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# Role of the Capsular Polysaccharide-Like Serotype-Specific Antigen in Resistance of *Actinobacillus actinomycetemcomitans* to Phagocytosis by Human Polymorphonuclear Leukocytes

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**Serotype b-specific polysaccharide antigen (SPA) of *Actinobacillus actinomycetemcomitans* Y4 consists of D-fucose and L-rhamnose. To clarify the role of SPA in phagocytosis of the organism by human polymorphonuclear leukocytes (PMNs), monoclonal antibodies (MAbs) against SPA and SPA-defective mutants, which were constructed by inserting the transposon Tn*916* into strain Y4, were used in a chemiluminescence (CL) assay and a phagocytic killing assay. The CL responses of human PMNs to strain Y4 were very low, and the organism was not killed by PMNs. In contrast, SPA-defective mutants induced strong CL responses. The addition of immunoglobulin G MAbs against Y4 SPA enhanced significantly both the CL responses to strain Y4 and the killing of the organism in the presence of complement. The CL responses to SPA-defective mutants were little affected by the addition of these MAbs. We conclude that SPA of *A. actinomycetemcomitans* plays an important role in the resistance to host defenses by PMNs.**

*Actinobacillus actinomycetemcomitans* has been implicated as one of the causative organisms of localized juvenile periodontitis (12, 41), adult periodontitis (5), and severe nonoral human infections (20). *A. actinomycetemcomitans* strains from the human oral cavity were divided into five serotypes, a, b, c, d, and e (18, 30, 42). The most common serotype isolated in subjects with localized juvenile periodontitis is serotype b (5, 42). In addition, patients with localized juvenile periodontitis exhibit elevated serum antibody levels to a serotype b-specific antigen of *A. actinomycetemcomitans* (10, 33). We reported previously that the serotype b-specific antigen of *A. actinomycetemcomitans* is a capsular polysaccharide-like antigen consisting of a disaccharide repeating unit, →3)-α-D-fucopyranosyl-(1→2)-β-L-rhamnopyranosyl-(1→ (3). However, little is known about the periodontopathogenic mechanism of the serotype b-specific polysaccharide antigen (SPA).

Phagocytic cells such as neutrophils and monocytes are considered to play a major role in preventing bacterial infections in periodontitis (25). Neutrophils in patients with localized juvenile periodontitis exhibit functional abnormalities such as defective chemotaxis, increased $O_2$ production, increased adherence, and reduced bactericidal activity (2, 37).

We have recently inserted the transposon Tn*916* into serotype b *A. actinomycetemcomitans* Y4 to construct its SPA-defective mutants (32). In this study, we compared the chemiluminescence (CL) responses of human polymorphonuclear leukocytes (PMNs), which are dependent on cellular metabolism of molecular oxygen (14, 19), to these SPA-defective mutants with those to the parent strain. Furthermore, we determined the opsonic activity of monoclonal antibodies (MAbs) against several antigens of *A. actinomycetemcomitans* in CL and killing assays.

## MATERIALS AND METHODS

**Bacteria.** *A. actinomycetemcomitans* Y4 (serotype b) was obtained from Y. Yamamoto (Sunstar Corp., Osaka, Japan). The strain was grown in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) supplemented with 1% (wt/vol) yeast extract (THY broth) at 37°C in a 5% $CO_2$ atmosphere. Four SPA-defective mutants of *A. actinomycetemcomitans* Y4 (strains ST1, ST2, ST3, and ST5) were constructed by inserting the transposon Tn*916* as described previously (32). Plasmid pAM120 (pGL101::Tn*916*) was isolated from *Escherichia coli* DH1 containing the plasmid and purified twice by CsCl-ethidium bromide gradient centrifugation. *A. actinomycetemcomitans* Y4 was grown overnight in THY broth. The overnight culture (0.5 ml) was diluted into 5 ml of fresh THY broth and incubated at 37°C in a 5% $CO_2$ atmosphere for about 4 h. Samples (0.5 ml) were gently combined with 1 μg of pAM120 DNA. The mixtures were diluted with 1 ml of fresh THY broth and allowed to stand at 37°C in a 5% $CO_2$ atmosphere for 4 h. The cultures were plated on THY agar containing tetracycline (2 μg/ml) and incubated at 37°C for 3 days. The expression of SPA in colonies was determined by colony immunoblotting with MAb directed against SPA of *A. actinomycetemcomitans* Y4 (MAb S5) (32). Colonies that failed to react with MAb S5 were isolated as SPA-defective mutants. These mutants were grown in THY broth containing tetracycline (2 μg/ml). The cells were harvested at the mid-logarithmic phase of growth ($A_{580}$, ca. 0.2), washed twice with gelatin–Veronal-buffered saline (pH 7.5), supplemented with 2.5% glucose, 1 mM $MgCl_2$, and 0.15 mM $CaCl_2$ ($GGVB^{++}$) (17), and suspended in $GGVB^{++}$.

**Preparation of antigens.** SPA was extracted from whole cells of *A. actinomycetemcomitans* Y4 by autoclaving. The extract was purified by chromatography on DEAE-Sephadex A-25 (Pharmacia, Uppsala, Sweden) and Sephacryl S-300 (Pharmacia) as described by Amano et al. (3). Lipopolysaccharide (LPS) was extracted from whole cells of *A. actinomycetemcomitans* Y4 by the hot phenol-water procedure, treated with nuclease, and washed extensively with pyrogen-free water by ultrafiltration (34). The crude Y4 LPS was purified by chromatography on Sephadex G-200 (Pharmacia) equilibrated with 10 mM Tris HCl (pH 8.0) containing 0.2 M NaCl, 0.25% deoxycholate, 1 mM EDTA, and 0.02% sodium azide (21). The GroEL-like 64-kDa protein was extracted from whole cells of *A. actinomycetemcomitans* Y4 by mechanical destruction and purified by chromatography on DEAE-Sepharose CL-6B (Pharmacia), Sephadex G-100 (Pharmacia), and hydroxyapatite (BDH, Poole, England) as described by Nakano et al. (28).

**MAbs.** MAbs to SPA, LPS, and the 64-kDa protein of *A. actinomycetemcomitans* strain Y4 were elaborated by polyethylene glycol-induced fusion of SP2/0-Ag14 mouse myeloma cells and spleen cells from BALB/c mice immunized with

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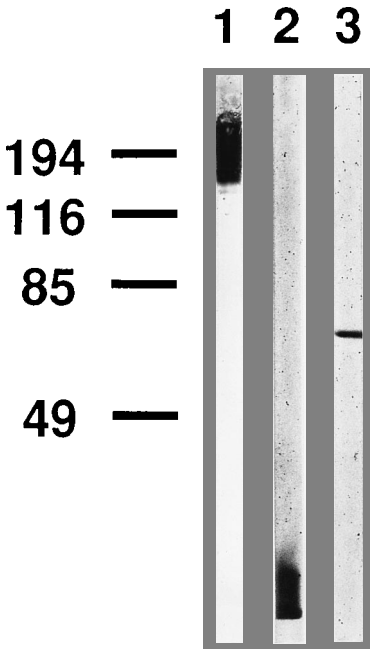


FIG. 1. Western blot of SPA (lane 1), LPS (lane 2), and the 64-kDa protein (lane 3) from *A. actinomycetemcomitans* Y4 developed with MAb S5 (100 μg/ml), MAb L2 (100 μg/ml), and MAb P3 (100 μg/ml), respectively. Sizes of proteins (in kilodaltons) are shown on the left.

whole cells of *A. actinomycetemcomitans* Y4 (22). Hybridoma cells ($5 \times 10^6$) were inoculated into BALB/c mice which had been primed 7 to 10 days earlier by an intraperitoneal injection of pristane. Ascitic fluids were taken starting 7 days after inoculation of the hybridoma cells. After the ascitic fluids were centrifuged, ammonium sulfate was added to the supernatants to a 50% saturation. The precipitates were dissolved in phosphate-buffered saline (pH 7.2; PBS) and dialyzed against PBS. These ascitic fluids were used as MAb preparations in all experiments. The antigenic specificities of these MAbs were determined by Western blotting as described below. The subclass of MAb was determined by mouse monoclonal antibody isotyping kit (Amersham Corp., Arlington Heights, Ill.). Protein concentrations of MAbs were determined by the method of Lowry et al. (24).

**Preparation of complement.** Lyophilized cells of *A. actinomycetemcomitans* Y4 (0.5 g [dry weight]) were suspended in 50 ml of PBS and washed three times with PBS. The washed cells were resuspended in 50 ml of fresh human sera from periodontally healthy adult volunteers with undetectable anti-SPA antibody (determined by an enzyme-linked immunosorbent assay) (31). The suspension was incubated for 1 h at 4°C. The cells were removed by centrifugation, and then the supernatant was passaged through a 0.45-μm membrane filter (Millipore Corp., Bedford, Mass.). The absorption was repeated twice. The absorbed sera served as the complement source and were stored in 1-ml aliquots at −70°C until used.

**Preparation of PMNs.** Heparinized blood was obtained from healthy adult volunteers. PMNs were isolated with Mono-Poly resolving medium (ICN Biomedicals Japan Co., Tokyo, Japan), washed with PBS, and suspended in $GGVB^{++}$.

**CL assay.** CL was measured as described by Mundi et al. (27) and China et al. (11). Reaction mixtures contained human PMNs ($10^6$ cells), 20 μl of luminol solution (2 mg/ml), and 10 μl of complement in a total volume of 980 μl of $GGVB^{++}$. In the assay for determining the opsonic activity of MAbs, each MAb was added to the reaction mixture at 100 μg of protein per ml. After the mixtures were allowed to equilibrate at 37°C for 10 min, 20 μl of bacterial suspension ($2 \times 10^8$ CFU/ml) was added to activate the system. The light emission was recorded continuously for 1 h with a six-channel Biolumat LB9505 luminometer (Berthold, Wildbad, Germany). Data are expressed as the mean of CL peak values ($10^5$ cpm/$10^6$ PMNs) ± standard deviation for three different experiments.

**Phagocytic killing assay.** The killing assay was carried out as described by Baltimore et al. (7). Briefly, bacteria ($2 \times 10^6$ CFU) were incubated with PMNs ($2 \times 10^6$ cells) and 50 μl of complement in 1 ml of $GGVB^{++}$ in sterile plastic tubes. For determining the opsonic activity of MAbs in the killing assay, each MAb was added to the reaction mixture described above at a final concentration of 100 μg of protein per ml. After incubation at 37°C for 1 or 2 h, aliquots were removed, diluted immediately in cold sterile water, and spread on THY agar plates for quantitative culture at zero time and after 1 or 2 h of incubation. Percent survival was calculated as [(CFU per milliliter after 1 or 2 h)/(CFU per milliliter at zero time)] × 100. Data are expressed as the mean percent survival ± standard error for three different experiments performed in triplicate.

**Western blotting (immunoblotting).** SPA (16 mg), LPS (1 mg), or the 64-kDa protein (1 mg) from *A. actinomycetemcomitans* Y4 was suspended in 1 ml of 10 mM Tris HCl buffer (pH 6.8), containing 1% sodium dodecyl sulfate (SDS), 1% mercaptoethanol, and 20% glycerol, and heated at 100°C for 5 min. The samples (10 μl) were electrophoresed at 25 mA per gel at room temperature for 60 min with a 12.5% resolving gel and a 4% stacking polyacrylamide gel (90 by 80 by 1 mm) containing 0.1% SDS. After SDS-polyacrylamide gel electrophoresis, the gels were transferred to a nitrocellulose sheet by the Western blotting technique (9). The sheet was treated with MAb diluted 1:100 in PBS containing 0.05% Tween 20 and 1% bovine serum albumin (PBST-BSA). The antibodies bound to the immobilized replica antigens on the sheet were detected by a solid-phase immunoassay with alkaline phosphatase-conjugated goat anti-mouse immunoglobulins (Zymed Laboratories, South San Francisco, Calif.) diluted 1:1,000 in PBST-BSA. Prestained protein standards (Bio-Rad Laboratories, Richmond, Calif.) were used for the estimation of the molecular weights of specific bands.

**Statistical analysis.** Differences in the peak CL responses and the percent survival values were determined by Student's *t* test.

## RESULTS

**Effects of various MAbs on CL responses.** To determine the opsonic activity of MAbs against antigens of *A. actinomycetemcomitans* strain Y4, we used 10 MAbs in this study. The specificity of MAbs was determined by Western blotting with antigens extracted from whole cells of *A. actinomycetemcomitans* Y4. Five MAbs (S2, S4, S5, S8, and S9), three MAbs (L2, L3, and L4), and two MAbs (P2 and P3) reacted specifically with SPA, LPS, and the 64-kDa protein, respectively, from the organism (Fig. 1). The opsonic activity of these MAbs was determined by CL assay. The CL response of human PMNs to *A. actinomycetemcomitans* Y4 in the presence of complement was weak, and the peak CL response was reached after about 30 min of incubation. The addition of MAb S5 at concentrations of 1, 10, and 100 μg/ml increased the peak values by about 2, 3, and 6 times, respectively (Fig. 2). The degree of enhancement of the CL response by the addition of MAb S5 was very weak in the absence of complement.

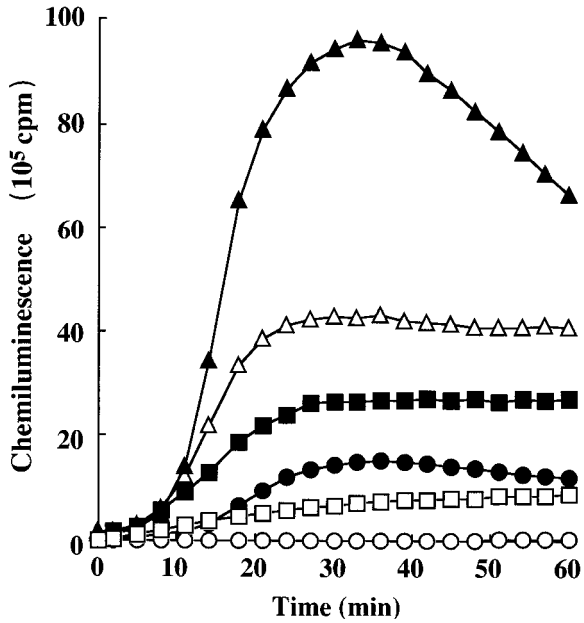


FIG. 2. Effects of MAb against SPA of *A. actinomycetemcomitans* Y4 on CL responses of human PMNs. Human PMNs ($10^6$ cells) pretreated with $GGVB^{++}$ (○), 1% complement alone (●), MAb S5 (100 μg/ml) alone (□), or 1% complement plus MAb S5 (at 1 [■], 10 [△], or 100 [▲] μg/ml) were stimulated at 37°C for 1 h with *A. actinomycetemcomitans* Y4 ($4 \times 10^6$ CFU). The experiments were performed three times, and similar results were obtained in each experiment.

TABLE 1. Effects of various MAbs on CL responses of human PMNs induced by phagocytosis of *A. actinomycetemcomitans* Y4[a]

| MAb | Class or subclass | CL peak ($10^5$ cpm/$10^6$ PMNs) |
|---|---|---|
| Anti-SPA | | |
| S2 | IgG1 | 63.7 ± 16.6[b] |
| S4 | IgG2a | 33.0 ± 6.9[b] |
| S5 | IgG3 | 98.5 ± 4.0[c] |
| S8 | IgG3 | 56.6 ± 6.3[c] |
| S9 | IgM | 2.7 ± 1.3 |
| Anti-LPS | | |
| L2 | IgG2b | 4.8 ± 0.9 |
| L3 | IgG3 | 6.9 ± 0.7 |
| L4 | IgG3 | 4.7 ± 0.2 |
| Anti-64-kDa protein | | |
| P2 | IgG2a | 2.4 ± 0.9 |
| P3 | IgG2a | 2.7 ± 1.6 |
| Normal ascites | | 3.7 ± 1.3 |

[a] Human PMNs ($10^6$ cells) pretreated with 1% complement and MAb at a final concentration of 100 μg/ml were stimulated at 37°C for 1 h with *A. actinomycetemcomitans* Y4 ($4 \times 10^6$ CFU). The data show the mean of CL peak values ± standard deviation for three different experiments.
[b] $P < 0.01$ versus the normal ascites group.
[c] $P < 0.001$ versus the normal ascites group.

Table 1 shows the effects of MAbs of different classes and subclasses against antigens of *A. actinomycetemcomitans* on CL responses of human PMNs. The addition of immunoglobulin G1 (IgG1), IgG2a, and IgG3 anti-SPA MAbs to the CL assay exhibited significantly higher peak CL values than that of normal ascites. In contrast, the addition of IgM anti-SPA MAb and IgG2a anti-64-kDa protein MAb did not affect the CL responses. IgG2b and IgG3 anti-LPS MAbs weakly enhanced the responses.

**PMN CL response to SPA-defective mutants.** The ability of *A. actinomycetemcomitans* Y4 and its SPA-defective variants to elicit the CL response of human PMNs in the presence of complement was examined. Figure 3A shows typical patterns of the CL responses of PMNs induced by *A. actinomycetemcomitans* strains. The peak CL values induced by strains Y4, ST1, ST2, ST3, and ST5 were $40.3 \times 10^5 \pm 17.7 \times 10^5$ cpm (mean ± standard deviation for three independent experiments), $82.1 \times 10^5 \pm 19.9 \times 10^5$ cpm, $71.8 \times 10^5 \pm 13.2 \times 10^5$ cpm, $100.3 \times 10^5 \pm 21.8 \times 10^5$ cpm, and $93.1 \times 10^5 \pm 14.0 \times 10^5$ cpm, respectively. There was a statistically significant difference between the peak CL response induced by strain Y4 and that induced by strain ST1 ($P < 0.1$), ST2 ($P < 0.1$), ST3 ($P < 0.05$), or ST5 ($P < 0.05$). PMNs did not produce any CL responses in the absence of bacteria (Fig. 3A). We examined the effects of IgG3 MAb S5 on the CL responses of PMNs induced by strain Y4 and SPA-defective mutants. The addition of MAb S5 (100 μg/ml) markedly enhanced the CL responses induced by strain Y4, but it had little effect upon the CL responses induced by SPA-defective mutants (Fig. 3B). These mutants did not generate any CL responses of human PMNs in the absence of complement and MAb S5 (data not shown).

**Killing of *A. actinomycetemcomitans* by PMNs.** The effect of MAbs against antigens of *A. actinomycetemcomitans* on opsonophagocytic killing by human PMNs in the presence of complement was examined. *A. actinomycetemcomitans* Y4 was resistant to the killing by human PMNs in the presence of complement (Fig. 4). The addition of IgG3 MAb against Y4 SPA (MAb S5) reduced the bacterial viability by 92% after 2 h of incubation, whereas the addition of IgG2b MAb against Y4

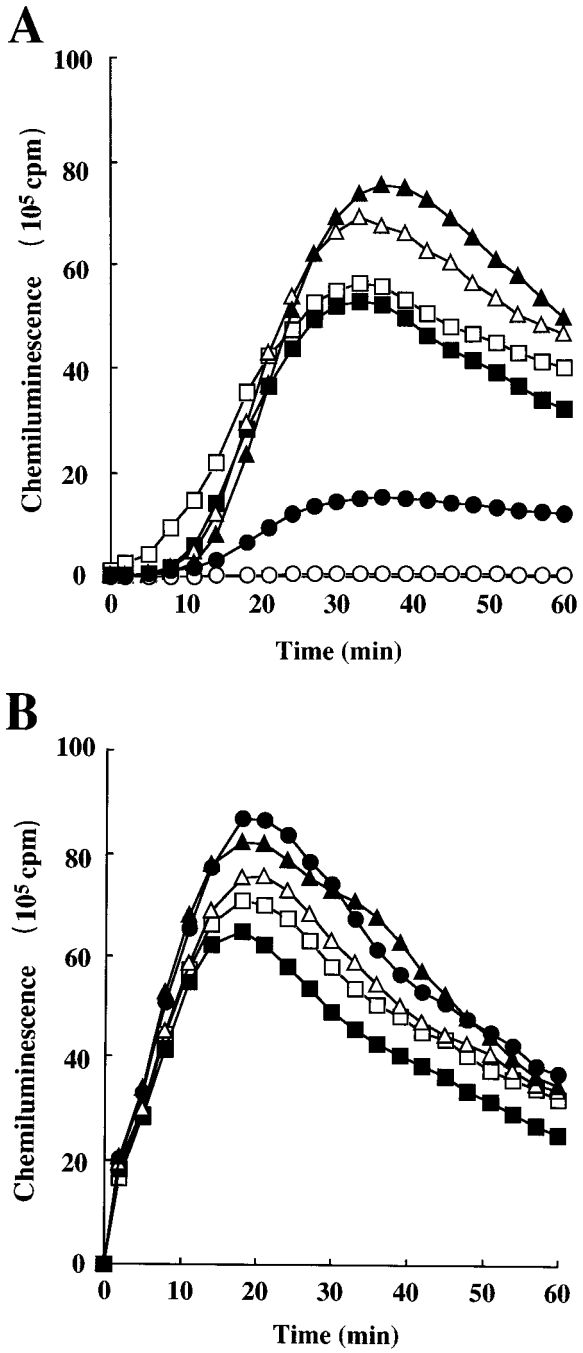


FIG. 3. Representative CL responses of human PMNs to *A. actinomycetemcomitans* strains. Human PMNs ($10^6$ cells) pretreated with 1% complement (A) or 1% complement plus MAb S5 (100 μg/ml) (B) were unstimulated (○) or stimulated at 37°C for 1 h with *A. actinomycetemcomitans* Y4 (●), ST1 (□), ST2 (■), ST3 (△), or ST5 (▲) ($4 \times 10^6$ CFU). The experiments were performed three times, and similar results were obtained in each experiment.

LPS (MAb L2) did so by 31%. The viability was not affected by the addition of normal ascites. We examined the role of complement in the enhancement of PMN killing of *A. actinomycetemcomitans* Y4 by the addition of MAb S5. The presence of complement only or complement plus human PMNs did not have any significant effect on the viability of *A. actinomycetemcomitans* Y4 (Table 2). The viability of the organism after 2 h

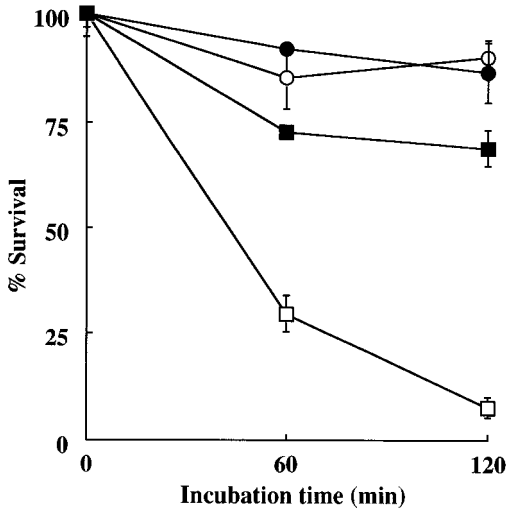


FIG. 4. Effects of MAb on opsonophagocytic killing of *A. actinomycetemcomitans* Y4 by human PMNs. *A. actinomycetemcomitans* Y4 ($2 \times 10^6$ CFU) was incubated with human PMNs ($2 \times 10^6$ cells) in the presence of 5% complement (○), 5% complement plus normal ascites (100 μg/ml) (●), 5% complement plus MAb S5 (100 μg/ml) (□), or 5% complement plus MAb L2 (100 μg/ml) (■). Data are the mean percent survival ± standard error for three different experiments performed in triplicate.

of incubation in the presence of MAb S5 only, complement plus MAb S5, or human PMNs plus MAb S5 was about 50%. The reduction of bacterial viability might result from cell aggregation of *A. actinomycetemcomitans* induced with MAb S5. In fact, all MAbs against Y4 SPA, including MAb S5, induced the aggregation of the organism, whereas MAb L2 and MAb P2 did not (data not shown). These results indicate that the opsonic activity of MAb against Y4 SPA depends upon the coexistence of complement. Moreover, the susceptibility of SPA-defective mutants (strains ST1, ST2, ST3, and ST5) of *A. actinomycetemcomitans* Y4 to human PMNs in the presence of complement was determined. Percent survival values of the mutants were less than 10% after 2 h of incubation at 37°C with human PMNs in the presence of complement (data not shown). However, there was a 60 to 80% decrease in bacterial viable numbers of the mutants after 2 h of incubation at 37°C in $GGVB^{++}$ only. All of the mutants, but not strain Y4, were observed under a microscope to clump in $GGVB^{++}$, suggesting that the assay used in this study is unsuitable for determining phagocytic killing of SPA-defective mutants.

## DISCUSSION

Several investigators have shown that patients with localized and generalized juvenile periodontitis have a greater frequency of occurrence and a higher level of antibodies to the SPA of *A. actinomycetemcomitans* (10, 15, 33, 40). Wilson and Schifferle (40) and Page et al. (29) suggested that the serotype b-specific immunodominant antigen resides in the polysaccharide side chain of LPS. On the other hand, we have shown that the serotype b-specific antigen is a capsular polysaccharide-like antigen consisting of a disaccharide repeating unit, →3)-α-D-fucopyranosyl-(1→2)-β-L-rhamnopyranosyl-(1→, but not the polysaccharide moiety of LPS (3, 32). It has not yet been concluded whether the serotype b-specific antigen is derived from LPS or from capsule. In addition, the function of antibodies to the polysaccharide antigen developed in juvenile periodontitis patients is poorly understood.

To clarify the functional role of antibodies to the serotype b-specific antigen in periodontitis, we determined whether MAbs against SPA of *A. actinomycetemcomitans* Y4 enhanced CL responses of human PMNs to the organism and killing of the organism by human PMNs. IgG1, IgG2a, and IgG3 MAbs against SPA significantly enhanced the CL responses of human PMNs to *A. actinomycetemcomitans* Y4 in the presence of complement, but IgM MAb against SPA did not. Phagocytes are known to have Fc receptors for IgG but not IgM (8). The opsonic activity of IgG2a MAb to SPA was lower than that of IgG1 and IgG3 MAbs to the antigen. In this regard, Amir et al. (4) indicated that human IgG1 anti-*Haemophilus influenzae* type b polysaccharide is functionally more effective than human IgG2 antibody. This difference of opsonic activity among IgG subclasses is considered to result from the difference in the binding efficiency of antibodies to the Fcγ receptors on PMNs and the ability to fix complement (23). Although it is necessary to take into account the difference between human IgG subclasses and murine IgG subclasses, it is possible that the difference of opsonic activity among murine IgG subclasses may be attributable to the difference in the binding efficiency of MAbs on PMNs and/or their ability to fix complement.

Ling et al. (23) reported that IgG2 antibodies predominate in the IgG responses of juvenile periodontitis patients to cell-surface antigens of *A. actinomycetemcomitans*. Several investigators showed that IgG antibodies purified from the sera of juvenile periodontitis patients but not from the sera of periodontally healthy subjects promote phagocytosis and killing of *A. actinomycetemcomitans* (6, 36, 38). IgG antibodies to SPA that are developed in juvenile periodontitis patients may play an important role in promoting PMN-mediated host defense.

IgG2b and IgG3 MAbs against LPS weakly enhanced the CL responses of human PMNs to *A. actinomycetemcomitans* Y4 (Table 1). Moreover, the addition of IgG2b MAb against LPS reduced the bacterial viability by about 30% in the killing assay using human PMNs, complement, and *A. actinomycetemcomitans* Y4 (Fig. 4). These results indicate that antibodies against LPS as well as antibodies against SPA enhance host defenses against the organism by PMNs. However, the present study demonstrated that IgG MAbs against LPS enhanced to a lesser degree the CL responses of human PMNs induced by phagocytosis with *A. actinomycetemcomitans* than IgG MAbs against SPA did (Table 1). The SPA of *A. actinomycetemcomitans* is considered to be a capsule that covers the surface of the organism (3). On the other hand, LPS is a component of the outer membrane. Western blotting analysis suggested that

TABLE 2. Role of complement in opsonophagocytic killing of *A. actinomycetemcomitans* Y4 by human PMNs

| Group with: | | | | % Survival[a] |
|---|---|---|---|---|
| Bacteria ($2 \times 10^6$) | Complement (5%) | MAb S5 (100 μg/ml) | PMNs ($2 \times 10^6$) | |
| + | − | − | − | 89.0 ± 1.1 |
| + | + | − | − | 97.4 ± 1.6 |
| + | − | + | − | 47.3 ± 4.9 |
| + | + | + | − | 38.8 ± 1.3 |
| + | − | − | + | 78.1 ± 5.8 |
| + | + | − | + | 89.8 ± 4.3 |
| + | − | + | + | 49.5 ± 3.3 |
| + | + | + | + | 7.7 ± 2.3[b] |

[a] Percent survival was calculated as [(CFU per milliliter after 2 h)/(CFU per milliliter at zero time)] × 100. Data are the mean percent survival ± standard error for three different experiments performed in triplicate.

[b] $P < 0.001$ versus the group with bacteria only and the group with bacteria plus MAb S5 plus PMNs.

MAbs against LPS used in this study are directed to the core region of LPS, which may not be surface accessible in an intact organism. MAbs against LPS might be less effectively bound to the surface of the organism as compared with MAbs against SPA. It is possible that the difference in binding efficiency to the cell surface between MAbs against LPS and MAbs against SPA creates a difference between the opsonic activities of both types of MAbs in the CL assay.

It is known that certain gram-negative bacteria have the ability to activate complement in the absence of antibody and to induce the complement-mediated cytolytic destruction of themselves (13). In the present study, the viability of *A. actinomycetemcomitans* Y4 was not affected by incubation with complement only. When the cells of strain Y4 were incubated with MAb S5 alone or with MAb S5 and complement, about a 50% decrease in CFU occurred after 2 h. In our microscopic examination, all of the MAbs specific for SPA, including MAb S5, induced the cell aggregation of strain Y4, suggesting that the decrease of CFU in the presence of MAb S5 might result from this aggregation. The finding that *A. actinomycetemcomitans* is resistant to complement-mediated serum bactericidal mechanisms was previously reported by Miyasaki et al. (26) and Wilson and Genco (39).

Sato et al. (32) inserted the transposon Tn*916* into *A. actinomycetemcomitans* Y4 to construct mutants defective in SPA consisting of D-fucose and L-rhamnose. The hydrolysates of autoclaved extracts from strains ST1 and ST2 contain a trace amount of rhamnose but not fucose. Those of autoclaved extracts from strains ST3 and ST5 contain a trace amount of fucose but not rhamnose (32). Whole cells of strains ST1 and ST2 lack reactivity with MAb against Y4 SPA (MAb S5) in an enzyme-linked immunosorbent assay, but those of strains ST3 and ST5 react weakly with MAb S5 (32). In this study, four SPA-defective mutants (strains ST1, ST2, ST3, and ST5), as compared with the parent strain Y4, were more sensitive to CL responses by human PMNs, suggesting that the resistance of *A. actinomycetemcomitans* to the complement-mediated killing by human PMNs might be attributed to the presence of SPA. However, strains ST3 and ST5 that react weakly with MAb S5 induced slightly stronger CL responses of PMNs, when compared with strains ST1 and ST2 that lack the reactivity with MAb S5. The CL emission does not assess phagocytosis and killing per se but does measure membrane perturbation and the ensuing respiratory burst (14, 19). It has been known that bacterial hydrophobicity plays an important role in the interaction between bacteria and phagocytic cells (1, 16). In fact, the cell hydrophobicity of SPA-defective mutants is significantly higher than that of strain Y4, and the hydrophobicity of strains ST1 and ST2 is slightly lower than that of strains ST3 and ST5 (32).

In conclusion, the present study suggests that SPA of *A. actinomycetemcomitans* plays an important role in the resistance to phagocytosis and killing by human PMNs. IgG MAb against SPA effectively opsonized *A. actinomycetemcomitans* in both in vitro CL and killing assays. We have recently found that intranasal or subcutaneous immunization with SPA coupled with bovine serum albumin induced serum IgG responses to the polysaccharide antigen (35). This conjugate vaccine designed to induce an anti-SPA immune response may protect humans against *A. actinomycetemcomitans* infections.

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