin B1 receptors were calculated through a 10-fold serial dilution of Pg-LPS-treated rat paw cDNA (not shown); the slope values of log input amounts plotted against ΔCt (mean Ct receptor − mean Ct β-actin) for both, target gene and endogenous control, were found to be within acceptable values, making it suitable for the use of the 2^ΔΔCt method. Expression of the target genes was calibrated against conditions found in naive animals.

In a separate series of experiments, different groups of animals were pretreated with dexamethasone or resolvin E1, at the same doses and time periods indicated earlier. Other groups of rats received dexamethasone, infliximab, resolvin E1, or vehicle, at the same doses and time periods indicated earlier. The biochemical assay for determining MPO activity was the same as described in this section. The tissues were collected at 3 h, because the increase of MPO activity peaked between 3 and 12 h after Pg-LPS administration.

**Measurement of TNF-α Levels in the Rat Paw.** TNF-α production in the rat paw was measured as described by Passos et al. (2004). The animals were locally treated with Pg-LPS (3 μg/paw; 1–3 h before euthanasia), and had the subcutaneous tissue of the right hindpaw removed and placed on a PBS solution containing 0.05% Tween 20, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine chloride, 10 mM EDTA, and 20 KI units of aprotinin A. PBS-treated paws were used as control. Tissues were homogenized and centrifuged at 5000g for 10 min, and the supernatant was collected and stored at −80°C for further analysis. The levels of TNF-α were evaluated via a standard sandwich ELISA protocol (DuoSet Kit; R&D Systems, Minneapolis, MN).

Separate experimental groups were pretreated with dexamethasone, infliximab, resolvin E1, or vehicle solution, at the same doses and intervals of time, as described earlier. After 2 h of dexamethasone administration or 15 min of treatment with resolvin E1, the animals received an injection of Pg-LPS (3 μg/paw i.d.), and they were euthanized 1 h later. This time point was selected considering the maximal increase of TNF-α levels, after local treatment with Pg-LPS. The tissue processing and ELISA experiments were performed as described previously.

**Neutrophil Myeloperoxidase Assay.** Neutrophil recruitment to the rat paw was measured by means of tissue myeloperoxidase (MPO) activity, determined according to Passos et al. (2004). Animals received an injection of Pg-LPS (3 μg/paw i.d.) in the right paw and were euthanized at different intervals of time (1–36 h). PBS-treated paws were used as control. The subcutaneous tissue of the paws was removed, homogenized at 5% (w/v) in EDTA/NaCl buffer, pH 4.7, and centrifuged at 5000g for 20 min at 4°C. The pellet was resuspended in 0.5% hexadecyltrimethyl ammonium bromide buffer, pH 5.4, and the samples were frozen. Upon thawing, the samples were recentrifuged and 25 μl of the supernatant were used for MPO assay. The enzymatic reaction was assessed with 1.6 mM tetramethylbenzidine, 80 mM NaPO₄, and 0.3 mM hydrogen peroxide. The absorbance was measured at 595 nm, and the results are expressed as optical density per milligram of tissue.

To determine some of the mechanisms responsible for the increased MPO activity after Pg-LPS intradermal administration, other groups of rats received dexamethasone, infliximab, resolvin E1, or vehicle, at the same doses and time periods indicated earlier. The biochemical assay for determining MPO activity was the same as described in this section. The tissues were collected at 3 h, because the increase of MPO activity peaked between 3 and 12 h after Pg-LPS administration.

**Statistical Analysis.** Most results are presented as the mean ± S.E.M. of five to eight animals per group. For the real-time PCR experiments, the results are given as the mean ± S.E.M. of three independent experiments. Statistical comparison of the data was performed by one-way analysis of variance (ANOVA) followed by Bonferroni’s test, or unpaired Student’s t test when appropriate. P values smaller than 0.05 (P < 0.05) indicated significance. Total inhibitions of the edematogenic responses are given as the difference (in percentage) between the areas under the time-response curve (AUC) of the drug-treated group in relation to the corresponding control group. The area under the curve was also used for demonstrating the time-related effects of Pg-LPS treatment on des-Arg²-BK-induced edema formation.

**Results**

**Modulation of Kinin B₁ Receptors after Pg-LPS Treatment.** Our data show that intradermal injection of the selective kinin receptor agonist des-Arg²-BK (100 nmol/paw) in control animals produced a small increase in rat paw volume (0.24 ± 0.008 ml). In contrast, intradermal administration of des-Arg²-BK in animals locally pretreated with Pg-LPS (3 μg/paw; 3–72 h) resulted in marked formation of rat paw edema (Fig. 1A). The analysis of the AUC revealed that Pg-LPS effects on B₁ receptor-mediated edema present a time-related profile (Fig. 1B). Accordingly, des-Arg²-BK-evoked edema reached its peak between 6 and 36 h after Pg-LPS administration, decreasing after 72 h of Pg-LPS treatment. Therefore, the 24-h time point (57 ± 9% of in-
crease) was chosen for the subsequent studies of functional B1 receptor up-regulation.

In another set of experiments, to evaluate some of the mechanisms implicated in kinin B1 receptor regulation, we pretreated animals with the anti-inflammatory steroid dexamethasone (0.5 mg/kg s.c.) 2 h before Pg-LPS injection. The edema induced by des-Arg⁹-BK (100 nmol/paw) in rats pretreated with Pg-LPS (3 μg/paw, 24 h previously) was significantly inhibited by the systemic administration of dexamethasone (Fig. 2A). The percentage of inhibition observed for dexamethasone treatment was 39 ± 9%, as calculated on the basis of the AUC. To determine the possible involvement of TNF-α in Pg-LPS-induced B1 receptor up-regulation, we have used the anti-TNF-α chimeric monoclonal antibody infliximab (1 mg/kg s.c.), dosed 15 min before Pg-LPS. Data revealed that des-Arg⁹-BK (100 nmol/paw)-evoked edema after Pg-LPS treatment (3 μg/paw, 24 h) was partially, but significantly inhibited by systemic pretreatment with infliximab (Fig. 2B), with an inhibition percentage of 24 ± 5%, as estimated based on the AUC. Finally, we have evaluated to what extent the proresolution lipid mediator resolvin E1 might prevent Pg-LPS-induced functional B1 receptor up-regulation. For this purpose, animals received a single intraperitoneal injection of resolvin E1 (300 ng/animal), 30 min before Pg-LPS treatment (3 μg/paw), and the edema was induced 24 h later by the B1 receptor agonist des-Arg⁹-BK. This strategy was able to markedly decrease the edema formation elicited by des-Arg⁹-BK (100 nmol/paw) in rats injected with Pg-LPS (3 μg/paw) (Fig. 2C), with an inhibition percentage of 43 ± 7%, as calculated by use of the AUC.

B1 Receptor mRNA Expression. The changes in kinin B1 receptor mRNA expression after Pg-LPS local treatment are presented in Fig. 3A. Bar graphs represent variations relative to control animals; therefore, they reflect fold changes relative to basal levels of B1 receptor mRNA. The intraplantar injection of Pg-LPS (3 μg/paw) produced a marked and time-related increase of B1 receptor mRNA expression in the rat subcutaneous paw tissue, which was evident as early as at 1 h after LPS administration (5-fold increase), and reached its peak at 3 h after (17-fold increase), decreasing after 5 h. Therefore, in assessing Pg-LPS B1 receptor expression in response to different anti-inflammatory compounds we adopted the 3-h time point as the cutoff. Pretreatment of the animals with the glucocorticoid dexamethasone was able to reduce the expression of B1 mRNA by 85 ± 4%; infliximab reduced the expression of B1 mRNA by 61 ± 23%. Note that B1 receptor mRNA expression was virtually abolished by the proresolution lipid mediator resolvin E1 (96 ± 3%) (Fig. 3B).

TNF-α Levels. TNF-α is a proinflammatory cytokine with multiple biological actions, which is up-regulated by infectious stimuli (Rocha et al., 2006). The relevance of TNF-α production for the up-regulation of kinin B1 receptors in the rat paw was further assessed by ELISA analysis, at different intervals of time (1–5 h) after Pg-LPS local treatment. The results depicted in Fig. 4A indicate that Pg-LPS administration induced a significant increase in TNF-α levels in the subcutaneous paw tissue, which reached maximal effect at 1 h (approximately 8-fold). In contrast, undetectable or very low levels of TNF-α were found in control animals. The administration of dexamethasone (0.5 mg/kg s.c., 2 h before Pg-LPS), or resolvin E1 (300 ng/animal, 30 min before Pg-LPS) significantly reduced the augmentation of TNF-α levels in response to Pg-LPS (as shown in Fig. 4B); the percentages of inhibition were 54 ± 7.5% and 96 ± 4%, respectively. However, infliximab failed to significantly affect TNF-α production (results not shown).

Relevance of Neutrophil Influx. The migration of neutrophils to the rat paw in response to Pg-LPS (3 μg/paw) treatment was evaluated indirectly by means of MPO activ-
ity assay. As shown in Fig. 5A, Pg-LPS injection (1–36 h) was capable of inducing a time-related increase in MPO levels, reaching the maximal values between 3 and 12 h, with an approximately 2-fold augmentation compared with the control group. The increase in MPO activity at 3 h was significantly reduced by the pretreatment with dexamethasone (0.5 mg/kg s.c., 2 h) and infliximab (1 mg/kg s.c., 15 min), or resolvin E1 (300 ng/rat i.p., 30 min) on B1 receptor mRNA expression in rats pretreated with Pg-LPS (3 μg/paw, 3 h). Naive indicates no previous treatment with Pg-LPS. Each column represents the mean ± S.E.M. of three independent experiments, and the vertical lines are the S.E.M. **, p < 0.01, significantly different from control paws; ###, p < 0.01, significantly different from LPS-injected paws.

**Discussion**

Kinin B1 receptors are atypical G-protein-coupled receptors that are not constitutive in general, because they are highly up-regulated after stressful stimuli (Calixto et al., 2004). The induction of this receptor has been associated with generation of inflammatory cytokines, neutrophil migration, and activation of several signaling pathways (Passos et al., 2004; Medeiros et al., 2007). Compelling in vivo and in vitro evidence indicates that kinin B1 receptors can be up-regulated by infectious stimuli (Calixto et al., 2004; Campos et al., 2006). A few reports have linked periodontal pathogens and kinin B2 receptors (Griesbacher et al., 1994; Rubinstein et al., 2001; Hu et al., 2006; Brechter et al., 2008). Nevertheless, there is no evidence showing whether Pg-LPS might lead to the up-regulation of B1 receptors. Our study indicates, for the first time, that B1 receptors can be up-regulated after in vivo administration of Pg-LPS by mechanisms sensitive to the proresolving mediator resolvin E1.

Previous data from our group demonstrated that local treatment with E. coli LPS resulted in a functional up-regulation of B1 receptors in the rat paw, as assessed by an increase in des-Arg9-BK-induced edema (Passos et al., 2004).
In this publication, des-Arg9-BK-caused edema peaked at 12 h, and then decreased gradually between 24 and 36 h. The present results show that Pg-LPS can induce a marked increase in the rat paw edema elicited by des-Arg9-BK, an effect that was significant between h and 36 h. Literature data have suggested that LPS from *E. coli* preferentially activates TLR4, whereas Pg-LPS displays greater affinity for TLR2 receptors (Darveau et al., 2004; Muthukuru et al., 2005; Kikkert et al., 2007). Furthermore, some publications have pointed to the activation of differential signaling pathways after TLR2 and TLR4 stimulation (Burns et al., 2006; Zhou et al., 2007; Hajishengallis et al., 2008). Concerning B1 receptors, it seems that both Pg-LPS and *E. coli* LPS are able to produce their up-regulation, although slight temporal and intensity differences are observed.

Dexamethasone is a glucocorticoid that displays several anti-inflammatory actions via genomic and nongenomic means, and it might block TLR signaling by multiple mechanisms (Chinenov and Rogatsky, 2007; Stahn and Buttgerleit, 2008). Several studies have demonstrated that dexamethasone is able to block B1 receptor up-regulation induced by infectious stimuli (Calixto et al., 2004, Passos et al., 2004). Our data show that dexamethasone markedly reduced des-Arg9-BK-induced edema after Pg-LPS treatment, suggesting that B1 receptor up-regulation is probably related to de novo protein synthesis. This idea was further confirmed by results showing that functional up-regulation of B1 receptors was preceded by an increase of B1 receptor mRNA expression (maximal at 3 h), which was markedly prevented by dexamethasone.

The chimeric anti-TNF-α antibody infliximab produced a partial, but significant inhibition of edema induced by des-Arg9-BK in Pg-LPS-pretreated rats. This suggests that B1 receptor functional up-regulation by Pg-LPS is a process that depends, at least in part, on the release of TNF-α. The relevance of TNF-α for up-regulation of B1 receptors has been also demonstrated elsewhere (Passos et al., 2004; Rocha et al., 2006). Extending our in vivo data, the increased B1 receptor mRNA expression after Pg-LPS treatment was sensitive to infliximab administration. Relevantly, it was demonstrated that TLR2 activation by Pg-LPS leads to TNF-α up-regulation by the stimulation of transduction pathways distinct from that of *E. coli* LPS; whereas TLR2/JNK is the main pathway for Pg-LPS, the induction by *E. coli* LPS is mediated by TLR4/NF-κB/p38MAPK pathways (Zhang et al., 2008).

It has been demonstrated that resolvin E1 orchestrates the resolution of inflammation by promoting tissue homeostasis (Serhan et al., 2008). As reviewed recently (Serhan et al., 2008; Van Dyke, 2008), resolvin E1 prevents neutrophil infiltration and stimulates the phagocytic activity of macrophages, increasing the exit of inflammatory cells from the inflamed site. Previous results on a rabbit model of periodontitis demonstrated an important role for resolvin E1 in the inhibition of alveolar bone resorption (Hasturk et al., 2007). A recent in vitro study demonstrated that resolvin E1 is able to limit osteoclast growth and bone resorption (Herrera et al., 2008). In our article, we describe further mechanisms for resolvin E1 actions in the responses evoked by periodontal pathogens. We demonstrate, for the first time, the ability of resolvin E1 in reducing des-Arg9-BK-evoked paw edema in animals pretreated with Pg-LPS. In addition, the administration of a single dose of resolvin E1 almost abolished the increase of B1 receptor mRNA expression elicited by Pg-LPS. It is possible to propose that resolvin E1 prevents the inflammatory responses evoked by the selective B1 receptor agonist des-Arg9-BK, mainly by reducing B1 receptor mRNA expression. Of course, we cannot rule out that resolvin E1 might interfere in other levels of B1 receptor regulation.

It would be very pertinent to determine whether resolvin E1 is more potent than dexamethasone or infliximab in preventing B1 receptor up-regulation. Pilot experiments (data not shown) revealed that no clear dose-response effects were observed when additional doses of dexamethasone (2 mg/kg s.c.), infliximab (10 mg/kg s.c.), or resolvin E1 (100 and 500 ng/rat) were tested on B1 receptor-mediated edema. A lack of dose-related effects for these inhibitors had been shown previously (Campos et al., 1996; Triantafillidis et al., 2005; Haas-Stepleton et al., 2007; Schwab et al., 2007; Seadi Pereira et al., 2009). Therefore, although the results suggest that resolvin E1 is more potent than dexamethasone and infliximab in preventing B1 receptor up-regulation, a comparison at the level of ID50 values is not possible.

Our data show that Pg-LPS injection into the rat paw...
resulted in a marked and time-related increase of TNF-α production, which was maximal at 1 h. This temporal profile was very similar to that observed after E. coli LPS treatment, where TNF-α levels were maximal after 1 h in a process sensitive to dexamethasone treatment (Passos et al., 2004). In our study, the increase of TNF-α levels in response to Pg-LPS injection was significantly diminished by dexamethasone and resolvin E1. It was demonstrated that resolvin E1 reduced the leukocyte infiltration, and prevented TNF-α gene expression in a mouse model of colitis (Arita et al., 2005). In this context, we believe that part of the inhibitory effects of Resolvin E1 on B1 receptors rely on the reduction of TNF-α production. However, the treatment with infliximab failed to significantly reduce the increase of TNF-α levels 1 h after Pg-LPS injection, at least in our experimental conditions and according to evaluation by ELISA experiments after Pg-LPS injection, at least in our experimental conditions (Fernandes et al., 2005), which demonstrated that TNF-α receptor up-regulation by platelet-activating factor (Passos et al., 2004; Fernandes et al., 2005). Previous data demonstrate that injection E. coli LPS into the rat paw induced a time-related increase in MPO levels, which peaked at 12 h and lasted for up to 36 h (Passos et al., 2004). The results provided herein show that Pg-LPS induced a marked and earlier increase of MPO activity, which was found significant at 3 h, and remained increased for up to 12 h. The temporal profile for increased MPO activity by Pg-LPS was consistent with the up-regulation of B1 receptor-mediated edema, which suggests that neutrophils might provide signals for B1 receptor modulation. Interestingly, the elevation of MPO activity induced by Pg-LPS was significantly reduced by pretreatment with dexamethasone, although infliximab failed to significantly affect this parameter. It is possible to surmise that TNF-α increase and neutrophil influx independently contribute to the up-regulation of B1 receptors by Pg-LPS. Our findings extend previous literature data (Fernandes et al., 2005), which demonstrated that TNF-α production and neutrophil migration are independent events which are leading to B1 receptor up-regulation by platelet-activating factor in the rat paw.

In our study, resolvin E1 markedly reduced the increase of MPO activity in response to Pg-LPS injection. These results are consistent with previous evidence showing that resolvin E1 is able to prevent neutrophil infiltration (Hasturk et al., 2007; Serhan et al., 2008). It was demonstrated that resolvin E1 rapidly reduces the leukocyte rolling in mouse venules and it was also found effective in preventing transepithelial migration of isolated human neutrophils (Campbell et al., 2007; Dona et al., 2008). It is alluring to propose that the inhibitory effects of resolvin E1 on B1 receptor modulation are possibly related to the inhibition of neutrophil migration.

Altogether, our data suggest the following sequence of events leading to B1 receptor up-regulation by Pg-LPS in vivo: there is a rapid increase of TNF-α levels, followed by increased neutrophil migration, leading to the up-regulation of B1 receptors. Of interest, the proresolvent mediator resolvin E1 is able to down-regulate B1 receptor expression by reducing both TNF-α release and neutrophil influx. These findings shed new light on the mechanisms underlying B1 receptor modulation, indicating that this receptor subtype might represent a relevant pathway for the inflammatory responses evoked by periodontal pathogens.

Acknowledgments

We thank Juliano Soares for excellent technical assistance.

References


Nishida E, Hara Y, Kaneko T, Ikeda Y, Uki T, and Kato I (2001) Bone resorption and local interleukin-1α and interleukin-1β synthesis induced by Actinobac-

Downloaded from jpet.aspetjournals.org at ASPET Journals on January 22, 2018


Address correspondence to: Dr. Maria Martha Campos, School of Dentistry, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6881, Partenon, 90619-900, Porto Alegre, Brazil. E-mail: camposmartha@yahoo.com or maria.campos@pucrs.br.