

Hydrophobicity of *Streptococcus pyogenes* is Responsible for the Phagocytic Reaction of Human Phagocytes

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ABSTRACT. Stimulation of the phagocytic response of human phagocytes depends on the surface properties of bacteria. We studied what properties of *Streptococcus pyogenes* stimulate a phagocytic reaction, focusing on hydrophobicity and the Fc receptor. From Fc receptor-positive hydrophobic parent streptococcus, hydrophilic mutant and Fc receptor-deficient mutants were isolated. The mutant and parent strains were tested for stimulating activity for phagocytosis by measuring the chemiluminescence (CL) response of human phagocytes. The phagocytic response caused by the hydrophilic strain was significantly lower than that of the hydrophobic parent; while no difference in the stimulation of the phagocytic response was observed between Fc receptor-positive and -negative strains. These results showed that the hydrophobicity of the cell surface is responsible for the stimulation of the phagocytic response.

Key words : chemiluminescence — hydrophobicity — Fc receptor — phagocytes — *Streptococcus pyogenes*

It is known that the cell surface properties of bacteria are critical for the phagocytic reaction of host phagocytes.¹⁻³⁾ We previously found the hydrophobicity of a staphylococcal surface is important for the phagocytic response by human phagocytes.⁴⁾ Jonsson and Wadström reported that the hydrophobicity of *Staphylococcus aureus* (*S. aureus*) was correlated to the presence of protein A⁵⁾ an Fc receptor of *S. aureus* that binds to the Fc region of immunoglobulin G (IgG).⁶⁻¹²⁾ Some strains of *Streptococcus pyogenes* (*S. pyogenes*) possess an Fc receptor on their cell surface.¹³⁻²⁰⁾ However, the biological significance of the streptococcal Fc receptor and its relationship to hydrophobicity are not clear. It is also unknown whether the hydrophobicity of streptococci is responsible for stimulation of human phagocytes. *S. pyogenes* causes a variety of suppurative diseases, such as pharyngitis, scarlet fever and pyoderma.²¹⁾ The susceptibility of *S. pyogenes* to phagocytosis should affect virulence. In this study, we examined the relationships of hydrophobicity, Fc receptor and the phagocytic reaction by human phagocytes. From a hydrophobic and Fc receptor-positive parent *S. pyogenes* strain, we isolated hydrophilic and Fc receptor-deficient mutants. With these bacteria, we assayed the stimulating activity for a phagocytic reaction of human phagocytes by measuring the chemiluminescence (CL)

response, which is known to closely correlate to the phagocytic reaction.²²⁻²⁴⁾ We found that the hydrophobicity of streptococci was responsible for the stimulation of the phagocytic reaction, and that the presence of an Fc receptor had no effect on it.

MATERIALS AND METHODS

Bacteria and culture conditions

S. pyogenes strain IP-28 was used as the parent Fc receptor-positive hydrophobic strain. The IP-28 strain was previously cloned in our laboratory from a clinical strain,²⁵⁾ which had been isolated in the Clinical Laboratory of Kawasaki Medical School Hospital. The bacteria were cultured for 17 hr at 37°C by stationary cultivation in Todd-Hewitt broth (THB) (Difco Laboratories, Detroit, Mich., USA).

Isolation of hydrophilic strain

A hydrophilic IPP-2 strain was isolated from the parent hydrophobic IP-28 strain as previously reported.⁴⁾ Briefly, the bacteria and *n*-octane were vigorously vortexed for 3 min and then settled for 20 min. The aqueous phase was collected and cultivated in brain heart infusion broth (BHIB: Nissui Pharmaceutical Co., Tokyo). After overnight cultivation, the bacteria were again treated with *n*-octane. This cycle was repeated five times and the final aqueous phase plated on brain heart infusion agar (BHIA). Several colonies were selected and the hydrophobicity of the bacteria was assayed as described below.

Isolation of Fc receptor-deficient strain

Fc receptor-deficient strain IPF-22 was isolated from strain IP-28 by a previously reported method.^{12,26)} Briefly, the bacterial cells were mixed with heavily sensitized sheep red blood cells (SRBC), which were densely coated with IgG, incubated at room temperature for 10 min and then the mixture was spun down at $2,300 \times g$ for 1 min to remove the aggregates of Fc receptor-positive bacterial cells and heavily-SRBC. The bacterial cells in the supernatant were cultivated in BHIB at 37°C overnight. This procedure was repeated five times and the final supernatant was appropriately diluted and placed onto a BHIA plate. Some colonies were then collected after overnight incubation. Negative hemagglutination indicated that the bacterial cells were Fc receptor-free coccus. The Fc receptor-deficiency of the IPF-22 strain had been stably maintained.

Assay of hydrophobicity

The hydrophobicity of the bacteria was assayed by the method of Rosenberg *et al*²⁷⁾ with slight modification.⁴⁾ The relative hydrophobicity was scored by subtracting the optical density (O.D.) at 400 nm of the organic phase after mixing with *n*-octane from the initial bacterial suspension, which had been adjusted to O.D. 1.0.

Assay of Fc receptor activity

Streptococcal Fc receptor activity was assayed by quantifying the

agglutination with IgG-sensitized SRBC as previously reported.^{4,12,28,29} The bacterial cells were counted in a Coulter Counter (ZM, Coulter Electronics, Inc., Hialeah, FL, USA) and adjusted to 5×10^9 cells/ml with phosphate-buffered saline. To inactivate hemolytic activity, the bacterial suspension was incubated at 56°C for 30 min, and then applied to a hemagglutination (HA) test with sensitized SRBC. The HA titer was defined as the reciprocal of the highest dilution of the bacterial suspension showing visible HA.

CL assay

It is known that CL of luminol added to the mixture of bacteria and phagocytes reflects the phagocytic reaction.²²⁻²⁴ Luminol-dependent CL was measured in a light-detecting instrument, a lumiphotometer TD-4000 (Labo Science, Tokyo) as previously described.^{4,30} Briefly, human phagocytes (1×10^5 cells of polymorphonuclear leukocytes) in 0.2 mM luminol solution was added with the bacterial suspension (1×10^8 cells). After mixing, the mixture was immediately transferred to the lumiphotometer chamber, and CL was then measured every 1 min for 20 min. The magnitude of the CL response was scored by the peak height and the total integral as the relative light units (R.L.U.).

RESULTS

Isolation of hydrophilic mutant

A hydrophilic mutant, IPP-2, was isolated from the IP-28 parent strain as described in above. The hydrophobicity of the mutant and the parent strain is shown in Fig 1. It is evident that the mutant IPP-2 is highly hydrophobic. The HA activity of the IPP-2 mutant to sensitized SRBC remained unchanged (Table 1).

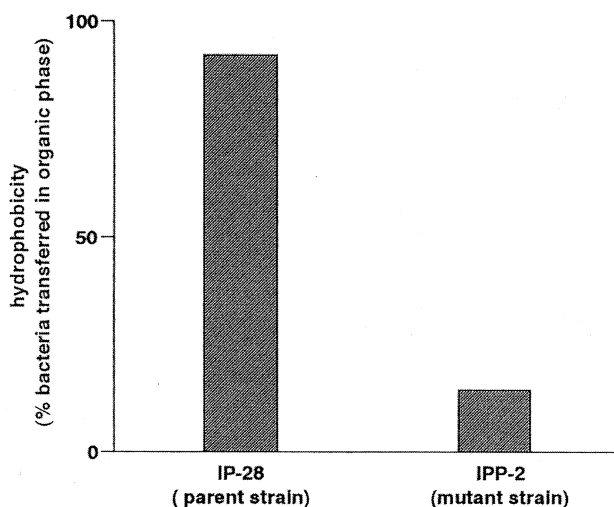


Fig 1. Hydrophobicity of parent and mutant strains.

Hydrophobicity (■) is indicated by the percentage of bacteria transferred to an organic phase after mixing with *n*-octane.

TABLE 1. Evaluation of Fc receptor on *S. pyogenes* with HA titer

strain	HA titer ^{a)} (log ₂)
IP-28 (hydrophobic parent strain)	3.8
IPP-2 (hydrophilic mutant strain)	4.3

^{a)}HA titer was defined as the reciprocal of the highest dilution of the bacterial suspension showing visible HA with sensitized SRBC.

Isolation of Fc receptor-deficient mutant

To examine whether the Fc receptor of streptococci has any effect on stimulation of the phagocytic response, we isolated an Fc receptor-deficient mutant from the parent IP-28 strain, as described above.

As shown in Table 2, we obtained an Fc receptor-deficient mutant, the IPF-22 strain. The hydrophobicity of the mutant was then compared to that of the parent. No significant difference in hydrophobicity was observed between the Fc receptor-deficient mutant and the Fc receptor-positive parent strain (Fig 2). Thus no direct correlation was observed between the presence of Fc receptor and the hydrophobicity of streptococci. This is consistent with our previous result with staphylococcal protein A.⁴⁾

TABLE 2. Evaluation of Fc receptor on *S. pyogenes* with HA titer

strain	HA titer ^{a)} (log ₂)
IP-28 (parent strain)	3.6
IPF-22 (mutant strain)	<1

^{a)}HA titer was defined as the reciprocal of the highest dilution of the bacterial suspension showing visible HA with sensitized SRBC.

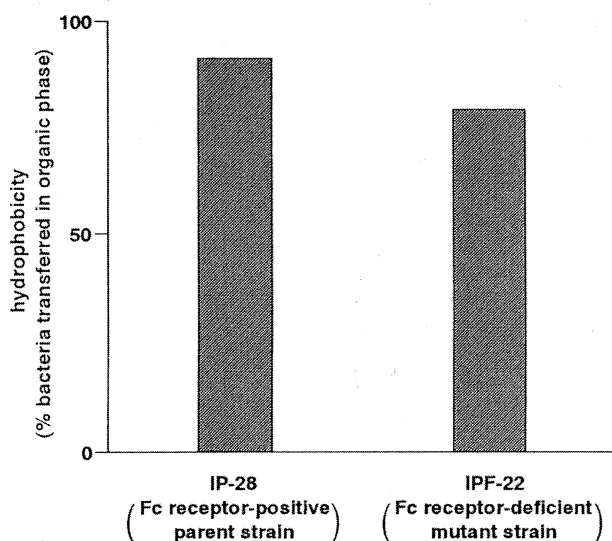


Fig 2. Loss of hydrophobicity in the mutant isolated from the streptococcal hydrophobic parent strain.

Hydrophobicity (■) is indicated by the percentage of bacteria transferred to an organic phase after mixing with *n*-octane.

Streptococcal factor(s) stimulating phagocytic response of phagocytes

Finally we examined streptococcal factor(s) stimulating the phagocytic response of human phagocytes. As a marker of phagocytosis, the CL response was measured. CL response with the hydrophilic IPP-2 strain was significantly lower than that with the parent hydrophobic IP-28 strain in either peak height or in the total integral of the CL response of phagocytes (Table 3). On the other hand, the parent and the Fc receptor-deficient strain IPF-22 did not differ in their CL responses. There was thus no clear relationship between Fc binding activity and the CL response.

A similar result as above was obtained with another hydrophilic mutant ARP-1 strain (data not shown). These results indicated that the hydrophobicity of streptococci was directly responsible for the phagocytic reaction of human phagocytes.

TABLE 3. Phagocytic CL response (by peak height and total integral) caused by *S. pyogenes* hydrophilic strain and by *S. pyogenes* Fc receptor-deficient strain

Fc receptor-positive, hydrophobic parent strain	strain		CL response	
	hydrophilic mutant strain	Fc receptor-deficient mutant strain	peak height ^{a)}	total integral ^{b)}
IP-28			18.12±1.27	7.14±1.21
	IPP-2		3.42±1.67***	1.88±0.39***
		IPF-22	14.97±2.57	4.02±2.18

a) R.L.U. (mean±1SD). b) $\times 10^3$ R.L.U. (mean±1SD).
*** $P < 0.01$ (paired t-test)

DISCUSSION

The present study has indicated that hydrophobicity of streptococci is a determinant for the stimulation of phagocytes, and therefore is very important for clearance of bacteria from an infected region. Thus, this property may be closely correlated to the virulence of streptococci. This fact is consistent with our previous finding that the phagocytic response of human phagocytes to staphylococci depends on the hydrophobicity of cell surface.⁴⁾ Van Oss and Gillman³¹⁾ and Van Oss³⁾ proposed that the more hydrophobic bacterial species are, the more easily they should be ingested by phagocytes. Ofek and Sharon,³²⁾ and Absolom³³⁾ reported that the efficiency of bacterial binding to phagocytes might depend on the hydrophobicity of the bacteria. The present study has shown that this concept extends to streptococci. The CL response observed in the present study was caused by superoxide, hydrogen peroxide and hydroxyl radicals, which are produced during phagocytosis.²²⁻²⁴⁾ Therefore, the CL assay was more specific and sensitive to detect any phagocytic reaction than an assay with ingestion.

The present study showed that an Fc receptor on streptococcal cell surface does not directly correlate with phagocytosis. This fact is consistent with staphylococcal protein A.⁴⁾ As protein A is common in *S. aureus* strains,⁶⁻¹²⁾ it was expected that it might interfere with the immunological binding of IgG and thereby interfere with the opsonic activity of IgG,

against *S. aureus*. However, we previously found that its presence on a staphylococcal surface had no significant interference on the opsonic activity of IgG.³⁰⁾ Therefore, the biological significance of the Fc receptor on *S. pyogenes* remains unknown. In a future study, it would be important to clarify whether the Fc receptor interferes with the opsonic activity of IgG against streptococci. Membrane protein, lipoprotein, lipoteichoic acid, M protein and hyaluronic acid capsule are known as substances responsible for the hydrophobicity of *S. pyogenes*.³⁴⁻³⁶⁾ It would also be important to clarify the main factor responsible for the streptococci and the CL response using our mutants.

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