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What is This?
Kinin Generation in the Gingival Inflammatory Response to Topically Applied Bacterial Lipopolysaccharides

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A water-soluble lipopolysaccharide from Salmonella enteritidis and a phenol-soluble lipopolysaccharide from Leptotrichia buccalis were applied topically to the healthy marginal gingiva of beagle dogs. Saline was applied to contralateral areas as an internal control. Increases in vascular permeability were monitored by measurement of gingival fluid, and the collected gingival fluid samples were assayed for kininogenase and kinin activities. Both lipopolysaccharides induced an inflammatory response, as indicated by increased gingival fluid flow. Kininogenase-kinin activities paralleled the increases in gingival fluid flow, with the highest values being associated with peak increases in gingival fluid. The results indicate that both lipopolysaccharides, although different in lipid solubility, penetrate healthy sulcular epithelium and initiate an inflammatory response which is mediated in part by the kallikrein-kinin system. Interrelationships between this system and other inflammatory mediators suggest that kinin generation not only plays a role in the early phases of acute gingival inflammation, but may also contribute to the activation of other mediators appearing later in the response and in chronic inflammatory lesions.


Introduction.

Bacterial lipopolysaccharide (LPS), the endotoxin moiety associated with Gram-negative bacteria, has long been implicated in inflammatory periodontal disease in humans and other animals (Daly et al., 1980). The initiation and increasing severity of gingival inflammation have been correlated with a change in the subgingival dental plaque from that of a predominately Gram-positive bacterial flora to an endotoxin-containing Gram-negative population (Theilade et al., 1966; Socransky, 1977). In addition, a close correlation between the levels of endotoxin activity in subgingival plaque and severity of gingival disease has been reported by several investigators (Simon et al., 1971; Simon et al., 1972; Shapiro et al., 1972; Fine et al., 1978).

These macromolecules are multipotent initiators of inflammation. Lipopolysaccharides have direct effects on platelets, neutrophils, mast cells, monocytes, macrophages, and endothelial cells, and also affect at least two major humoral systems [complement and coagulation (Morrison and Ulevitch, 1978)]. LPS has been shown to penetrate healthy intact gingival tissue and to initiate vascular changes indicative of an acute inflammatory response (Ranney and Montgomery, 1973). Although all of these activities could be expressed in the gingival tissues and could contribute to inflammatory periodontal disease, the role of LPS in the pathogenesis of periodontal disease has not been completely defined. It was the purpose of this investigation to study the acute gingival inflammatory response following topical application of LPS extracted from Salmonella enteritidis and Leptotrichia buccalis by assessing the changes in gingival fluid flow and to evaluate the role of a kinin-generating system in the development of this response.

Materials and methods.

Beagle dog gingiva model. — Male beagle dogs, each from 10 to 12 months old, with minimal gingival pigmentation were used to assess the inflammatory activity of the topically applied lipopolysaccharides. Initial assessment of the gingival health of the newly received animals indicated slight marginal gingivitis and supra-gingival deposits of plaque and calculus, but there were no pockets or signs of more severe periodontal involvement. After the animals' teeth were scaled and polished, their gingival health was maintained by means of a hard diet (Wayne Dog Chow, Chunk Size²) and by daily toothbrushing. The animals were used for experimentation after clinically healthy gingiva had been maintained for at least a four-week period. Determination of gingival health was based on gross appearance and initial control measurements of gingival fluid.

The dogs were anesthetized with sodium pentobarbital (Nembutal® Sodium²), 30 mg/kg body weight, administered intravenously. An in-dwelling catheter was used to provide a continuous infusion of physiological saline, and supplementary doses of pentobarbital were given as necessary to provide an uninterrupted light anesthesia. Patency of the airway was maintained by endotracheal intubation. Body temperature was monitored rectally and supported by heating pads and blankets as needed. Jaws were held open by a bite block in the incisor area, and the mandibular molar areas were isolated by placement of gauze packs beneath the tongue and into the buccal vestibule. The lower lip was retracted to provide a continuously dry field in the posterior mandibular quadrants.

Increase in vascular permeability was assessed by collection of the gingival fluid with filter paper strips (2 mm width by 5 mm length; Whatman Chromatography paper, No. 1)² placed at the gingival margin. The filter paper strips were changed every ten minutes. The volume of gingival fluid was determined by measuring the height of rise, to the nearest 0.5 mm, of fluid on the paper strips and converting this figure to microliters using a conversion factor based on the height of rise of specific volumes of dog plasma. The amount of gingival fluid collected was totaled for each 60-minute period during the experiment. The permanent lower first molar of one side served as the experimental application site, while the contralateral area served as an internal control site. This contralateral control served not only as a vehicle control, but also as an indicator of any changes in the degree of gingival inflammation resulting from insertion of the filter paper strips, drying effects on the gingiva, and/or exposure to other environmental factors.

An initial control collection of gingival fluid for 60 minutes was performed so that the clinical health of the gingiva could

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1Allied Mills, Inc., Chicago, IL
2Abbott Pharmaceuticals, Inc., North Chicago, IL
3Whatman, Inc., Clifton, NJ
be assessed. In all of the experiments completed, this initial control value for gingival fluid was less than 1 μl/hr and was zero in most cases. After the initial 60-minute control collection of gingival fluid, test or control solutions were applied topically with a Hamilton4 microsyringe and a 30-gauge needle. The solutions were applied in a bead (approximately 5 μl) on the tooth surface superior to the gingival margin and allowed to flow into the gingival sulcus. The application site was kept moist by applying the solution at a rate approximately equal to its disappearance until the total volume had been applied. After application and absorption of the experimental and control samples, gingival fluid was collected, as described above, beginning at the end of the second hour after application and continuing for the duration of the experiment. The filter paper strips from each hourly collection period were placed into 1.0 ml of de Jalon solution (Gaddum and Lembeck, 1949), pH 6.5, containing 1,10 phenanthroline (o-phenanthroline)5, 10−3 M, to inhibit kininase activity.

Application of lipopolysaccharides. — A commercial preparation of Salmonella enteritidis lipopolysaccharide6 was dissolved in 0.9% saline at a concentration of 1 mg/ml. Fifty microliters of this solution were applied topically to the buccal aspect of the first mandibular molar on one side as described above. An equal volume of 0.9% saline was applied to the contralateral molar. After absorption, the increase in vascular permeability was monitored by determination of gingival fluid flow rates as described above.

In another series of experiments, a phenol-soluble lipopolysaccharide prepared from Leptotrichia buccalis was tested (Knox and Parker, 1973). The compound was freed from phenol by exhaustive dialysis and re-dissolved in 0.9% saline at a concentration of 1 mg/ml. Fifty microliters of this solution were applied to the permanent first lower molar as described above. Fifty microliters of 0.9% saline were applied to the contralateral region. After absorption, gingival fluid flow rates were monitored and totaled hourly as described above.

Kininogenase activity. — The filter paper strips were removed from the de Jalon solution, and the samples were centrifuged. Fifty-microliter aliquots of the sample were assayed for kininogenase (esterase) activity according to the spectrophotofluorimetric procedure of Trautschold et al. (1974), with benzoyl-l-arginine ethyl ester (BAEE)7 as the substrate. The procedure measures the alkaline hydrolysis fluorescent product of NADH generated by the action of alcohol dehydrogenase and NAD+ on the cleaved ethanol from BAEE hydrolysis. Enzyme activity is expressed in milliunits, where one milliunit represents the hydrolysis of one nanomole of BAEE per minute at 25° C.

Kinin activity. — Assay of kinin activity was performed using the isolated rat uterus bioassay technique as modified by Krivoy and Kroeger (1964). Uterine strips from virgin Sprague-Dawley rats were suspended in a 1.5 ml bath, 30° C, containing de Jalon solution and atropine sulfate, 1.4 × 10−9 M, and pyrilamine maleate, 2.5 × 10−8 M, to inhibit the actions of acetylcholine and histamine, respectively. A two-dose bracketed assay was used in which two doses of a standard solution of bradykinin triacetate8 and two doses of gingival fluid sample were applied in a random order. The volumes of sample added to the bath ranged between 0.02 and 0.09 ml, and the final bath volume was adjusted to 1.5 ml. The contraction response was recorded using a linear motion transducer9 and chart recorder10. Kinin levels were determined by measuring the height of contraction after determining that the curves for the samples and standards were parallel (Krivoy and Kroeger, 1964). Only uterine strips sensitive to a bradykinin standard, 9.4 × 10−10 M or less, were used for the kinin bioassay. Further proof of kinin-like activity was accomplished by incubating the samples for one hour at 37° C with alpha-chymotrypsin11, 0.5 units/ml final concentration, or carboxypeptidase B11, 10−4 units/ml, final concentration, in 0.08 M Tris buffer, pH 7.4.

Results.

In all experiments, the initial control value for gingival fluid was less than 1 μl/hr; in most experiments this value was zero. The time required for application of the LPS and saline control solutions ranged between one and two hours. Saline control values of gingival fluid flow ranged between 1 and 2 microliters per hour.

Salmonella enteritidis lipopolysaccharide. — The acute gingival inflammatory response resulting from the topical application of 50 micrograms of S. enteritidis LPS is shown in Fig. 1. Each value on the curve represents the average of four animals and is expressed as the difference from the contralateral control. Therefore, the response indicated is that resulting from the effects of the LPS and not due to filter paper strips, vehicle, or drying effects on the oral environment. The degree of vascular permeability, as indicated by the increased volume of gingival fluid collected, showed a peak between five and six hours following the topical application of LPS. Gingival fluid flow began to diminish between seven and nine hours after LPS application, but was still elevated above baseline levels. A second increase in gingival fluid flow occurred between 10 and 12 hours after LPS application. The time course for kinin-like activity closely corresponded with that for vascular permeability, with peak kinin levels occurring at six hours and again ten hours after initial LPS application.

Leptotrichia buccalis lipopolysaccharide. — The acute inflammatory response of the gingiva following the topical application of 50 μg (50 μl) of a phenol-soluble L. buccalis LPS is shown in Fig. 2. Each value represents the average of eight experiments (seven animals) and is expressed as the difference from the saline control. Although there are some similarities to the response with S. enteritidis endotoxin, the time course of the response to L. buccalis LPS was monophasic, with peak increase in gingival fluid flow occurring eight hours after initial application. Kinin activity in the gingival fluid samples paralleled the increase in gingival fluid flow, with the highest kinin activity appearing after nine hours. Kininogenase activity was present in all of the experimental samples, with highest values occurring at three hours and eight hours after LPS application. The value at three hours preceded a sharp increase in kinin activity and gingival fluid flow. Likewise, the highest kininogenase value, occurring at eight hours, preceded the highest kinin activity found in the samples. There was a marked decrease in kinin activity after 13 hours, while gingival fluid rates were still elevated.

Discussion.

Bacterial lipopolysaccharides have been implicated repeatedly in periodontal disease, and the results of this study are

4Curtin Matheson Scientific, Inc., Houston, TX
5Sigma Chemical, St. Louis, MO
6Difco Laboratories, Detroit, MI
7Sigma Chemical, St. Louis, MO
8Calbiochem-Behring, San Diego, CA
9Phips and Bird, Inc., Richmond, VA
10Rikadenki, Soltec, Encino, CA
11Worthington Biochemical, Freehold, NJ
Kinin- and endotoxin-induced gingival inflammation

Fig. 1 — Time course of the gingival inflammatory response to topically applied Salmonella enteritidis lipopolysaccharide. Each open circle on the line graph is the mean of four experiments and indicates the amount of gingival fluid collected for each 60-minute period. Closed bars, kinin activity (nanograms per hour); vertical lines, S.E.M.; N = 4.

Fig. 2 — Time course of the gingival inflammatory response to topically applied Leptotrichia buccalis lipopolysaccharide. Each open circle on the line graph is the mean of eight experiments and indicates the amount of gingival fluid collected for each hour. Solid bars, kinin activity (nanograms/hour); open bars, kallikrein (kininogenase) activity (milliunits/hour); vertical lines, S.E.M.; N = 8.
consistent with the involvement of these compounds in this disease process. A number of reports indicate that LPS and other macromolecules can penetrate intact non-keratinized gingival sulcular epithelium (Schwartz et al., 1972; Ranney and Montgomery, 1973; Cimasoni, 1983). In the present investigation, two chemically different LPSs were studied—a classic water-soluble S. enteritidis LPS, and a phenol-soluble L. buccalis LPS. The former is postulated to cross cell membranes by virtue of the hydrophobic lipid moiety. L. buccalis LPS possesses hydrophobic deoxy-sugars which increase its lipid solubility and should facilitate its passage across cellular membranes (Knox and Parker, 1973). The results of the present study confirm that both types of lipopolysaccharide penetrate the intact gingival sulcular epithelium. This is indicated by the fact that topical application of these LPSs resulted in an increase in gingival fluid flow, which is primarily related to increased vascular permeability (Cimasoni, 1983), and the appearance of kinin activity.

Once LPS has entered the gingival tissue, it may induce numerous biological effects relevant to periodontal disease. It may be directly toxic (Chedid and Parant, 1971; De Renzis and Chen, 1983), stimulate macrophages to release collagenase (WahI et al., 1974), induce bone resorption (Hausmann, 1974), activate complement by the classic and alternative pathways (Morrison and Kline, 1977), act as a polyclonal B-cell activator (Smith et al., 1980) and activate Hageman factor, which in turn activates the clotting system and the plasma kallikrein-kinin system (Morrison and Cochrane, 1974). Activation of complement results in the appearance of kinin-like activity and products which are chemotactic for polymorphonuclear leukocytes (PMNs) and macrophages and are toxic to fibroblasts. Factors released from PMNs and macrophages attracted to the area may result in the breakdown of collagen and connective tissue ground substance and in the fibrogenesis seen in chronic inflammatory periodontal disease (Allison et al., 1976).

The results of the present investigation show that a kininogenous-kinin system was activated by these two LPSs. The observations that increases in kininogenase and kinin activities were associated with increases in gingival fluid flow for L. buccalis LPS and kinin activity for S. enteritidis LPS, and that kinin activity was highest at the times coinciding with peak gingival fluid flow rates, suggest a cause-and-effect relationship between this mediatory system and the increase in vascular permeability.

The finding that LPS from S. enteritidis consistently produced a biphasic time course of gingival fluid flow, in contrast to the monophasic response to L. buccalis LPS, may be due to the differences in potencies of LPS from the different species, a fact which is well-documented (Knox and Parker, 1973; Sven, 1977; Daly et al., 1980). However, these differences may be the result of activation of different host mechanisms involved in the inflammatory response. The plasma kallikrein-kinin system was instrumental in producing the response to both LPSs studied, since kinin activity quantitatively paralleled the gingival fluid flow. This does not preclude other factors and endogenous inflammatory mediators being involved in the response at various time periods, and the relative differences in degree of activation or interaction of these factors with other systems would influence the pattern of the response obtained. This is exemplified by the fact that gingival fluid flow remained elevated despite a marked decrease in kinin activity 13 hours after L. buccalis LPS application. This suggests activation of other endogenous inflammatory mediators. A sequential activation of histamine, kinins, and prostaglandins has been shown in certain types of acute inflammatory responses (Di Rosa et al., 1971; Di Rosa, 1972; Pedata et al., 1976).

Several reports indicate that bradykinin can directly activate phospholipase A2, which can then cleave arachidonic acid from membrane phospholipids (Hong and Levine, 1976; Juan, 1977). Metabolism of arachidonic acid by the cyclo-oxygenase pathway results in the formation of prostaglandins, and elevated levels of prostaglandin E2 have been found in inflamed gingival tissue and exudates (Goodson et al., 1974; ElAttar, 1976). Metabolism of arachidonic acid by the lipoxygenase pathway has been shown to be particularly active in inflamed gingiva, the major end-product being 12-hydroxy-eicosatetraenoic acid (12-HETE), a neutrophil chemotactic factor (ElAttar and Lin, 1983; Sidhagen et al., 1982). It is likely that a sequential activation of histamine, kinins, prostaglandins, and arachidonic acid metabolites via the lipoxygenase pathway is involved in the progression of gingival inflammation. Thus, the kallikrein-kinin system is not only involved in the earlier phases of acute gingival inflammation as shown by this investigation, but also may be contributory to the activation of other inflammatory mediators characteristic of chronic inflammatory lesions.

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