INFECTION AND IMMUNITY, Aug. 1996, p. 2904–2910 Vol. 64, No. 8
0019-9567/96/$04.00+0

The Major Surface Protein Complex of *Treponema denticola* Depolarizes and Induces Ion Channels in HeLa Cell Membranes

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Received 3 November 1995/Returned for modification 7 February 1996/Accepted 20 May 1996

The oral spirochete *Treponema denticola* is closely associated with periodontal diseases in humans. The 53-kDa major surface protein (Msp) located in the outer membrane of *T. denticola* serovar *a* (ATCC 35405) has both pore-forming activity and adhesin activity. We have used standard patch clamp recording methods to study the effects of a partially purified outer membrane complex containing Msp on HeLa cells. The Msp complex was free of the chymotrypsin-like proteinase also found in the outer membrane of *T. denticola*. Msp bound to several HeLa cell proteins, including a 65-kDa surface protein and a 96-kDa cytoplasmic protein. The Msp complex depolarized and increased the conductance of the HeLa cell membrane in a manner which was not strongly selective for Na^+, K^+, Ca^{2+}, and Cl^- ions. Cell-attached patches of HeLa cell membrane exposed to Msp complex exhibited short-lived channels with a slope conductance of 0.4 nS in physiologically normal saline. These studies show that Msp binds both a putative epithelial cell surface receptor and cytoplasmic proteins and that the Msp complex can form large conductance ion channels in the cytoplasmic membrane of epithelial cells. These properties may contribute to the cytopathic effects of *T. denticola* on host epithelial cells.

Oral spirochetes of the genus *Treponema* are believed to play an important role in the progression of periodontal disease in humans (36, 45). A member of this genus, *Treponema denticola*, has been implicated in a number of potentially pathogenic processes, including altered fibroblast (6) and lymphocyte function (44), enhanced vascular exudation (29), and depressed endothelial cell proliferation (46). Despite the well-documented association of spirochetes with periodontal lesions, particularly acute necrotizing ulcerative gingivitis (31, 32), little is known about the effects of these oral pathogens on host cells.

T. denticola possesses several potential virulence factors which may be important in the pathogenesis of periodontal disease (23). The major outer sheath protein (Msp) is a 53-kDa peptide that forms detergent-stable oligomers visible as a hexagonal array on the surface of *T. denticola* cells by electron microscopy (11). Both intact *T. denticola* and Msp have been shown to bind extracellular matrix (ECM) components associated with periodontal epithelium (13, 19). Whole *T. denticola* cells have been shown to induce cytopathic morphological changes in epithelial cells (51) and fibroblasts (8). A surface-located chymotrypsin-like proteinase which copurifies with Msp (17, 50) has been associated both with adherence and with cytopathic effects of *T. denticola* on periodontal epithelial cells (28, 51). Other potential virulence factors include various hydrolytic enzymes (33, 38, 40) and hemagglutinating or hemolytic factors (16, 35).

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Recently, Msp has been shown to have pore-forming properties similar to those of outer membrane porins of gram-negative bacteria, suggesting that Msp may have functions in addition to mediating attachment to ECM components. *T. denticola* Msp was shown to form very large conductance ion channels in the black lipid bilayer model membrane system (11). The channels formed by Msp in this system were significantly larger than those formed by any other bacterial porin. Possible roles of the Msp pore-forming activity include the following: nutrient uptake, as in classical bacterial porins (24, 42); translocation of bacterial products into infected host cells, as with diphtheria toxin (12); or mediation of host cell killing, as in the case of staphylococcal alpha-toxin (25).

Translocation of bacterial porins to eukaryotic cell membranes has been reported for several species, with diverse biological effects that may be related to perturbation of membrane permeability. The class 1 porin of *Neisseria gonorrhoeae* interacted with neutrophils, causing inhibition of granule exocytosis (21). The class 2 porin of *Neisseria meningitidis* was mitogenic to lymphocytes and increased membrane permeability in erythrocytes and lymphocytes, eventually resulting in cell lysis (52). The porins of *Salmonella typhimurium* induced the release of several cytokines by human monocytes and lymphocytes (15). In two organisms associated with periodontal diseases, outer membrane components with porin-like activity have been shown to have marked effects on eukaryotic cells. *Porphyromonas gingivalis* heat-stable outer membrane components depolarized membranes of polymorphonuclear leukocytes by a mechanism consistent with pore formation and without activation of a respiratory burst (39). Low concentrations of *Eikenella corrodens* porin induced cytopathic changes and cytotoxicity in murine macrophages, accompanied by selective release of lysosomal enzymes (49).

TABLE 1. Compositions of saline solutions used in patch clamp experiments[a]

Solution	Concn (mM) of component										
	KCl	NaCl	$CaCl_2$	$MgCl_2$	K gluconate	Ca gluconate	Mg gluconate	K-ATP	EGTA[b]	HEPES[c]	Glucose
a	4	135	1.8	1						10	5
b					150	1.8	1			15	20
c	135	5	2.2	1					3	10	
d					150	0.005	1	2	1	5	5

[a] All saline solutions were buffered to a pH of 7.2 to 7.4.
[b] EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.
[c] HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

The electrophysiological behavior of bacterial porins in model membrane systems has been well characterized (for a review, see reference 24). These techniques have not been employed in studying the behavior of bacterial porins interacting with eukaryotic cells. In order to clarify the role of Msp in *T. denticola*-mediated pathogenesis, we have investigated the channel-forming activity of Msp in an intact biological membrane. Using standard patch clamp recording methods, we here describe the interaction of the *T. denticola* outer membrane complex containing Msp with HeLa cells, a human epitheloid cell line (43).

MATERIALS AND METHODS

Culture of HeLa cells. The HeLa cell line was maintained in modified Eagle's medium (Gibco, Grand Island, N.Y.) containing 10% fetal bovine serum (Gibco) (MEM–10% FBS), 10,000 U of penicillin G per ml, 10 mg of streptomycin per ml, and 30 μg of amphotericin B per ml. Cell cultures were kept in a humidified, 5% CO_2 atmosphere at 37°C.

Preparation of the Msp complex. *T. denticola* serovar *a* (ATCC 35405) was grown in NOS broth medium (19). Detergent extraction of Msp complex from a total of 60 liters of batch cultures proceeded as previously described (11), with minor modifications. All procedures were carried out at 4°C unless specified. Approximately 8 g (wet weight) of cells per 4-liter culture in late logarithmic growth phase was harvested by centrifugation at 5,000 × *g* for 1 h. The cells were washed twice with phosphate-buffered saline (PBS) (pH 7.3) and once with double-distilled water and then suspended in 25 ml of 20 mM Tris (pH 7.5)–1 mM dithiothreitol–2 mM EDTA–0.1% deoxycholate per liter of culture. The mixture was stirred gently overnight, and undissolved material was collected by centrifugation at 200,000 × *g* for 45 min. The deoxycholate extraction was repeated twice. The pellet was then suspended in the same volume of 10 mM Tris (pH 8.0) containing 1% *n*-octyl-polyoxyethylene (Octyl-POE; Bachem, King of Prussia, Pa.) and stirred gently overnight. The Octyl-POE extraction supernatant enriched for Msp was collected after centrifugation at 200,000 × *g* for 45 min at 25°C. The solution was then incubated for 24 to 48 h at 37°C until proteinase activity could no longer be detected with anti-95 kDa proteinase immunoglobulin G (IgG) (17) and chromogenic substrates for trypsin- and chymotrypsin-like proteinases (50). The protein solution was then concentrated approximately 30-fold by Amicon ultrafiltration with an XM50 filter (Amicon Inc., Beverly, Mass). Detergent was removed from the concentrated protein solution by passing 3,500 ml of 10 mM Tris (pH 8.0) through the Amicon unit. The solubilized Msp with the detergent washed out precipitated inside the Amicon unit. The precipitate was then washed three times with double-distilled water and twice with Ringer's solution (pH 7.4) and centrifuged at 200,000 × *g* for 2 h at 25°C to remove any possible trace of detergent. The washed Msp complex, in a small volume of Ringer's solution or MEM, was mildly ultrasonicated with a ultrasonic processor XL (5-min pulse sonication at 20% duty cycle and output of 2; Heat Systems Inc., Farmingdale, N.Y.) before use in assays. Protein content of the specimen was measured by Bradford assay (Bio-Rad, Richmond, Calif.).

Immunoprecipitation of HeLa cell proteins with the Msp complex. HeLa cells were incubated with 10 μCi of [^{35}S]methionine (Translabel; ICN) per ml for 24 h. Alternatively, HeLa cell surface proteins were labeled with Sulfo-NHS-LC-biotin (Pierce, Rockford, Ill.) by the method of Lisanti et al. (30). After labeling, the cell layer was washed extensively with PBS. Cell membrane and cytoplasmic proteins were extracted at 4°C with 2% Triton X-100 in PBS containing 2 mM phenylmethylsulfonyl fluoride and 15 μg of leupeptin per ml (41). After centrifugation at 10,000 × *g* for 5 min, the supernatant containing solubilized protein was mixed with rabbit immunoglobulins conjugated to protein A Sepharose (Sigma) to remove nonspecifically binding material. Following another centrifugation, the supernatant was mixed with Msp complex for 1 h at room temperature. Anti-Msp immunoglobulins conjugated to protein A Sepharose (13) which had been preincubated with unlabeled HeLa cell proteins (14) were added to the labeled HeLa protein-Msp complex mixture. For additional controls, immunoprecipitation of [^{35}S]methionine-labeled HeLa cell proteins was carried out under conditions in which Msp complex was omitted or in which anti-*P. gingivalis* IgG was substituted for anti-Msp IgG. The resulting Msp-HeLa protein aggregates were collected by centrifugation and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to detect radiolabeled proteins or by SDS-PAGE and immunoblotting to detect biotinylated proteins.

Gel electrophoresis, blotting, and autoradiography. SDS-PAGE was done by the method of Laemmli (26) with a Mini Protean II electrophoresis apparatus (Bio-Rad). Proteins or peptides were solubilized in sample buffer, heated at 100°C for 5 min unless stated otherwise, electrophoresed at 200 V, and detected by Coomassie blue staining. For radiolabeled samples, gels were treated with fluorography amplification reagent and dried at 80°C under vacuum. Autoradiography was done at −70°C with Kodak X-OMAT film. To detect biotinylated surface proteins, proteins were transferred electrophoretically from SDS-polyacrylamide gels to nitrocellulose membranes as described by Towbin et al. (48). Following transfer, the membranes were blocked with 3% bovine serum albumin (BSA) in Tris-buffered saline (20 mM Tris [pH 7.5], 0.5 M NaCl) and then incubated with streptavidin-conjugated alkaline phosphatase (Bethesda Research Laboratories, Gaithersburg, Md.). The membranes were developed with 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium (Sigma).

Patch clamp recordings from HeLa cells. Patch clamp recordings from HeLa cells were carried out at room temperature (21 to 23°C) using a List EPC-7 amplifier with electrodes with a resistance of 5 to 10 MΩ. Membrane currents were filtered at a direct current bandwidth of 2 kHz. For single-channel analysis of data obtained in the cell-attached mode (22), membrane current and voltage were digitized at 8 kHz and analyzed on an Atari Mega 4 computer using commercial software (Instrutech Corp, Elmont, N.Y.). Channel conductance was estimated from the single-channel current-voltage relationship.

For analysis of whole-cell, voltage clamp currents (22), 400-ms-duration voltage clamp command pulses were applied from a holding potential of −57 or 0 mV, and corresponding whole-cell currents were stored and analyzed with an Atari Mega 4 computer running commercial software (Instrutech Corp). Fast and slow capacitance neutralization and series resistance compensation were employed to minimize artifacts associated with whole-cell voltage clamp. These recordings utilized isolated, spherical cells to avoid artifacts associated with cell-cell coupling and complex cell geometries.

The compositions of bath and pipette saline solutions used are given in Table 1. For whole-cell, current clamp determination of resting membrane potentials, pipettes contained solution *c* and cells were bathed in solution *a*. During whole-cell, voltage clamp recordings, intrinsic membrane currents of HeLa cells were normally suppressed by using pipettes filled with solution *d* while cells were bathed in solution *b* (47). In cell-attached recordings, both the bath and pipette contained solution *a*, and Msp complex was applied by adding the complex to the patch electrode solution.

RESULTS

Composition of the Msp complex. The Msp complex obtained by sequential detergent extraction and autoproteolysis of *T. denticola* outer membrane material contained primarily Msp, which migrated as an oligomer of 110 to 150 kDa on SDS-polyacrylamide gels when an unheated sample was analyzed (Fig. 1, lane 1) and as a 53-kDa monomer when a heated sample was analyzed (Fig. 1, lane 2). The 95-kDa chymotrypsin-like proteinase that copurified with Msp in the initial detergent extraction was not visible in the final Msp complex stained with Coomassie blue (Fig. 1, lane 1). The 95-kDa chymotrypsin-like proteinase was not detected in the final Msp complex by silver-stained SDS-PAGE or Western immuno-

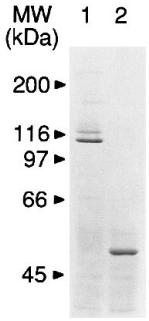

FIG. 1. *T. denticola* Msp complex used in this study. The *T. denticola* Msp complex was prepared as described in the text, subjected to SDS-PAGE and stained with Coomassie blue. Lanes: 1, Msp complex that was not heated; 2, Msp complex heated to 100°C.

blotting, nor was there any chymotrypsin-like enzyme activity (data not shown).

Msp binding to HeLa cell proteins. SDS-polyacrylamide gels of HeLa cell proteins showed [^{35}S]methionine labeling at many molecular masses over the range from 30 to 200 kDa (Fig. 2A, lane 1). A [^{35}S]methionine-labeled HeLa protein of 96 kDa was immunoprecipitated by anti-Msp IgG after exposure to the Msp complex, with additional reactivity at several other bands including bands of 50 to 53, 65, and 75 kDa (Fig. 2A, lane 2). Control gels in which the Msp complex was omitted or in which anti-*P. gingivalis* IgG replaced anti-Msp IgG showed negligible labeling (Fig. 2A, lanes 3 and 4). A biotinylated HeLa cell surface protein of 65 kDa was immunoprecipitated by anti-Msp IgG after exposure to the Msp complex (Fig. 2B). The biotinylated protein recognized by Msp had the same relative molecular weight as one of the [^{35}S]methionine-labeled HeLa proteins recognized by Msp.

The Msp complex depolarized HeLa cell membranes. HeLa cells incubated for 24 h at 37°C with the Msp complex (39 μg/ml) in MEM–10% FBS were found to have a significantly lower mean membrane potential than was observed in cells incubated in MEM–10% FBS alone. The mean resting potential was -51 ± 2.5 mV for untreated cells ($n = 23$) and -24 ± 2.9 mV for Msp-treated cells ($n = 11$) ($P < 0.001$, Student's t test).

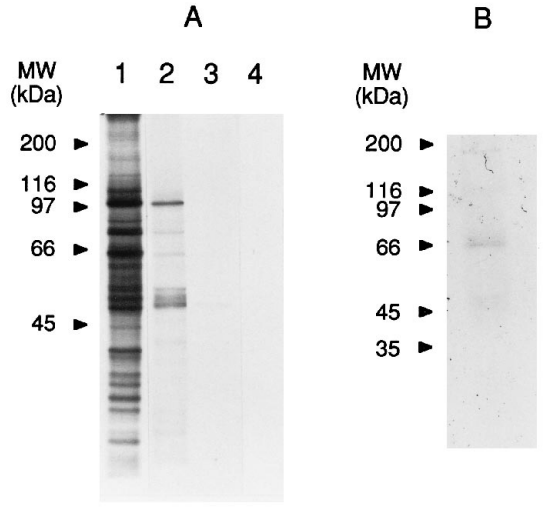

FIG. 2. SDS-polyacrylamide gel showing HeLa cell proteins immunoprecipitated by anti-Msp IgG after incubation with Msp complex. (A) [^{35}S]methionine-labeled HeLa cell proteins. Lanes: 1, total HeLa cell proteins showing incorporation of [^{35}S]methionine label; 2, [^{35}S]methionine-labeled HeLa cell proteins binding Msp complex and immunoprecipitated by anti-Msp IgG; 3, as in lane 2 except anti-*P. gingivalis* IgG replaced anti-Msp IgG; 4, as in lane 2 except the cells were not exposed to Msp complex. (B) Biotin-labeled HeLa cell proteins binding Msp complex and immunoprecipitated by anti-Msp IgG.

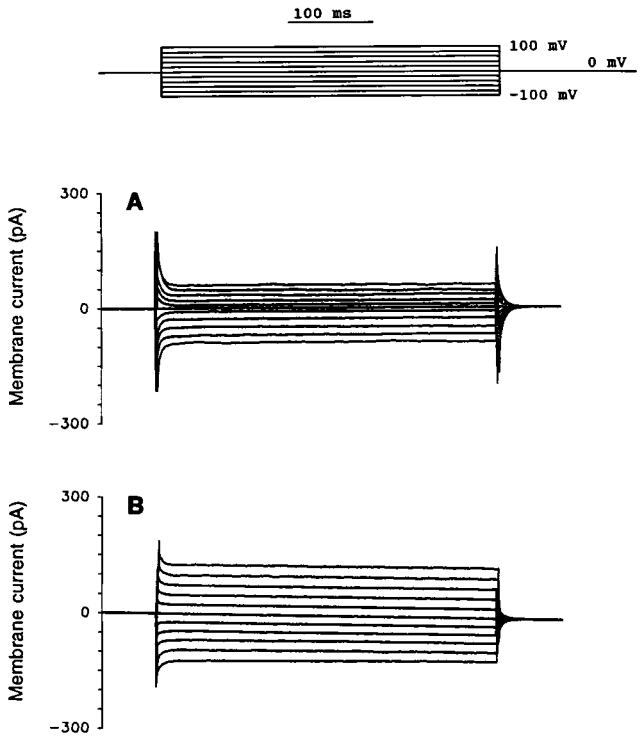

FIG. 3. Effects of Msp complex on whole-cell currents in HeLa cell membranes recorded in potassium gluconate saline solutions. Bathing and pipette solutions contained 150 mM potassium gluconate (solutions *b* and *d*, respectively [Table 1]) with a free intracellular Ca^{2+} concentration of 1 nM. Currents were generated in response to 400-ms-duration voltage commands applied from a holding potential of 0 mV. (A) Currents obtained from a representative cell incubated in MEM–10% FBS without Msp complex. (B) Currents obtained from a representative cell incubated for 30 min at 37°C in MEM–10% FBS containing 39 μg of Msp complex per ml.

The Msp complex increased membrane conductance in HeLa cells. To determine if the Msp complex induced a conductance change in the HeLa cell membrane, whole-cell, voltage clamp recordings were performed. In the first series of these experiments, patch electrodes contained solution *d* (Table 1), while cells were bathed in solution *b*. In these solutions, Na^+ was absent, K^+ was the predominant cation, and Cl^- was replaced by gluconate, which cannot pass through most anion channels in eukaryotic cell membranes. Sample recordings obtained during this work are shown in Fig. 3. Under these conditions, cells incubated with MEM–10% FBS alone had current-voltage (*I-V*) curves showing slight inward rectification (Fig. 4A). In cells incubated for 30 min at 37°C in the presence of 39 μg of the Msp complex per ml, the *I-V* curve became almost linear, and the mean slope conductance measured at positive membrane potentials was significantly higher than in the control ($P < 0.05$, analysis of variance [ANOVA] (Fig. 4A). These experiments were then repeated in physiologically relevant saline solutions. Cells were bathed in solution *a*, while patch pipettes contained solution *c* (Table 1). Sample recordings from this work are shown in Fig. 5. Under these conditions, the *I-V* curves of untreated cells were almost linear (Fig.

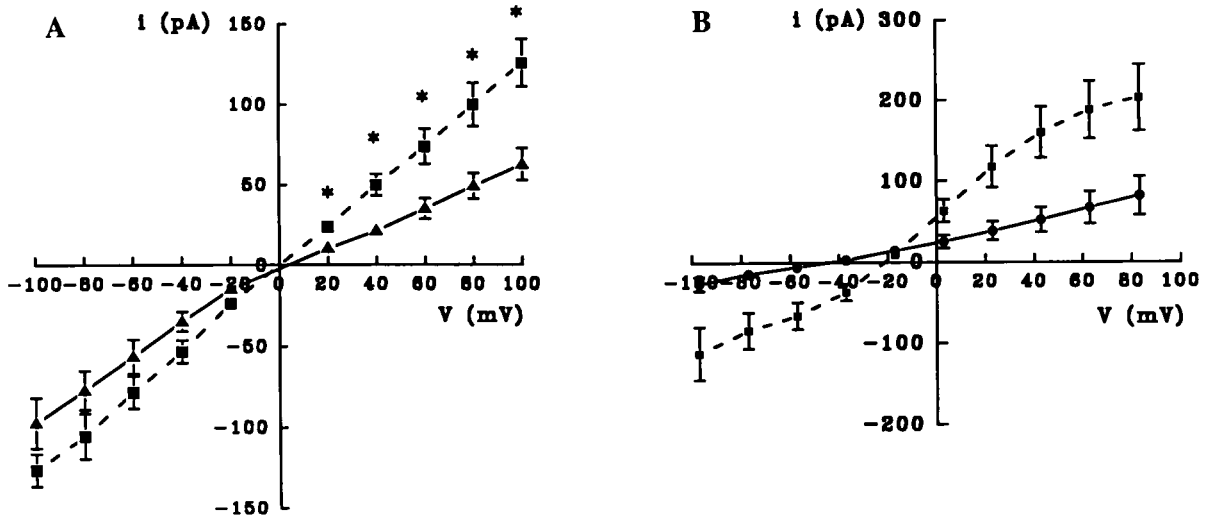

FIG. 4. Effects of Msp complex on the current-voltage (*I-V*) relationship of the HeLa cell membrane. Data were obtained as described in the legends to Fig. 3 and 5. (A) Data from cells exposed to solution *b* extracellularly and solution *d* intracellularly. Datum points indicate the values (mean ± standard error of the mean) for currents for 8 cells incubated in MEM–10% FBS only (triangles) and for 12 cells incubated in MEM–10% FBS with 39 μg of Msp complex per ml (squares). Asterisks indicate pairs of datum points which were significantly different ($P < 0.05$, ANOVA). (B) Data from cells exposed to solution *a* extracellularly and solution *c* intracellularly. Datum points indicate the values (mean ± standard error of the mean) for currents obtained from 14 cells incubated in MEM–10% FBS only (circles) and from 11 cells incubated in MEM–10% FBS with 39 μg of Msp complex per ml (squares). All pairs of datum points were significantly different except that obtained at a membrane potential of −20 mV ($P < 0.05$, ANOVA).

4B). In contrast, the *I-V* graph for cells incubated with 39 μg of Msp complex per ml showed marked outward rectification and displayed increased slope conductances for both inward and outward membrane currents ($P < 0.05$ with control, ANOVA) (Fig. 4B). The *I-V* curves of control and Msp-treated cells intercepted at a membrane potential of −18 mV (Fig. 4B). This voltage presumably represented the equilibrium potential for Msp-induced membrane currents (12).

The Msp complex induced large conductance ion channels in the HeLa cell membrane. The depolarization and conductance increase caused by Msp complex could reflect incorporation or activation of ion channels in the HeLa cell membrane. To test this possibility, we recorded current from cell-attached patches of HeLa membrane with electrodes containing 39 μg of Msp complex per ml dissolved in solution *a* (Table 1). These patch pipettes also contained 0.03% Octyl-POE as the vehicle and 5% BSA to minimize binding of the Msp complex to glass. The remainder of the cell membrane was bathed in normal solution *a*. No ionic channels with a slope conductance of 200 pS or greater were observed when patch electrodes containing Octyl-POE and BSA but lacking Msp complex were applied to the cell membrane (0 of 14 patches on 14 cells). In contrast, abrupt, square-wave changes in current consistent with the gating of large conductance ion channels were seen in 18 of 51 patches exposed to Msp complex, Octyl-POE, and BSA ($P < 0.05$, Chi-square test) (Fig. 6). Msp-induced channels had a mean slope conductance of 386 ± 49 pS (17 patches). When recorded at the normal resting potential of the HeLa cell, current through open channels was inwardly directed. Channels remained closed most of the time, occasionally undergoing transitions to a short-lived open state.

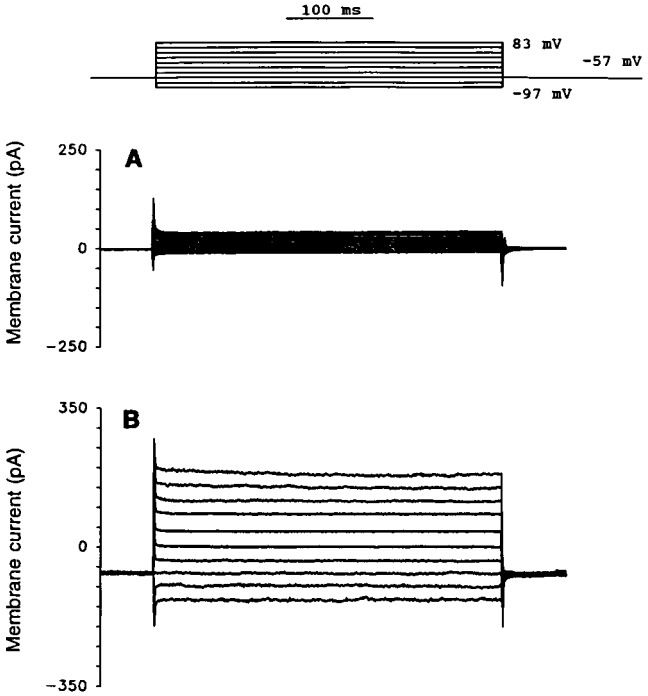

FIG. 5. Effects of Msp complex on whole-cell currents in HeLa cells bathed in physiologically relevant solutions. Patch pipettes contained 135 mM KCl (solution *c* [Table 1]), while cells were bathed in saline containing 135 mM NaCl (solution *a* [Table 1]). The intracellular free Ca^{2+} concentration was 140 nM. Currents were generated in response to 400-ms-duration voltage commands delivered from a holding potential of −57 mV. (A) Currents obtained from a representative cell incubated in MEM–10% FBS without Msp complex. (B) Currents obtained from a representative cell incubated for 30 min at 37°C in MEM–10% FBS containing 39 μg of Msp complex per ml.

DISCUSSION

The interaction between *T. denticola* and periodontal tissue is a complex process. Initial adhesion events involve carbohydrate receptors on the host cell surface (54) and Msp-mediated adhesion to ECM components including laminin, fibronectin,

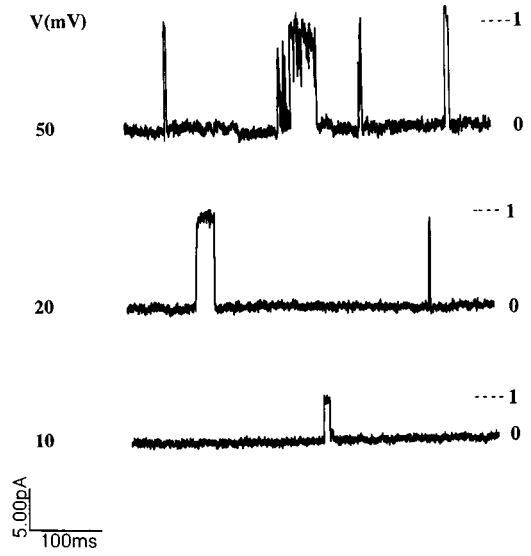

FIG. 6. Currents consistent with the gating of a large conductance ion channel recorded from a cell-attached patch of HeLa cell membrane. The patch electrode contained solution *a* with 39 μg of Msp complex per ml, 5% BSA, and 0.03% Octyl-POE. Solution *a* alone bathed the remainder of the cell surface. The traces show currents obtained at various membrane potentials (V). V was calculated by subtracting the pipette potential from the cell resting potential, −51 mV, obtained by whole-cell recording at the end of the experiment. The closed-channel current level is denoted by 0, while 1 indicates the open-channel current level. Downward deflection from baseline indicates inward membrane current. The direct current bandwidth of recording is 800 Hz.

fibrinogen, collagen, and hyaluronan (13, 18–20). Later events include induction of F-actin rearrangement and induction of membrane blebbing under adherent bacteria (1, 51). The present study showed that Msp binds certain epithelial cell surface and cytoplasmic proteins and depolarizes the epithelial cell membrane.

Outer membrane components of *T. denticola*, including the Msp complex, are released to the environment under normal growth conditions and during cell lysis. As with other bacterial porins, the Msp complex was resistant to proteolytic degradation and extended incubation at 37°C. These conditions resemble those found in the periodontium during inflammatory periodontal disease episodes, in which very high concentrations of spirochetes are often present (31, 32). Partial purification of Msp complex under these conditions yielded material which was electrophoretically similar to Msp purified by fast protein liquid chromatography (19).

Modified immunoprecipitation experiments were done to identify potential Msp receptor(s) on epithelial cells. Msp present in the Msp complex preparation bound at least one biotin-labeled HeLa cell surface protein of 65 kDa, as well as several other presumably cytoplasmic proteins including a strongly reactive protein of 96 kDa. While a specific receptor was not necessary for Msp pore formation in the black lipid bilayer system (11), it is likely that specific interactions between bacterial outer membrane components and epithelial cell surface components are important for the insertion of a bacterial porin into an epithelial cell membrane. The 65-kDa protein, a likely Msp receptor, remains to be further characterized. The strong signal due to binding of Msp to the [^{35}S]methionine-labeled 96-kDa protein is intriguing and suggests that Msp may also have specific interactions with cytoplasmic proteins. Experiments are in progress to further characterize the Msp-binding proteins of epithelial cells.

Msp is a multifunctional outer membrane protein, with pore-forming ability and adhesin activity toward a number of ECM components. A specific role for the Msp porin activity in *T. denticola* metabolism has not yet been determined. The cytopathic and cytotoxic effects ascribed to numerous purified bacterial porin molecules (15, 49, 52) suggested a series of experiments designed to assay the interaction between *T. denticola* Msp and epithelial cells in culture. To our knowledge, this is the first report of the use of patch clamp techniques to study electrophysiological responses of epithelial cells to challenge by a bacterial porin.

The Msp complex was cytotoxic to HeLa cells at doses similar to those at which other purified bacterial porins have shown cytotoxic activity (15, 49, 52; unpublished observations). At these concentrations, the Msp complex depolarized and increased the conductance of the HeLa cell membrane. This increase was observed even after substitution of Cl^- by gluconate, an anion that cannot pass through HeLa cell chloride channels (47). The effect of Msp complex is therefore unlikely to result from the activation of intrinsic chloride channels in the HeLa cell membrane. Complete replacement of Na^+ by K^+ in the extracellular solution also failed to block the Msp-induced conductance increase. In physiologically normal solutions, the conductance induced by Msp complex showed a reversal potential of −18 mV, a value which did not correspond to the Nernstian potential of any single ionic species present.

These observations indicate that the Msp complex increased membrane conductance in a relatively nonspecific manner. The current-voltage relationship of Msp-treated cells showed a greater enhancement in outward current than was seen for inward membrane current, indicating a moderate voltage dependency for Msp-induced conductance.

The relatively nonspecific, weakly voltage-dependent nature of Msp-induced conductance is consistent with the Msp complex inducing porin-like channels in the HeLa cell membrane (5, 27, 37). This hypothesis was strengthened by direct observation of channel-like currents in membrane patches exposed to the complex in the patch pipette solution. The slope conductance of these channels was about 0.4 nS in a total salt concentration of 0.3 M. This value is lower than the 1.8 nS reported for Msp reconstituted in black lipid bilayers (11) but remains within the range typical of bacterial porins (2–4). The smaller conductance value obtained in the present study may reflect a dependence of channel conductance on the reconstitution medium employed, as previously reported for the OmpC porin of *Escherichia coli* (9) and for channels formed by diphtheria toxin (12).

Channels induced by the Msp complex in HeLa cell membranes exhibited only brief excursions into a short-lived open state. In contrast, Msp reconstituted in black lipid bilayers formed channels showing very long duration openings with no evidence of closing transitions (11). Porins reconstituted in artificial membranes exist mainly in the open state, leading to the suggestion that these channels impart sieve-like properties to the bacterial outer membrane (2, 34, 56). However, more recent cell-attached recordings from intact bacteria suggest that the majority of native porin channels are normally closed, perhaps as a result of the presence of intracellular modulators of channel function (7, 10).

The present results support the view that porin channels open infrequently when studied in intact biological membranes. However, determination of the kinetic and conductance properties of native Msp channels in *T. denticola* awaits patch clamp recordings from the intact outer membrane of *T. denticola*. This may be possible using the giant spherical bodies

which form when the spirochete is cultured under conditions of osmotic stress (53, 55).

ACKNOWLEDGMENTS

This study was supported by the Medical Research Council of Canada and the Canadian Bacterial Diseases Network.

REFERENCES

1. **Baehni, P. C., M. Song, C. A. G. McCulloch, and R. P. Ellen.** 1992. *Treponema denticola* induces actin rearrangement and detachment of human gingival fibroblasts. Infect. Immun. **60:**3360–3368.
2. **Benz, R.** 1988. Structure and function of porins from Gram-negative bacteria. Annu. Rev. Microbiol. **42:**359–393.
3. **Benz, R., and K. Bauer.** 1988. Permeation of hydrophilic molecules through the outer membrane of Gram-negative bacteria. Eur. J. Biochem. **176:**1–19.
4. **Berrier, C., A. Coulombe, C. Houssin, and A. Ghazi.** 1992. Fast and slow kinetics of porin channels from *Escherichia coli* reconstituted into giant liposomes and studied by patch clamp. FEBS Lett. **306:**251–256.
5. **Berrier, C., A. Coulombe, C. Houssin, and A. Ghazi.** 1993. Voltage-dependent cationic channel of *Escherichia coli*. J. Membr. Biol. **133:**119–127.
6. **Boehringer, H., N. S. Taichman, and B. J. Schenker.** 1984. Suppression of fibroblast proliferation by oral spirochetes. Infect. Immun. **45:**155–159.
7. **Buechner, M., A. H. Delceur, B. Martinac, J. Adler, and C. Kung.** 1990. Ion channel activities in the *Escherichia coli* outer membrane. Biochim. Biophys. Acta **1024:**111–121.
8. **Dawson, J. R., and R. P. Ellen.** 1990. Tip-oriented adherence of *Treponema denticola* to fibronectin. Infect. Immun. **58:**3924–3928.
9. **Delcour, A. H., J. Adler, and C. Kung.** 1991. A single-amino acid substitution alters conductance and gating of OmpC porin of *Escherichia coli*. J. Membr. Biol. **119:**267–275.
10. **Delcour, A. H., J. Adler, C. Kung, and B. Martinac.** 1992. Membrane-derived oligosaccharides (MDOs) promote closing of an *E. coli* porin channel. FEBS Lett. **304:**216–220.
11. **Egli, C., W. K. Leung, K.-H. Müller, R. E. W. Hancock, and B. C. McBride.** 1993. Pore-forming properties of the major 53-kilodalton surface antigen from the outer sheath of *Treponema denticola*. Infect. Immun. **61:**1694–1699.
12. **Eriksen, S., S. Olsnes, K. Sandvig, and O. Sand.** 1994. Diphtheria toxin at low pH depolarizes the membrane, increases the membrane conductance and induces a new type of ion channel in Vero cells. EMBO J. **13:**4433–4439.
13. **Fenno, J. C., K.-H. Müller, and B. C. McBride.** 1996. Sequence analysis, expression, and binding activity of recombinant major outer sheath protein (Msp) of *Treponema denticola*. J. Bacteriol. **178:**2489–2497.
14. **Firestone, G. L., and S. D. Winguth.** 1990. Immunoprecipitation of proteins. Methods Enzymol. **182:**688–700.
15. **Galdiero, F., G. Cippollaro de L'Ero, N. Benedetto, M. Galdiero, and M. A. Tufano.** 1993. Release of cytokines induced by *Salmonella typhimurium* porins. Infect. Immun. **61:**155–161.
16. **Grenier, D.** 1991. Characteristics of hemolytic and hemagglutinating activities of *Treponema denticola*. Oral Microbiol. Immunol. **6:**246–249.
17. **Grenier, D., V.-J. Uitto, and B. C. McBride.** 1990. Cellular location of a *Treponema denticola* chymotrypsin-like protease and importance of the protease in migration through the basement membrane. Infect. Immun. **58:**347–351.
18. **Haapasalo, M., P. Hannam, B. C. McBride, and V.-J. Uitto.** Hyaluronan, a possible ligand mediating *Treponema denticola* binding to periodontal tissue. Oral Microbiol. Immunol., in press.
19. **Haapasalo, M., K.-H. Müller, V.-J. Uitto, W. K. Leung, and B. C. McBride.** 1992. Characterization, cloning, and binding properties of the major 53-kilodalton *Treponema denticola* surface antigen. Infect. Immun. **60:**2058–2065.
20. **Haapasalo, M., U. Singh, B. C. McBride, and V.-J. Uitto.** 1991. Sulfhydryl-dependent attachment of *Treponema denticola* to laminin and other proteins. Infect. Immun. **59:**4230–4237.
21. **Haines, K. A., L. Yeh, M. S. Blake, P. Cristello, H. Korchak, and G. Weissmann.** 1988. Protein I, a translocatable ion channel from *Neisseria gonorrhoeae*, selectively inhibits exocytosis from human neutrophils without inhibiting O_2^- generation. J. Biol. Chem. **263:**945–951.
22. **Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth.** 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflüg. Arch. Eur. J. Physiol. **391:**85–100.
23. **Holt, S. C., and T. E. Bramanti.** 1991. Factors in virulence expression and their role in periodontal disease pathogenesis. Crit. Rev. Oral Biol. Med. **2:**177–281.
24. **Jap, B. K., and P. J. Walian.** 1990. Biophysics of the structure and function of porins. Q. Rev. Biophys. **23:**367–403.
25. **Jonas, D., I. Walev, T. Berger, M. Liebetrau, M. Palmer, and S. Bhakdi.** 1994. Novel path to apoptosis: small transmembrane pores created by staphylococcal alpha-toxin in T lymphocytes evoke internucleosomal DNA degradation. Infect. Immun. **62:**1304–1312.
26. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
27. **Lakey, J. H., and F. Pattus.** 1989. The voltage-dependent activity of *Escherichia coli* porins in different planar bilayer reconstitutions. Eur. J. Biochem. **186:**303–308.
28. **Leung, W. K., M. Haapasalo, V.-J. Uitto, P. M. Hannam, and B. C. McBride.** 1996. The surface proteinase of *Treponema denticola* may mediate attachment of the bacteria to epithelial cells. Anaerobe **2:**39–46.
29. **Lindhe, J., and S. S. Socransky.** 1979. Chemotaxis and vascular permeability produced by human periodontopathic bacteria. J. Periodontal Res. **14:**138–146.
30. **Lisanti, M. P., M. Sargiacomo, L. Graeve, A. R. Saltiel, and E. Rodriguez-Boulan.** 1988. Polarized apical distribution of glycosylphosphatidylinositol-anchored proteins in a renal epithelial cell line. Proc. Natl. Acad. Sci. USA **85:**9557–9561.
31. **Listgarten, M. A.** 1965. Electron microscopic observation of the bacterial flora of acute necrotizing ulcerative gingivitis. J. Periodontol. **36:**328–339.
32. **Loesche, W. J., A. S. Syed, B. E. Laughon, and J. Stoll.** 1982. The bacteriology of acute necrotizing gingivitis. J. Periodontol. **53:**223–230.
33. **Mäkinen, K. K., P.-L. Mäkinen, and S. A. Syed.** 1992. Purification and substrate specificity of an endopeptidase from the human oral spirochete *Treponema denticola* ATCC 35405, active on furylacryloyl-Leu-Gly-Pro-Ala and bradykinin. J. Biol. Chem. **267:**14285–14293.
34. **Mauro, A., M. Blake, and P. Labarca.** 1988. Voltage gating of conductance in lipid bilayers induced by porin from outer membrane of Neisseria gonorrhoeae. Proc. Natl. Acad. Sci. USA **85:**1071–1075.
35. **Mikx, F. H. M., and R. A. C. Keulers.** 1992. Hemagglutination activity of *Treponema denticola* grown in serum-free medium in continuous culture. Infect. Immun. **60:**1761–1766.
36. **Moore, W. E. C., L. H. Moore, R. R. Ranney, J. A. Burmeister, R. M. Smibert, and H. A. Schenkein.** 1991. The microflora of periodontal sites showing active destructive progression. J. Clin. Periodontol. **18:**729–739.
37. **Morgan, H., J. T. Lonsdale, and G. Adler.** 1990. Polarity-dependent voltage-gated porin channels from *Escherichia coli* in lipid bilayer membranes. Biochim. Biophys. Acta **1021:**175–181.
38. **Nitzan, D., J. F. Sperry, and T. D. Wilkins.** 1978. Fibrinolytic activity of oral anaerobic bacteria. Arch. Oral Biol. **23:**465–470.
39. **Novak, M. J., and H. J. Cohen.** 1991. Depolarization of polymorphonuclear leukocytes by *Porphyromonas* (*Bacteriodes*) *gingivalis* 381 in the absence of respiratory burst activation. Infect. Immun. **59:**3134–3142.
40. **Ohta, K., K. K. Mäkinen, and W. J. Loesche.** 1986. Purification and characterization of an enzyme produced by *Treponema denticola* capable of hydrolyzing synthetic trypsin substrates. Infect. Immun. **53:**213–220.
41. **Roberts, C. J., T. Birkenmeier, J. McQuillan, S. Akiyama, S. Yamada, W.-T. Chen, K. Yamada, and J. McDonald.** 1988. Transforming growth factor β stimulates the expression of fibronectin and of both subunits of the human fibronectin receptor by cultured human lung fibroblasts. J. Biol. Chem. **263:**4586–4592.
42. **Rosenbusch, J. P.** 1990. Structural and functional properties of porin channels in *E. coli* outer membranes. Experientia **46:**167–173.
43. **Sauve, R., C. Simmoneau, R. Monette, and G. Roy.** 1986. Single-channel analysis of the potassium permeability in HeLa cancer cells: evidence for a calcium-activated potassium channel of small unitary conductance. J. Membr. Biol. **92:**269–282.
44. **Shenker, B. J., M. A. Listgarten, and N. S. Taichman.** 1984. Suppression of human lymphocyte responses by oral spirochetes: a monocyte-dependent phenomenon. J. Immunol. **132:**2039–2045.
45. **Socransky, S. S., and A. D. Haffajee.** 1991. Microbial mechanisms in the pathogenesis of destructive periodontal diseases: a critical assessment. J. Periodontal Res. **26:**195–212.
46. **Taichman, N. S., J. E. Klass, B. J. Shenler, E. J. Macarak, H. Boehringer, and C.-C. Tsai.** 1984. Suspected periodontopathic organisms alter *in vitro* proliferation of endothelial cells. J. Periodontal Res. **19:**583–586.
47. **Takahashi, A., H. Yamaguchi, and H. Miyamoto.** 1993. Change in K^+ current of HeLa cells with progression of the cell cycle studied by patch clamp technique. Am. J. Physiol. **265:**C328–C336.
48. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
49. **Tufano, M. A., L. Sommese, and F. Galdiero.** 1986. Some biological activities of *Eikenella corrodens* major outer membrane proteins. Eur. J. Epidemiol. **2:**305–311.
50. **Uitto, V.-J., D. Grenier, E. C. S. Chan, and B. C. McBride.** 1988. Isolation of a chymotrypsin-like enzyme from *Treponema denticola*. Infect. Immun. **56:**2717–2722.
51. **Uitto, V.-J., Y.-M. Pan, W. K. Leung, H. Larjava, R. P. Ellen, B. B. Finlay,**

and B. C. McBride. 1995. Cytopathic effects of *Treponema denticola* chymotrypsin-like proteinase on migrating and stratified epithelial cells. Infect. Immun. **63:**3401–3410.

52. **Ulmer, J. B., C. J. Burke, C. Shi, A. Friedman, J. J. Donnelly, and M. A. Liu.** 1992. Pore formation and mitogenicity in blood cells by the class 2 protein of *Neisseria meningitidis*. J. Biol. Chem. **267:**19266–19271.
53. **Umemoto, T., and I. Namikawa.** 1980. Electron microscopy of the spherical bodies of oral spirochetes *in vitro*. Microbiol. Immunol. **24:**321–334.
54. **Weinberg, A., and S. C. Holt.** 1990. Interaction of *Treponema denticola* TD-4, GM-1, and MS25 with human gingival fibroblasts. Infect. Immun. **58:**1720–1729.
55. **Wolf, V., R. Lange, and J. Wecke.** 1993. Development of quasi-multicellular bodies of *Treponema denticola*. Arch. Microbiol. **160:**206–213.
56. **Xu, G. Z., B. Shi, E. J. MacGroarty, and H. T. Tien.** 1986. Channel-closing activity of porins from *Escherichia coli* in bilayer lipid membranes. Biochim. Biophys. Acta **862:**57–64.

Editor: J. R. McGhee