

Type 2 Fimbrial Lectin-Mediated Phagocytosis of Oral *Actinomyces* spp. by Polymorphonuclear Leukocytes

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Phagocytosis of *Actinomyces viscosus* T14V and *A. naeslundii* WVU45 by human polymorphonuclear leukocytes in the absence of antibody or complement was mediated by the lectin associated with the type 2 fimbriae of these bacteria. This effect was markedly enhanced by exogenous sialidase, an enzyme also secreted by these actinomyces. Since sialidase treatment of the bacteria did not result in increased phagocytosis, this enzyme presumably acts by unmasking receptors for the fimbrial lectin on phagocytic cells. The viability of *A. viscosus* T14V, which possesses type 1 and type 2 fimbriae (1⁺2⁺), and *A. naeslundii* WVU45, which possesses only type 2 fimbriae (2⁺), was decreased by at least 98% following incubation with polymorphonuclear leukocytes in the presence of sialidase. Entirely analogous findings were obtained with a 1⁻2⁺ mutant of *A. viscosus* T14V. In contrast, the phagocytosis of 1⁺2⁻ and 1⁻2⁻ mutants of *A. viscosus* T14V and a 2⁻ mutant of *A. naeslundii* WVU45 was minimal or absent. Lactose and β-methylgalactoside inhibited the destruction of the bacteria, whereas cellobiose and α-methylgalactoside were ineffective. Thus, the type 2 fimbriae of the oral actinomyces recognize galactose-containing receptors on polymorphonuclear leukocytes which have been exposed by the removal of sialic acid, an interaction that is followed by internalization and subsequent killing of the bacteria.

Certain strains of oral actinomyces are susceptible to the bactericidal effects of polymorphonuclear leukocytes, and these bacteria concomitantly or independently induce the degranulation of phagocytic cells (20), a process which may contribute to inflammation of the surrounding tissues. Since phagocytosis of the actinomyces can occur in the absence of antibody or complement or both (15), this event must be initiated by the recognition of receptors on phagocytic cells by bacterial adhesins.

Accumulating evidence indicates that the adherence of these bacteria to other cells and tissues is mediated by a series of highly specific interactions involving the actinomyces fimbriae. These interactions appear to be instrumental in determining the site of colonization within the oral cavity by different strains of actinomyces. Thus, *Actinomyces viscosus* T14V, an organism that possesses two antigenically distinct types of fimbriae (type 1 and type 2) (5), predominantly colonizes the tooth surface (19). This property of *A. viscosus* T14V is attributable to the type 1 fimbriae. Antibodies monospecific for the type 1 fimbriae but not the type 2 fimbriae inhibit bacterial attachment to saliva-treated hydroxyapatite, an *in vitro* model of the acquired pellicle (8). In contrast, *A. naeslundii* WVU45, an organism that possesses only type 2 fimbriae (6), is more commonly found on epithelial surfaces (10, 17). This interaction is dependent on the recognition of glycoprotein receptors on epithelial cells by the lectin associated with the type 2 fimbriae (3).

The type 2 fimbriae of the actinomyces also recognize receptors on other bacteria, thereby contributing to the establishment of oral microbial communities. An investigation of the interaction of *A. viscosus* T14V or *A. naeslundii* WVU45 with *Streptococcus sanguis* 34 provided the initial description of the lectin activity associated with the

actinomyces (14), and subsequent studies associated this activity with the type 2 fimbriae (5).

The definition of these distinct attachment properties of the type 1 and type 2 actinomyces fimbriae has provided a basis for examining their possible participation in phagocytosis by polymorphonuclear leukocytes. The present studies were therefore designed to determine if the functional activities of these fimbriae include not only adherence but also stimulation of a phagocytic cell population.

MATERIALS AND METHODS

Materials. Sialidase (neuraminidase from *Clostridium perfringens*) type X and saccharides were purchased from Sigma Chemical Co., St. Louis, Mo.

Microorganisms. *A. viscosus* T14V and *A. naeslundii* WVU45 were obtained and grown as previously described (7). Mutants of these bacteria which lacked specific fimbriae were selected by their failure to react with monospecific antisera against type 1 or type 2 fimbriae (6; J. O. Cisar, manuscript in preparation).

Bactericidal assay. Polymorphonuclear leukocytes were obtained by Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, N.J.) separation of human peripheral blood. Erythrocytes were lysed with NH₄Cl lysing buffer (National Institutes of Health Media Unit, Bethesda, Md.). Assay mixtures of 1.5 ml contained 6 × 10⁶ polymorphonuclear leukocytes and 10⁷ bacteria in RPMI 1640 (Quality Biological, Inc., Gaithersburg, Md.) supplemented with 9 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 0.15 mM CaCl₂, 1 mM MgCl₂ and 1% bovine serum albumin. As indicated below, 0.05 U of sialidase per ml, was included. These mixtures were incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 15 min, sealed with Parafilm, placed on a rotating wheel at 37°C for various times, and sonicated with a Micro Ultrasonic Cell

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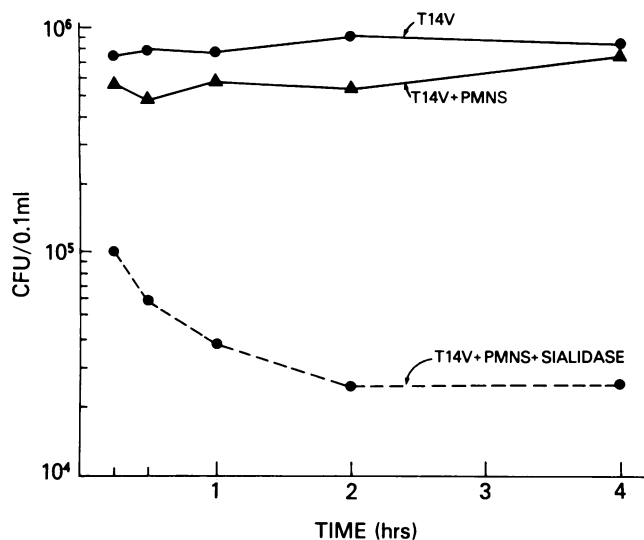


FIG. 1. Bactericidal activity of polymorphonuclear leukocytes (PMNS) against *A. viscosus* T14V (T14V). Bacteria (10⁷) and 6 × 10⁶ PMNS were incubated for the indicated times at 37°C in the absence or presence of 0.05 U of sialidase per ml. Reaction mixtures were sonicated, and aliquots were diluted and plated. Data represent the means of at least two dilutions of duplicate incubation mixtures.

Disruptor (Kontes, Vineland, N.J.) for 30 s. Disruption of the polymorphonuclear leukocytes was verified by microscopic examination. Aliquots were diluted in RPMI 1640 supplemented as indicated above and, in addition, with 0.05% Tween 20 and were plated on brain heart infusion agar (National Institutes of Health Media Unit), and the colonies were counted after incubation of the plates for 3 days at 37°C. Plates containing *A. naeslundii* WVU45 were incubated in anaerobic culture jars. The data presented are representative of at least five experiments.

Electron microscopy. In some experiments, Karnovsky fixative containing 1% tannic acid was added to the assay mixtures. The samples were postfixed in 0.1 M sodium cacodylate buffer (pH 7.3) containing 1% osmium tetroxide, dehydrated in ethanol, and infiltrated with Epon (M. J. Karnovsky, *J. Cell Biol.* 27:137A, 1965).

RESULTS

Effect of sialidase on the destruction of *A. viscosus* T14V by polymorphonuclear leukocytes. Some bactericidal activity was detected in incubation mixtures containing *A. viscosus*

TABLE 1. Bactericidal activity of polymorphonuclear leukocytes against *A. viscosus* T14V pretreated with medium or sialidase

Incubation mixture ^a	Viable bacteria (10 ⁵ CFU/0.1 ml) ^b	
	Medium	Sialidase
Bacteria	10.8	13.2
Bacteria + PMNs	10.1	14.8
Bacteria + PMNs + sialidase	1.1	1.4

^a Mixtures containing 10⁷ bacteria, 10⁷ bacteria and 6 × 10⁶ polymorphonuclear leukocytes (PMNs), or 10⁷ bacteria, 6 × 10⁶ PMNs, and 0.05 U of sialidase per ml were incubated for 2 h at 37°C and sonicated, and aliquots were diluted and plated.

^b Data represent the averages of at least two dilutions of duplicate incubation mixtures. Bacteria (10⁸) were incubated with medium or medium containing 0.05 U of sialidase per ml for 1 h at 37°C, washed twice, and resuspended prior to the addition of PMNs.

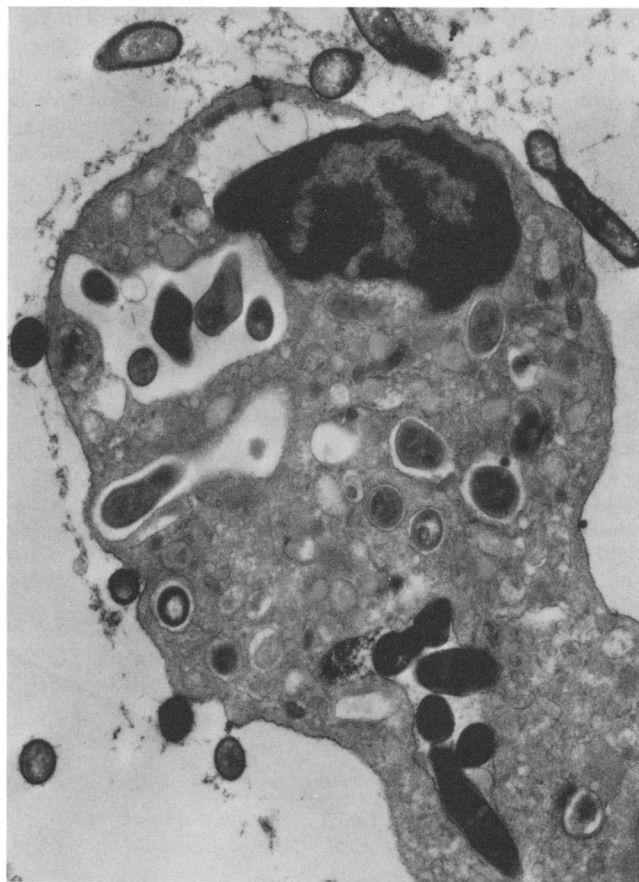


FIG. 2. Electron micrograph of polymorphonuclear leukocytes incubated with *A. viscosus* T14V for 30 min at 37°C in the presence of 0.05 U of sialidase per ml. ×11,600.

T14V and polymorphonuclear leukocytes at a ratio of 1.7:1 during a 4-h time period (Fig. 1). However, the addition of sialidase, an enzyme produced by the actinomyces, to these mixtures markedly enhanced the polymorphonuclear leukocyte-mediated destruction of the bacteria. This effect was evident at 15 min and optimal at 2 h, after which no further decrease in the numbers of viable bacteria occurred. At least a 10-fold and usually a 30- to 100-fold reduction in CFU was routinely observed after incubation with sialidase and polymorphonuclear leukocytes. Treatment of the bacteria with sialidase prior to incubation with polymorphonuclear leukocytes did not affect the viability of the bacteria and failed to result in enhanced polymorphonuclear leukocyte-mediated bactericidal activity (Table 1). Electron-microscopic examination of the reaction mixtures suggested that the bactericidal activity could be attributed to phagocytosis (Fig. 2) but did not exclude the possible contribution of extracellular killing. Ingestion of the actinomyces was observed as early as 4 min after the addition of polymorphonuclear leukocytes.

Involvement of the type 2 fimbriae in the phagocytosis of the actinomyces. One-third of the *A. viscosus* T14V, which possesses both type 1 and type 2 fimbriae, was destroyed by polymorphonuclear leukocytes in the absence of exogenous sialidase (Fig. 3). The addition of sialidase resulted in a 98% reduction in the numbers of viable bacteria. Analogous results were obtained with a mutant of *A. viscosus* T14V which possesses the type 2 fimbriae but lacks the type 1

fimbriae. The polymorphonuclear leukocyte-dependent bactericidal activity against a mutant possessing the type 1 fimbriae but lacking the type 2 fimbriae and a mutant lacking both fimbriae was minimal or absent. In marked contrast to the results obtained with *A. viscosus* T14V and the mutant possessing only the type 2 fimbriae, the addition of sialidase did not enhance the phagocytosis of the two mutants which lacked the type 2 fimbriae. These findings were supported by electron-microscopic examination of reaction mixtures containing the parent strain or each of the three mutants. The parent strain and the mutant possessing only the type 2 fimbriae were readily detected in the phagosomes of polymorphonuclear leukocytes, whereas the mutant lacking the type 2 fimbriae but possessing the type 1 fimbriae and the mutant lacking both fimbriae were either absent or only occasionally observed within these vacuoles (data not shown).

Further evidence for the participation of the type 2 fimbriae in phagocytosis was obtained by examining the interaction of *A. naeslundii* WVU45 with polymorphonuclear leukocytes. This strain possesses only the type 2 fimbriae, and 44% phagocytosis was observed in the absence of sialidase (Fig. 4). The addition of sialidase resulted in a 99% reduction in CFU. A mutant selected for its lack of fimbriae was not phagocytosed in either the presence or the absence of sialidase. These findings clearly implicate the type 2 fimbriae in this interaction of the actinomyces with polymorphonuclear leukocytes.

Inhibition of the phagocytosis of *A. viscosus* T14V by saccharides. The addition of lactose or β -methylgalactoside to mixtures of *A. viscosus* T14V and polymorphonuclear leukocytes in the presence of sialidase inhibited the phago-

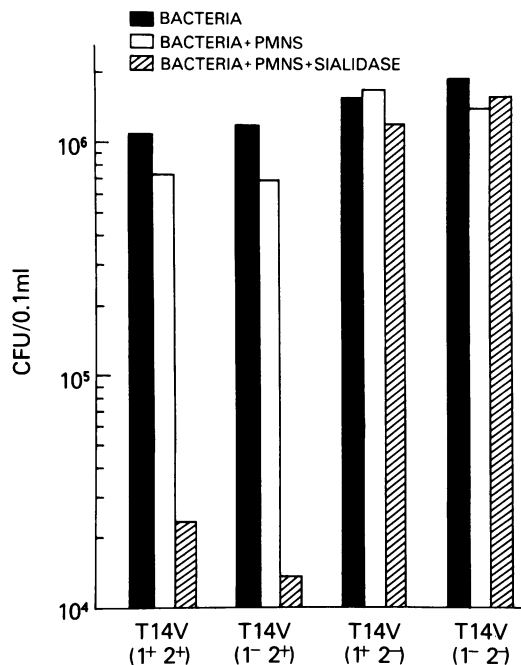


FIG. 3. Bactericidal activity of polymorphonuclear leukocytes (PMNS) against the parent and mutant strains of *A. viscosus* T14V (T14V). Bacteria (10^7) were incubated with 6×10^6 PMNS in the absence or presence of 0.05 U of sialidase per ml for 2 h at 37°C. Reaction mixtures were sonicated, and aliquots were diluted and plated. Data represent the averages of at least two dilutions of duplicate incubation mixtures. 1⁺, 2⁺, 1⁻, and 2⁻ indicate the presence or absence of type 1 and type 2 fimbriae.

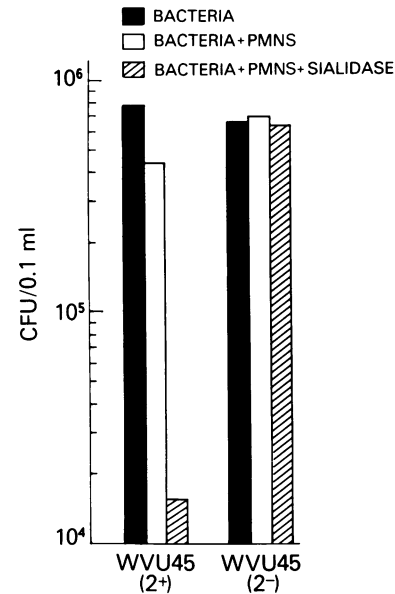


FIG. 4. Bactericidal activity of polymorphonuclear leukocytes (PMNS) against the parent and mutant strains of *A. naeslundii* WVU45 (WVU45). Bacteria (10^7) were incubated with 6×10^6 PMNS in the absence or presence of 0.05 U of sialidase per ml for 2 h at 37°C. Reaction mixtures were sonicated, and aliquots were diluted and plated. Data represent the averages of at least two dilutions of duplicate incubation mixtures. 2⁺ and 2⁻ indicate the presence or absence of type 2 fimbriae.

cytosis of the bacteria (Fig. 5). Neither α -methylgalactoside nor cellobiose was an effective inhibitor. These results indicate that the recognition of receptors on polymorphonuclear leukocytes by the type 2 fimbriae is dependent on the lectin activity associated with these bacterial structures.

DISCUSSION

These findings clearly implicate the type 2 fimbriae of *A. viscosus* T14V and *A. naeslundii* WVU45 in the phagocytosis and subsequent destruction of these bacteria by polymorphonuclear leukocytes. Moreover, this effect is attributable to the recognition of carbohydrate-containing receptors on phagocytic cells by the lectin associated with the type 2 fimbriae. Previous studies have demonstrated that the type 2 fimbrial lectin also mediates the attachment of the actinomyces to streptococci and epithelial cells (3, 5). However, the adherence of these bacteria to polymorphonuclear leukocytes is of major interest in that it is accompanied by the activation of these inflammatory cells, resulting in phagocytosis. Although this series of events provides a mechanism of host defense against the actinomyces, phagocytosis of these bacteria may also have detrimental consequences for host tissues by the release of leukocyte-derived inflammatory agents.

Some bactericidal activity (up to a 44% reduction in the numbers of viable bacteria) is observed in the absence of exogenous sialidase. However, the addition of this enzyme, which presumably unmasks the receptors for the type 2 fimbrial lectin on the polymorphonuclear leukocytes, markedly enhances this effect. The production of sialidase by actinomyces has been documented (9), and the exposure of receptors by this enzyme probably represents the initial event in the bactericidal process. Similarly, sialidase signifi-

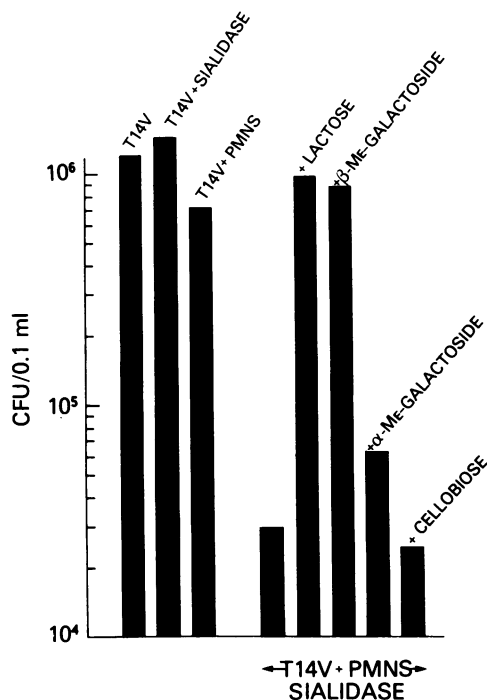


FIG. 5. Effect of saccharides and methyl (Me) galactosides on the bactericidal activity of polymorphonuclear leukocytes (PMNS) against *A. viscosus* T14V (T14V). Bacteria (10^7) were incubated with 6×10^6 PMNS in the presence or absence of 0.05 U of sialidase per ml. Saccharides were added at a final concentration of 30 mM as indicated, and reaction mixtures were incubated for 2 h at 37°C. Data represent the averages of at least two dilutions of duplicate reaction mixtures.

icantly elevates the attachment of actinomyces to epithelial cells (3), and its production would, therefore, facilitate colonization of epithelial surfaces. The exogenous sialidase used in these studies had no effect on the bacteria, since treatment of *A. viscosus* T14V with this enzyme prior to incubation of the bacteria with polymorphonuclear leukocytes did not affect bacterial viability or increase the susceptibility of the bacteria to the phagocytic cells.

Two strains of actinomyces, *A. viscosus* T14V and *A. naeslundii* WVU45, and mutants of these strains lacking specific fimbriae were used to assess the involvement of fimbriae in phagocytosis. The viability of both of these parent strains and their mutants possessing type 2 fimbriae was decreased by at least 98% after incubation with polymorphonuclear leukocytes in reaction mixtures containing sialidase. In contrast, mutants lacking type 2 fimbriae were not phagocytosed. The presence or absence of the type 1 fimbriae did not influence the bactericidal activity of polymorphonuclear leukocytes.

The initiation of phagocytosis by type 2 fimbriae is attributed to the lectin activity associated with these structures. Polymorphonuclear leukocyte-dependent destruction of *A. viscosus* T14V was completely inhibited by lactose and β -methylgalactoside but not by cellobiose or α -methylgalactoside. Thus, the interaction of the actinomyces with polymorphonuclear leukocytes apparently involves a galactose-containing carbohydrate receptor on phagocytic cells, and this saccharide may be in β linkage. The receptors for the type 2 fimbriae on streptococci and oral epithelial cells also contain galactose or *N*-acetylgalactosamine. The strep-

tococcal receptor has been purified and consists of hexasaccharide subunits with terminal *N*-acetylgalactosamine α 1-3 linked to rhamnose (13; F. C. McIntire, personal communication). Studies are in progress to purify and characterize the receptor for the type 2 fimbriae on the KB oral epithelial cell line (4). This receptor was identified by the utilization of plant lectins which inhibit attachment of the actinomyces to epithelial cells. Peanut agglutinin, which is specific for Gal β 3GalNAc, and *Bauhinia purpurea* lectin, which is specific for galactose or *N*-acetylgalactosamine, are effective inhibitors of this interaction. These lectins detect a 160-kilodalton glycoprotein in extracts of KB cells separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis a 160-kilodalton glycoprotein that can be both metabolically and surface labeled. Similar studies concerning the receptor for the actinomyces on polymorphonuclear leukocytes have been initiated. These investigations will facilitate the definition of the molecular basis for the initiation of phagocytosis of the actinomyces by polymorphonuclear leukocytes and will permit the comparison of this receptor with other actinomyces receptors on procaryotic and eucaryotic cells.

The interaction of certain strains of *Escherichia coli* with polymorphonuclear leukocytes is also mediated by a fimbria-associated lectin. Strains of *E. coli* which possess mannose-sensitive fimbriae attach to polymorphonuclear leukocytes (1, 2, 11), and adherence is inhibited by mannose (1, 11). This saccharide also blocks the subsequent phagocytosis of *E. coli* (16, 18) as well as the chemiluminescence of polymorphonuclear leukocytes incubated with these bacteria (12).

Clearly emerging from these studies is the definition of a series of specific interactions of the type 2 fimbriae on actinomyces with receptors on different oral surfaces and inflammatory cells. Through their reaction with similar but not necessarily identical receptors on other bacteria and mammalian cells, these fimbriae initiate the colonization of epithelial cells by actinomyces, participate in the accumulation of plaque, and mediate the destruction of the bacteria by phagocytic cells.

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