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The K1 Serotype Capsular Polysaccharide of *Porphyromonas gingivalis* Elicits Chemokine Production from Murine Macrophages That Facilitates Cell Migration▽

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***Porphyromonas gingivalis* is the principal organism associated with aggressive forms of generalized periodontal disease. Previous reports have suggested that encapsulated *P. gingivalis* strains are more virulent than unencapsulated strains; however, the contribution of capsular polysaccharide (CPS) to the virulence of this organism is poorly understood. Since periodontal disease presents with a complex inflammatory cell lesion comprised of neutrophils and monocytes, we cultured murine peritoneal macrophages with heat-killed *P. gingivalis* W83, CPS purified from *P. gingivalis* strain W83, and the seven known serotype-specific *P. gingivalis* CPS and assessed the ability of supernatant fluids produced by challenged macrophages to attract naïve inflammatory cells. We also defined JE/MCP-1, KC, MIP-2, and RANTES production in response to the *P. gingivalis* CPS antigens. We observed that supernatant fluids collected from macrophages incubated with *P. gingivalis* W83 and serotype K1 CPS stimulated the migration of naïve murine bone marrow-derived polymorphonuclear leukocytes in an in vitro cell migration chamber. CPS from W83 and the K1 serotype elicited potent chemokine secretion patterns for macrophages, while those specific to serotypes K2 to K7 were significantly less stimulatory. Reverse transcription-PCR and enzyme-linked immunosorbent assay revealed JE/MCP-1, KC, MIP-2, and RANTES expression from murine macrophages which had been challenged with purified *P. gingivalis* W83 CPS. Chemokine production appeared to be dependent on both the dose of and time of exposure to *P. gingivalis* W83 CPS. These data demonstrate that the *P. gingivalis* serotype K1 CPS elicits chemokine production from phagocytic cells. Furthermore, these data suggest that the host response to this antigen may contribute to the formation of the inflammatory cell lesion observed during *P. gingivalis*-elicited periodontal disease.**

Periodontal disease, an inflammatory disease that causes erosion of both the hard and soft tissues of the periodontium, is among the most common chronic infections in humans (2, 42). Analysis of human periodontal lesions has revealed a complex array of chemokines that are upregulated, including interleukin-8 (IL-8), monocyte chemoattractant protein 1 (MCP-1), regulated on activation of normal T cells, expressed and secreted (RANTES), and macrophage inflammatory protein 1 (MIP-1), at diseased sites (11, 13, 47). The cellular inflammatory lesion observed during adult periodontitis is complex and consists of neutrophils very early, followed by a predominantly mononuclear cell infiltrate characteristic of chronic infections (4, 16). *Porphyromonas gingivalis* is a gram-negative, encapsulated organism that is considered the principal organism associated with principal forms of periodontitis (23). This organism possesses a broad array of factors, including cysteine proteinases (gingipains) (25, 43), fimbriae (38, 41), hemagglutinins (35), lipopolysaccharide (LPS) (8, 9), and capsular polysaccharide (CPS) (51), that all play roles in the virulence of the organism.

The CPS of *P. gingivalis* is a poorly defined molecule. Schifferle et al. (44) reported that the sugar composition of *P. gingivalis* CPS consisted of glucose, glucosamine, galactosamine, and galactosaminuronic acid, while Farquharson et al. (12) reported that it was comprised of mannuronic acid, glucuronic acid, galacturonic acid, galactose, and 2-acetamido-2-deoxy-D-glucose. Comparative whole-genome-based microarray analysis of virulent and avirulent strains of *P. gingivalis* recently revealed that a putative polysaccharide biosynthesis locus found in a virulent *P. gingivalis* strain was lacking in an avirulent strain (6). Furthermore, deletions made in this locus resulted in nonencapsulated mutants of *P. gingivalis* strain W50 (1). In a murine model of subcutaneous abscesses, nonencapsulated strains of *P. gingivalis* have been reported to be less virulent than encapsulated strains (33). In further support of an important role for *P. gingivalis* CPS in disease, several reports have shown that *P. gingivalis* CPS-containing preparations function as immunogens (7) and that mice immunized with this antigen in pure form are protected from oral bone loss elicited by the organism (19). To date, there are at least seven types of *P. gingivalis* CPS based on serological evaluation (31, 45), and the virulence of strains with these serotype-specific CPS in mice has been assessed (33), as well as their incidence during human disease (5, 32).

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Bacterial capsular polysaccharides are classically reported to be T-cell-independent antigens that are involved primarily in immune evasion, as they are poorly opsonized and are weak stimulators of the host immune response (27, 36). However, recent observations challenge this classical description of bacterial capsules. Purified capsular polysaccharides of *Bacteroides fragilis* (17, 18, 49, 50) and *Staphylococcus aureus* (46) elicit inflammatory responses consisting of proinflammatory cytokines and chemokines when tested in vitro and in vivo. Since some bacterial capsular polysaccharides possess potent immunostimulatory activities and since the *P. gingivalis* capsular polysaccharide appears to be an important virulence factor for the organism, we challenged murine peritoneal macrophages with purified *P. gingivalis* CPS and assessed the chemokine expression profile of these cells in response to this antigen. Here we report that *P. gingivalis* W83 CPS, a K1 serotype antigen, stimulates the production of several chemokines and that these secreted chemokines contribute to polymorphonuclear leukocyte (PMN) migration. These observations suggest that the host innate immune response to *P. gingivalis* K1 CPS may play an important role in inflammatory cell activation and recruitment during *P. gingivalis*-elicited periodontal disease.

MATERIALS AND METHODS

Collection of peritoneal macrophages and bone marrow-derived PMNs. Six- to 8-week-old male C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and were cared for in accordance with NIH guidelines, using Boston University-approved IACUC procedures. Peritoneal macrophages were collected from mice following peritoneal injection of sterile thioglycolate broth. Harvested cells were adjusted to 5×10^5 cells/ml in RPMI 1640 plus 10% fetal bovine serum (FBS) and were cultured in 6- and 24-well plates (3 and 1 ml, respectively, in each well) for 3 days, with daily exchanges with fresh medium. Murine femur bone marrow cells harvested in RPMI 1640 plus 0.1% FBS were placed over a bed of Histopaque 1083 (Sigma, St. Louis, MO) and centrifuged for 30 min at $200 \times g$. The pelleted cells were isolated, and following erythrocyte lysis by ammonium chloride treatment, PMNs were washed and adjusted to 5×10^6 cells/ml in RPMI 1640 plus 0.1% FBS for cell migration studies. PMN purity was determined to be >90% by hematoxylin and eosin staining.

Cultivation of *P. gingivalis* and purification of *P. gingivalis* capsular polysaccharide. *P. gingivalis* strain W83 was grown for 3 to 5 days on brain heart infusion-yeast extract plates supplemented with hemin (5 μg/liter), menadione (1 μg/liter), and defibrinated sheep blood (50 ml/liter; Remel, Lenexa, KS) and used to seed brain heart infusion-yeast extract broth supplemented with hemin (5 μg/liter), menadione (1 μg/liter), and L-cysteine (0.75 mg/liter). *P. gingivalis* CPS was extracted using a previously described hot phenol-water method (18). In brief, crude polysaccharide-containing preparations present in the aqueous phase of the phenol-water extracts were treated sequentially with RNase, DNase, and proteinase K, dialyzed extensively with distilled water, precipitated with ice-cold ethanol, and separated by size-exclusion chromatography in deoxycholate buffer. Column fractions possessing high-molecular-mass capsular polysaccharide were pooled, dialyzed against water, lyophilized, assessed for purity, and stored at −80°C. Only CPS preparations that were determined to be free of nucleic acid, protein, and LPS, as described previously (19), were used in these assays. Purified *P. gingivalis* serotype-specific CPS antigens from strains W50 (K1), HG184 (K2), A7A1-28 (K3), ATCC 49417 (K4), HG1690 (K5), HG1691 (K6), and 34-4 (K7) were a kind gift provided by Robert Schifferle (Department of Oral Biology, SUNY, Buffalo, NY).

Macrophage challenge assays. Murine peritoneal macrophages in either 6- or 24-well cell culture plates were challenged with *P. gingivalis* W83 CPS (10 to 0.01 μg/ml) in the presence of polymyxin B (10 μg/ml), with heat-killed *P. gingivalis* strain W83 equivalent to a multiplicity of infection of 100, with *Escherichia coli* LPS (10 ng/ml) (serogroup O111:B4; Fluka, Buchs, Switzerland) in the presence or absence of polymyxin B (10 μg/ml), or with medium alone for 2, 6, or 24 h. In additional experiments, we cultured murine macrophages with 10 μg/ml of each of the *P. gingivalis* serotype-specific CPS antigens (specific for serotypes K1 to K7), and supernatant fluids were collected 24 h after the addition of CPS. Supernatant fluids harvested from these cells were stored at −80°C until enzyme-linked immunosorbent assays (ELISAs) were performed. The macrophage monolayers were then lysed, and total RNA was collected using RNeasy spin columns (QIAGEN, Valencia, CA) and stored at −20°C until reverse transcription-PCR (RT-PCR) assays were performed.

Cell migration assays. Supernatant fluids were collected from murine peritoneal macrophages that were stimulated for up to 24 h with medium, *P. gingivalis* W83 CPS in medium with polymyxin B, CPS from representative *P. gingivalis* K1 to K7 strains in medium with polymyxin B, or heat-killed *P. gingivalis* W83. These supernatant fluids were filtered through 0.2-μm low protein-binding filters and placed into the bottom wells of cell migration chambers (Becton Dickinson Labware, Franklin Lakes, NJ). Control stimuli applied to the bottom wells included fresh RPMI 1640 plus 0.1% FBS and also the same medium spiked with 10 ng/ml recombinant murine MIP-2 (R&D Systems, Minneapolis, MN). Freshly isolated murine PMNs (0.8 ml at 5×10^6 cells/ml) were added to 3-μm-pore-size filter well inserts (Becton Dickinson Labware) and placed into each culture well. Cell migration was allowed to occur for at least 1 h in a cell culture incubator at 37°C at 5% CO_2, and the total number of cells that migrated to the bottom well was determined using microscopic counts by averaging the numbers of cells counted in five random microscope fields (magnification, ×200) (17).

ELISA analysis of supernatant fluid levels of chemokines. Frozen cell culture supernatant fluids were thawed only once, filtered through 0.2-μm low protein-binding filters, and analyzed for levels of the chemokines JE/MCP-1 (CCL2), KC (CXCL1), MIP-2 (CXCL2), and RANTES (CCL5), using commercially available ELISA kits (R&D Systems, Minneapolis, MN).

RT-PCR of chemokines. Total RNA was quantified spectrophotometrically. RT-PCRs for murine JE/MCP-1, KC, MIP-2, and RANTES with specific primers were performed by using a single-step Superscript RT-PCR kit (Invitrogen, Carlsbad, CA), with RNAs normalized to β-actin levels. Primers were synthesized (Invitrogen) with the following sequences: for JE/MCP-1, 5′-ACTGAAG CCAGCTCTCTCTTCCTC-3′ (forward) and 5′-TTCCTTCTTGGGGTCAGC ACAGAC-3′ (reverse) (48); for KC, 5′-GGATTCACCTCAAGAACATCCAG AG-3′ (forward) and 5′-CACCCTTCTACTAGCACAGTGGTTG-3′ (reverse) (29); for MIP-2, 5′-GCTTCCTCGGGCACTCCAGAC-3′ (forward) and 5′-TT AGCCTTGCCTTTGTTCAGTAT-3′ (reverse) (37); for RANTES, 5′-CATAT GGCTCGGACACCACT-3′ (forward) and 5′-ACACACTTGGCGGTTCCTT C-3′ (reverse) (40); and for β-actin, 5′-TCATGAAGTGTGACGTTGACATC CGT-3′ (forward) and 5′-CCTAGAAGCATTTGCGGTGCACGATG-3′ (reverse) (53). A sample of each reaction was run in a 1.5% agarose gel and stained with ethidium bromide, and digital micrographs of the gels were recorded.

Statistical analysis. Data were obtained from at least two independent experiments and pooled for determinations of the median and range, and comparative statistical analysis was performed using the Prism statistical analysis software package (GraphPad Software Inc., San Diego, CA). Analysis included comparison to the medium control by the Mann-Whitney U test and analysis of variance (ANOVA) by the Kruskal-Wallis test with Dunn's multiple comparisons, as indicated. P values of <0.05 were considered significant.

RESULTS

Chemotaxis assays. Since periodontal disease presents with a complex cellular inflammatory lesion, we began to define the stimulatory effect of *P. gingivalis* CPS on cellular migration. Supernatant fluids were collected from murine peritoneal macrophages cultured with *P. gingivalis* CPS, and these fluids were placed into the bottom wells of cell migration chambers. To the upper chamber was added freshly isolated naïve murine bone marrow PMNs. After at least 1 h of incubation, the number of PMNs that migrated to the bottom chamber was determined. As expected, we observed that few PMNs migrated to the bottom wells of cell migration chambers that contained medium only, whereas vigorous PMN migration was observed in response to medium spiked with recombinant murine MIP-2 (Fig. 1). Supernatant fluids obtained from macrophages cultured in medium alone elicited PMN migration similar to that in unconditioned medium. We observed that supernatant fluids from macrophages challenged with heat-killed *P. gingivalis* stimulated robust PMN migration similar to that with recombinant murine MIP-2 (Fig. 1). Interestingly, supernatant fluids

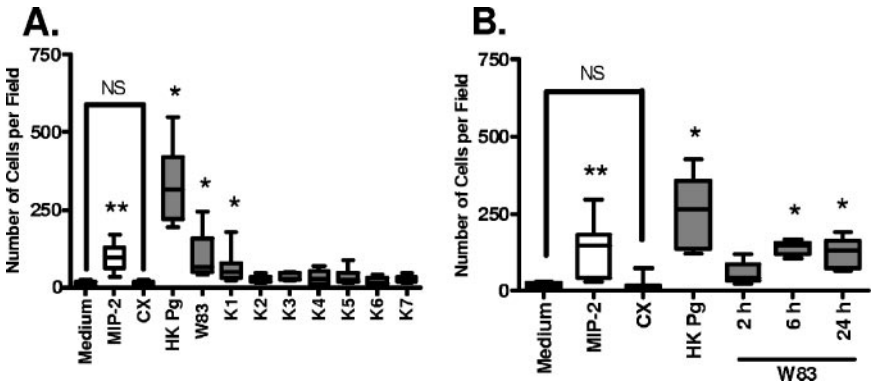

FIG. 1. Supernatant fluids of murine peritoneal macrophages challenged with W83 CPS or K1-specific CPS stimulate naïve PMN cell migration. Thioglycolate-elicited peritoneal macrophages were placed into either 24- or 6-well plates at 5×10^5 cells/ml and were stimulated with *P. gingivalis* strain W83 CPS (W83), CPS from representative K1 to K7 *P. gingivalis* strains (K1 to K7), or heat-killed *P. gingivalis* strain W83 (HK Pg). Collected cell-free supernatant fluids (gray bars) were passed through a 0.2-μm low protein-binding filter and were placed into the bottom wells of transwell systems. Control stimuli (white bars) included medium alone and medium spiked with recombinant murine MIP-2 (10 ng/ml). Naïve murine PMNs (4×10^6) were placed into the well inserts, and cell migration was allowed to proceed for at least 1 h. The relative number of PMNs that migrated to the bottom chamber was determined for each treatment by microscopic counts for five random fields (magnification, ×200) obtained from the well bottom. (A) Serotype-specific CPS induction of PMN migration. (B) Time-dependent induction of PMN migration by W83 CPS. Bars indicate quartiles. *, $P < 0.05$ (compared by Kruskal-Wallis ANOVA with Dunn's multiple comparisons to cells cultured in supernatants from macrophages incubated for 24 h with medium alone [CX]); **, $P < 0.05$ (compared by Kruskal-Wallis ANOVA with Dunn's multiple comparisons to cells cultured in medium alone). NS, not significantly different.

from macrophages stimulated with *P. gingivalis* W83 CPS or with serotype K1 CPS, from *P. gingivalis* W50, elicited significant PMN migration compared to that elicited by supernatant fluids transferred from macrophages incubated in medium only (Fig. 1A). No significant migration of PMNs was observed in response to supernatants from macrophages challenged with CPS from serotype K2 to K7 *P. gingivalis* strains. PMN migration was observed to be dependent on the time that macrophages were exposed to *P. gingivalis* W83 CPS (Fig. 1B). These data demonstrate that *P. gingivalis* CPS elicits a chemotactic stimulus from macrophages that can promote PMN migration.

Serotype-dependent chemokine secretion profiles. Since chemokines are known to elicit cell migration, capsule sero-

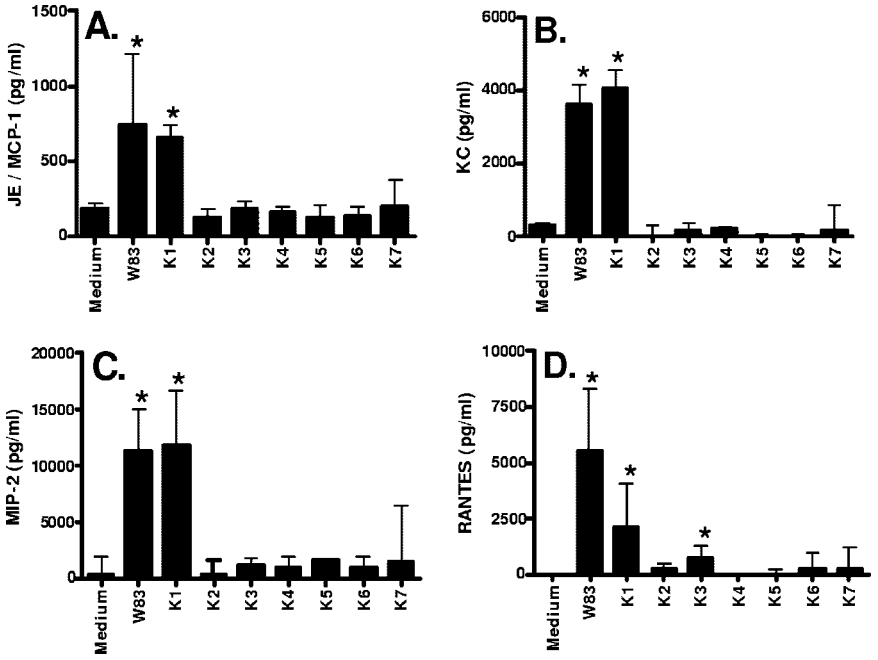

FIG. 2. Serogroup-specific expression of chemokines elicited from murine peritoneal macrophages by *P. gingivalis* capsular polysaccharide. Thioglycolate-elicited peritoneal macrophages were placed into either 24- or 6-well plates at 5×10^5 cells/ml and were stimulated with CPS isolated from *P. gingivalis* strains W83 (W83), W50 (K1), HG184 (K2), A7A1-28 (K3), ATCC 49417 (K4), HG1690 (K5), HG1691 (K6), and 34-4 (K7). Supernatant fluids collected from murine peritoneal macrophages were assessed by ELISA for (A) JE/MCP-1, (B) KC, (C) MIP-2, and (D) RANTES. Bars indicate medians plus ranges. *, $P < 0.05$ (compared to cells cultured in medium alone by the Mann-Whitney U test).

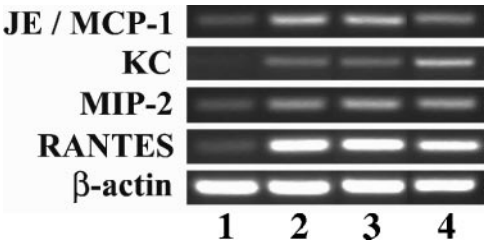

FIG. 3. RT-PCR analysis of chemokine gene transcription from murine peritoneal macrophages stimulated with *P. gingivalis* CPS. Thioglycolate-elicited peritoneal macrophages cultured in either 24- or 6-well plates were incubated with medium alone (lane 1), medium with *E. coli* LPS (lane 2), medium with *P. gingivalis* strain W83 CPS (lane 3), or medium containing heat-killed *P. gingivalis* strain W83 (lane 4). RT-PCRs with purified RNAs were performed using the chemokine-specific primers described in Materials and Methods.

type-dependent induction of the expression of chemokines JE/MCP-1, KC, MIP-2, and RANTES from macrophages was assessed next. Murine macrophages were cultured for 24 h with 10 μg/ml each of *P. gingivalis* W83 CPS and the seven representative *P. gingivalis* capsule serotype CPS antigens, and expression of the inflammatory mediators was determined by ELISA (Fig. 2). With the exception of W83 CPS and the K1 strain CPS, the remaining capsules to *P. gingivalis* serotypes K2 to K7 failed to elicit KC. Similar results were observed for JE/MCP-1 and MIP-2 expression from macrophages (Fig. 2). Interestingly, the CPS from W83 and the K1 and K3 strains were observed to elicit RANTES production by the murine peritoneal macrophages. These data demonstrate that K1 serotype capsules elicit the most potent chemokine response from murine macrophages.

RT-PCR analysis of murine peritoneal macrophage chemokine gene expression. Since periodontal disease presents with a complex cellular inflammatory lesion, we began to define the stimulatory effect of *P. gingivalis* CPS on macrophage chemokine expression. Murine peritoneal macrophages were incubated with *P. gingivalis* W83 CPS (10 μg/ml) or with heat-killed *P. gingivalis* W83 (equivalent to a multiplicity of infection of 100), and we defined the transcription of JE/MCP-1, KC, MIP-2, and RANTES (Fig. 3). All samples were normalized to β-actin mRNA expression levels. As expected, macrophages incubated in medium alone expressed little or no mRNA for the target genes of interest. We observed that macrophages incubated with heat-killed *P. gingivalis* W83 expressed elevated levels of chemokine-specific mRNAs (Fig. 2). Macrophages responded to *P. gingivalis* W83 CPS and expressed elevated levels of all chemokines tested. These data demonstrate that *P. gingivalis* capsular polysaccharide is a potent stimulator of gene transcription for several inflammatory chemokines.

***P. gingivalis* W83 CPS-dependent chemokine secretion profiles.** Next, we challenged murine macrophages with various doses of *P. gingivalis* W83 CPS and assessed chemokine production by ELISA. Murine macrophages incubated with medium alone expressed low levels of all chemokines assayed by ELISA (Fig. 4). *E. coli* LPS was a potent macrophage stimulator of chemokine secretion that was significantly affected by polymyxin B treatment. We observed that macrophages cultured with heat-killed *P. gingivalis* produced significantly greater levels of KC and MIP-2 than did uninfected control

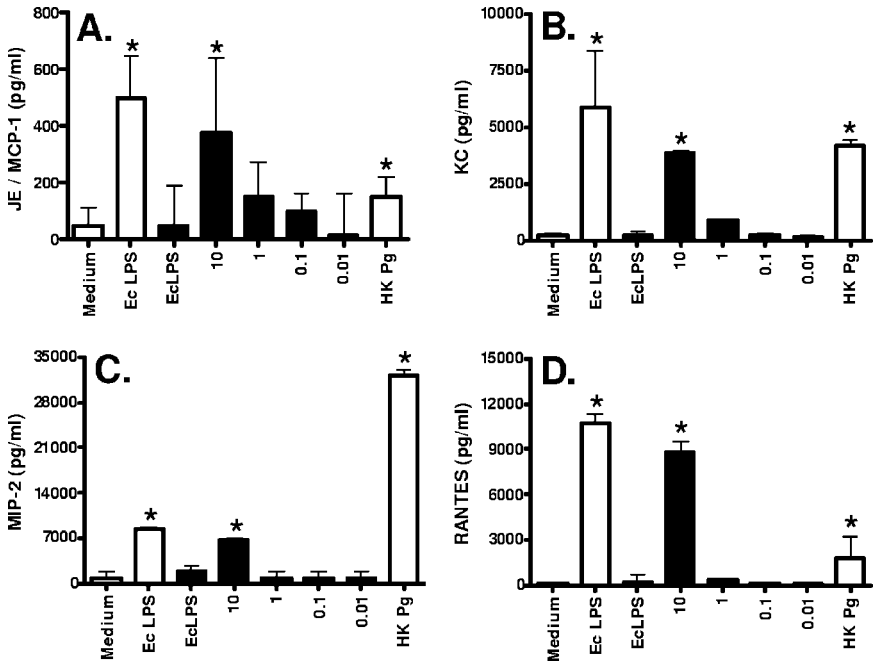

FIG. 4. Dose-dependent expression of chemokines elicited from murine peritoneal macrophages by *P. gingivalis* W83 capsular polysaccharide. Thioglycolate-elicited peritoneal macrophages were placed into either 24- or 6-well plates at 5×10^5 cells/ml and were stimulated with *E. coli* LPS, *P. gingivalis* strain W83 capsular polysaccharide, or heat-killed *P. gingivalis* W83. Supernatant fluids collected from murine peritoneal macrophages were assessed by ELISA for (A) JE/MCP-1, (B) KC, (C) MIP-2, and (D) RANTES. Bars indicate medians plus ranges. White bars, stimulation in the presence of polymyxin B; black bars, stimulation without polymyxin B. *, $P < 0.05$ (compared to cells cultured in medium alone by the Mann-Whitney U test).

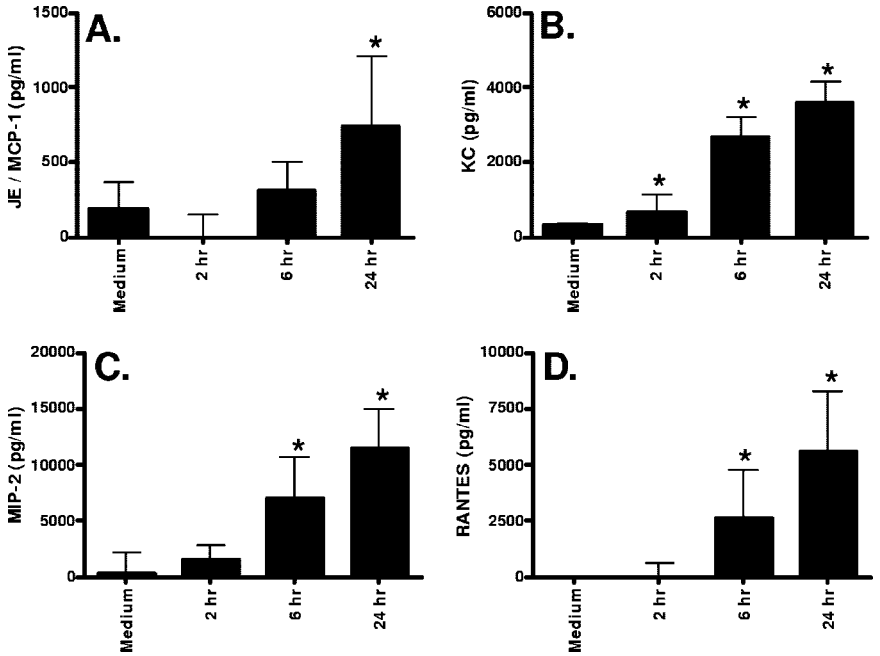

FIG. 5. Time-dependent expression of chemokines elicited from murine peritoneal macrophages by *P. gingivalis* W83 capsular polysaccharide. Thioglycolate-elicited peritoneal macrophages were placed into either 24- or 6-well plates at 5×10^5 cells/ml and were stimulated with *P. gingivalis* strain W83 capsular polysaccharide for either 2, 6, or 24 h. Supernatant fluids collected from murine peritoneal macrophages were assessed by ELISA for (A) JE/MCP-1, (B) KC, (C) MIP-2, and (D) RANTES. ELISA results for supernatants from cells incubated for 24 h with medium alone are presented as a control. Bars indicate medians plus ranges. *, $P < 0.05$ (compared to cells cultured in medium alone by the Mann-Whitney U test).

cells (Fig. 4). A significant increase in the expression of KC was observed at 10 μg/ml of CPS compared with that in untreated cells (Fig. 4); however, for 0.1 and 0.01 μg/ml CPS, we did not detect KC in supernatant fluids. Similar to the KC level, both MIP-2 and RANTES expression levels were significantly higher than those in untreated cells in response to *P. gingivalis* CPS, and these increases were lost at 0.1- and 0.01-μg/ml doses of antigen (Fig. 4). JE/MCP-1 levels were significantly higher in supernatant fluids of murine macrophages cultured only with a 10-μg/ml dose of antigen than that for untreated cells. For murine macrophages, these data were in agreement with the RT-PCR data and confirmed that various chemokines are elicited by *P. gingivalis* CPS.

The temporal expression of chemokines from *P. gingivalis* W83 CPS-challenged murine macrophages was studied last. Using the 10-μg/ml dose of purified *P. gingivalis* W83 CPS, only KC was detected at significant levels in supernatant fluids after 2 h of stimulation (Fig. 5). We observed significantly increased levels of KC and RANTES in the culture supernatant fluids after 6 h of stimulation (Fig. 5), while significant levels of JE/MCP-1 and MIP-2 were not achieved until 24 h after CPS addition (Fig. 5). These data demonstrate that there is a temporal sequence to chemokine expression from murine macrophages cultured with *P. gingivalis* CPS.

DISCUSSION

The present study observed a dose- and time-dependent expression of chemokines by murine macrophages stimulated with the capsular polysaccharide from *P. gingivalis* W83 and also showed the ability of fluids containing these secreted chemokines to attract murine bone marrow-derived PMNs. An important aspect of periodontal disease is a chronic cellular inflammatory infiltrate highlighted by an influx of PMNs and monocytes (16). A variety of cell culture models show that epithelial cells, fibroblasts, and mononuclear cells and PMNs express chemokines when cultured with *P. gingivalis* (26, 28, 30, 54). Indeed, increased expression of the chemokines MCP-1, MIP-1α, and RANTES has been reported for human and murine cells cultured with *P. gingivalis* (28, 55), just as MCP-1, RANTES, and IL-8 are found at elevated levels in the gingival crevicular fluid of diseased periodontal tissue (11, 14, 22). Various studies have focused on the contributions of specific antigens from *P. gingivalis* to inflammatory responses to the organism. Fimbriae, gingipains, hemagglutinins, and LPS of *P. gingivalis* are known to contribute to various aspects of the virulence of this organism (24, 34); however, these factors do not completely explain the inflammatory response elicited by this organism. The major fimbria, for instance, has been shown to elicit KC production from murine macrophages (21). Recently, however, it was reported that fimbriae played little role in the elicitation of MIP-2 in a calvaria model using CD-1 mice (20), where the *P. gingivalis* 381-derived FimA mutant DPG3 elicited MIP-2 as strongly as wild-type 381. We observed that KC and MIP-2 expression was similar for murine macrophages cultured with heat-killed *P. gingivalis* W83 or its purified CPS and that expression was dependent on both the time of exposure and the amount of purified CPS added to the macrophage cultures. Sonic extracts of surface-associated material from *P. gingivalis* have been shown to elicit IL-8 and MCP-1 from

human gingival epithelial cells more strongly than do purified *P. gingivalis* LPS and fimbriae (30). Another study with purified components found the polysaccharide fraction of *P. gingivalis* to be more strongly stimulatory of IL-8 expression from human gingival fibroblasts than the LPS or surface protein fractions (26). Zhou and Amar (56) reported good concordance in elevated expression levels of tumor necrosis factor alpha (TNF-α), IL-6, IL-10, MIP-1β, and MIP-3α observed for human monocytes cultured with live *P. gingivalis*, purified FimA protein, and purified LPS but observed increased expression of interferon-inducible protein 10 (IP-10), MCP-1, MCP-2, MCP-3, and RANTES only in response to the purified components. Similar to the study of Zhou et al. (55), who used murine peritoneal macrophages with live *P. gingivalis* and purified FimA, we observed that heat-killed *P. gingivalis* W83 and *P. gingivalis* W83 CPS elicited JE/MCP-1 and KC expression. Since gingipains are known to cleave cytokines and chemokines in the milieu of *P. gingivalis* infection (3, 25, 39), we used only heat-killed *P. gingivalis* W83 for this study to assess the antigenic potential of whole *P. gingivalis* cells. We observed that *P. gingivalis* W83 CPS stimulated murine macrophages to produce a broad array of inflammatory mediators, including JE/MCP-1, KC, MIP-2, and RANTES. These studies suggest that in addition to fimbriae and LPS, the polysaccharide capsule expressed by *P. gingivalis* W83 and the K1 serogroup likely contributes to the host chemokine response to these organisms.

The interaction of bacterial capsular polysaccharides with host cells is poorly understood. *S. aureus* CPS have been shown to elicit IL-6 and IL-8 from human endothelial cells and IL-6, IL-8, IL-1β, and TNF-α from human monocytes (46); moreover, *B. fragilis* CPS has been shown to elicit IL-8 from human monocytes and IL-1β and TNF-α from murine monocytes (18). Capsular material of *P. gingivalis* has been shown to elicit IL-8 and MCP-1 from human gingival epithelial macrophages (26, 30) and was shown here to elicit JE/MCP-1, KC, MIP-2, and RANTES from murine macrophages. Our study observed JE/MCP-1 and KC to be expressed with clear dose dependence within the range of concentrations of *P. gingivalis* W83 CPS assayed, while the expression of MIP-2 and RANTES was seen only at the highest concentration tested. The levels of all chemokines assayed were seen to increase with the duration of exposure of murine macrophages to *P. gingivalis* CPS, with KC and MIP-2 observed to be expressed earlier than JE/MCP-1 and RANTES. Our study began with a characterization of the capsular polysaccharide serotype specificity in *P. gingivalis* elicitation of chemokines from murine macrophages. Murine macrophages were found to have a strong response to purified CPS from serotype K1 strains of *P. gingivalis* but to be limited in their reactions to capsules from serotype K2 to K7 strains. Some expression of RANTES was observed from murine macrophages in response to K3-specific CPS. Although it was a significant level of expression, it was less than that in response to K1-specific CPS, and the levels of JE/MCP-1, KC, and MIP-2 were no greater in response to K3-specific CPS than those in unchallenged macrophages. *P. gingivalis* W83 CPS at 10 μg/ml was as strong a stimulator as heat-killed *P. gingivalis* in eliciting JE/MCP-1, KC, and RANTES expression from murine macrophages.

The chemokine profile elicited from macrophages cultured with *P. gingivalis* CPS was consistent with that required for recruitment of both neutrophils and mononuclear cells (14, 15). The observation that cell-free supernatant fluids from macrophages cultured with *P. gingivalis* or purified CPS facilitated inflammatory cell migration demonstrated that the chemokine response of inflammatory cells to this antigen functions as a gradient that is sensed by inflammatory cells, allowing for naïve inflammatory cell migration. The cell migration system used in this study has been utilized to assess the functional ability of the chemokines present in cell culture supernatant fluids elicited from inflammatory cells following *B. fragilis* capsule stimulation to facilitate cell migration (17). Our data are in agreement with the previous study, suggesting that chemokines elicited from inflammatory cells in response to bacterial CPS support cell migration. Based on our ELISA data, which indicated that supernatant fluids from macrophages cultured with live *P. gingivalis* expressed levels of MIP-2 that exceeded those induced by *P. gingivalis* W83 CPS and were similar to those induced by heat-killed *P. gingivalis* W83, it is conceivable that MIP-2 could play an important role in PMN recruitment to *P. gingivalis* and *P. gingivalis* CPS; however, it remains to be determined which are the precise molecules that promoted PMN migration in the present study. Studies are currently under way to define these molecules.

Studies have shown that patients with periodontal disease often develop antibody responses to all *P. gingivalis* serotypes (5, 10, 31). Califano et al. (5) reported that patients with either adult periodontitis or general early-onset periodontitis possessed immunoglobulin G (IgG) antibodies reactive to all six serotype-specific capsular polysaccharides and that the mean concentration of antibodies from the seropositive individuals ranged from 20 to 105 μg/ml. Interestingly, the subclass of IgG detected was primarily IgG2 (5). In addition, it has been reported that patients screened for capsular polysaccharide-specific antibodies often possess antibodies to multiple serotype-specific capsules (5, 31) or to nontypeable *P. gingivalis* strains (52). In light of our results and the serological data presented above, we are unsure why the difference in chemokine production and subsequent migration of naïve cells is exclusive to the K1-specific antigen. However, based on differences in capsular polysaccharide structures previously reported by Schifferle et al. (44) and Farquharson et al. (12), it is conceivable that the K1 serogroup possesses a different capsule structure from those of the other K serogroups and that these differences could result in a more immunostimulatory antigen. Studies to assess possible relationships between *P. gingivalis* capsular polysaccharide structure and function are needed.

In conclusion, our data demonstrate that in pure form, capsular polysaccharides from *P. gingivalis* W83 and K1 serotype strains elicit a potent inflammatory response from murine peritoneal macrophages. The chemokine profile elicited by CPS is consistent with that expected for an antigen that would be recognized by the host and would stimulate the recruitment of cells to the site of infection. Thus, these data suggest that the host response to *P. gingivalis* W83 CPS and K1 CPS plays a role in cell migration to the focus of a *P. gingivalis* infection and that this response could be involved in the establishment of the inflammatory lesion observed during periodontal disease.

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