Immunology 1984 **52** 427

Interaction between human polymorphonuclear leucocytes and *Staphylococcus aureus* in the presence and absence of opsonins

CHRISTINA M. J. E. VANDENBROUCKE-GRAULS, H. M. W. M. THIJSSEN & J. VERHOEF
Laboratory of Microbiology, State University of Utrecht, Utrecht, The Netherlands

Accepted for publication 23 January 1984

Summary. Phagocytosis of *Staphylococcus aureus* by human polymorphonuclear leucocytes (PMN) in the presence and absence of opsonins was studied with an assay which allows interaction between PMN and bacteria on a surface. The kinetics of uptake, the activity of the metabolic burst, and the degranulation during phagocytosis of opsonized and unopsonized bacteria were compared. Uptake of unopsonized *S. aureus* proceeded at a slower rate, but unopsonized staphylococci induced metabolic activity and degranulation in the PMN to the same extent as opsonized bacteria. Treatment of PMN with a metabolic inhibitor (2-deoxy-D-glucose) or with an inhibitor of microfilament function (cytochalasin B) totally inhibited the capacity of PMN to ingest unopsonized *S. aureus*, whereas uptake of opsonized bacteria was much less affected. Treatment of the PMN with pronase prevented uptake of unopsonized bacteria, but had no effect on the uptake of opsonized bacteria. Uptake was not inhibited by mannose. Recognition of *S. aureus* by the PMN was not dependent on the presence of the cell wall components protein A or teichoic acid. The presence of a capsule inhibited uptake.

Correspondence: C. M. J. E. Vandenbroucke-Grauls, Laboratory of Microbiology, Catharijnesingel 59, 3511 GG Utrecht, The Netherlands.

INTRODUCTION

Phagocytosis and killing of microorganisms by human polymorphonuclear leucocytes (PMN) is an important defence mechanism against infection. Classically, a number of steps is distinguished in the process of phagocytosis: opsonization of the bacteria and subsequent attachment to the PMN membrane, ingestion of the attached microorganisms, and finally intracellular killing of the ingested microorganisms. Intracellular killing involves two major mechanisms: degranulation of cytoplasmic granules (lysosomes) (Hirsch & Cohn, 1960) and generation of highly toxic oxygen products during the so-called metabolic burst (Babior, 1978).

The need for opsonization of the microorganisms for ingestion to occur has been extensively studied since Wright & Douglas (1903) first described the opsonic effect of serum (for review, see Horwitz, 1982). In contrast, in a classical paper from 1946, Wood, Smith & Watson reported that phagocytosis and killing can occur in the absence of opsonins when the bacteria are trapped by the PMN against a surface. This mechanism of phagocytosis was termed 'surface phagocytosis'. In more recent studies it was shown that the surface charge and hydrophobicity of the bacteria are important factors that determine whether microorganisms need opsonins or not for uptake by the PMN (Van Oss & Gillman, 1972a, b; Stendahl &

Edebo, 1972; Stendahl, Normann & Edebo, 1979). How unopsonized bacteria are ingested, and whether and how they can trigger degranulation and metabolic burst is still controversial and not well understood.

Therefore, we investigated the phagocytosis of *Staphylococcus aureus* in the presence and absence of opsonins and compared the kinetics of uptake, the activity of the metabolic burst and degranulation during phagocytosis of opsonized and unopsonized bacteria.

MATERIALS AND METHODS

Bacterial strains

Staphylococcus aureus Ev, a clinical isolate, was used throughout the study. In one series of experiments, seven additional staphylococcal strains were used: *S. aureus* Cowan I and its protein A deficient mutant Cowan EMS, *S. aureus* H and its teichoic acid deficient mutant 52A5, *S. aureus* M (encapsulated) and M variant (unencapsulated), and *S. aureus* Stephens, a clinical isolate. The bacteria were grown overnight in Müller-Hinton broth (Difco laboratories, Detroit, MI) at 37°. After washing three times with sterile isotonic phosphate-buffered saline (PBS, pH 7·4), bacterial concentrations were adjusted to a final concentration of 5×10^8 CFU/ml with a Klett-Summerson photoelectric colorimeter that was standardized by a pour-plate method (Verhoef, Peterson & Quie, 1977b). For phagocytosis experiments, bacteria were cultured and radiolabelled in Müller-Hinton broth, supplemented with 0·02 mCi [^{3}H]-thymidine (specific activity: 5 Ci/mmol; Radiochemical Centre, Amersham, U.K.) as previously described (Verhoef *et al.*, 1977b). Prior to use, bacteria were washed three times with PBS and resuspended in Hanks's balanced salt solution with 0·1% gelatin (Gel-HBSS) to a concentration of 5×10^8 CFU/ml. When needed, bacteria were opsonized by incubation on a rotator for 30 min at 37° in 5% human pooled serum in Gel-HBSS. The serum was removed by centrifugation (15 min at 1600 *g*), the opsonized bacteria were resuspended in Gel-HBSS, and kept at 4° until use.

Serum

Sera from 10 healthy donors, used for opsonization studies, were pooled and stored in 1 ml portions at −70°.

Isolation of human PMN

PMN were isolated by a method modified from Böyum (1968) as previously described (Verbrugh *et al.*, 1978). Venous blood samples from healthy adult donors were drawn into heparinized syringes and settled by gravity in 6% dextran (mol. wt. 70, 000; Pharmacia Fine Chemicals AB, Uppsala, Sweden) in normal saline (10 ml blood, 3 ml saline). The leucocyte-rich plasma was withdrawn and centrifuged at 160 *g* for 10 min. The pellet was resuspended in Eagle's minimal medium; 6 ml of the cell suspension was carefully layered onto 3 ml Ficoll-Paque (Pharmacia Fine Chemicals) and centrifuged at 160 *g* for 35 min. Mononuclear cells of the interface were removed. Residual erythrocytes in the pellet were lysed with ice-cold NH_4Cl (0·87% in sterile water). After centrifugation at 160 *g* for 5 min, PMN were washed twice with Gel-HBSS. Leukocytes were adjusted to a concentration of 10^7/ml Gel-HBSS.

Determination of surface phagocytosis

Phagocytosis assays were performed by a previously described method (Verhoef *et al.*, 1977b), modified to measure phagocytosis of bacteria on a surface.

Radiolabelled bacteria (0·1 ml) were centrifuged for 5 min at 1600 *g* in four flat-bottom glass cuvettes (8 mm × 47 mm, Payton, nr 0312, Scarborough, Canada). PMN (0·1 ml) were carefully added to the cuvettes to leave the bacterial layer intact. The cuvettes were incubated stationary at 37° and the PMN were allowed to settle on the sediment by gravity. After 5, 40 and 90 min, ice-cold PBS was added to the content of one cuvette to stop the interaction between PMN and bacteria. After careful resuspension, the mixture was transferred to a polypropylene biovial (10 mm × 55 mm; Biovials, Beckman, Chicago, IL), and the cuvette rinsed twice with additional ice-cold PBS. The two rinses were added to the biovial. In this biovial, the non-leucocyte-associated bacteria were removed by three cycles of differential centrifugation in PBS at 160 *g* for 5 min. To determine the total radioactivity (leucocyte-associated and non-leucocyte-associated radioactivity: a measure for the total number of bacteria present in each of the cuvettes) the content of one cuvette was transferred to a biovial and centrifuged at 1600 *g* for 15 min. Leucocyte pellets and 1600 *g* pellets were resuspended in 2·5 ml of scintillation liquid. Radioactivity was measured in a liquid scintillation counter, and phagocytosis was expressed as a percentage of the total amount of added radioactivity. In three experiments, lysostaphin (Schwarz-Mann, Orangeburg, NY, U.S.A.) was used to lyse extracellular staphylococci (Verhoef *et al.*, 1977b). To this

purpose, after the second cycle of differential centrifugation, the pellet of PMN and leucocyte-associated bacteria was resuspended in 2·5 ml of PBS with 1 μg/ml of lysostaphin, and incubated for 30 min at 37°. After two additional washes with PBS (160 *g*, 5 min), the pellets were resuspended in 2·5 ml of scintillation liquid.

Treatment of leucocytes with pronase, cytochalasin B, 2-deoxy-D-glucose and mannose

To determine the nature of the interaction between PMN and unopsonized bacteria, PMN were treated with pronase, mannose, cytochalasin B or 2-deoxy-D-glucose (2d-glu). For pronase treatment, PMN (5×10^6/ml) were incubated with 1 mg/ml pronase (Pronase E, 70.000 PVK, Merck, Darmstadt, West Germany) in Ca^{2+}- and Mg^{2+}-free Gel-HBSS for 30 min at 37°. For mannose treatment, PMN were preincubated with 10 mM mannose (Merck) in Gel-HBSS, for 20 min at 4°. After these incubations, PMN were washed in Gel-HBSS to remove pronase or unbound mannose and resuspended in Gel-HBSS (final concentration: 10^7 cells/ml). For cytochalasin B treatment, PMN (10^7/ml) were resuspended in Gel-HBSS containing 5 μg/ml cytochalasin B (Aldrich Chemical Company Inc., Milwaukee, WI, U.S.A.) and incubated for 5 min at 37°, prior to use in the phagocytosis assay. For 2d-glu treatment, PMN were preincubated with 50 mM 2d-glu (Serva, Heidelberg, West Germany) in Gel-HBSS, for 10 min at 37°. As a control, PMN were also preincubated with 50 mM glucose. During the phagocytosis assay, cytochalasin B, 2d-glu and glucose were not removed from the medium.

Measurement of lysozyme and lactate dehydrogenase

Lysozyme was measured as a marker for activity of cytoplasmic granules (lysosomes) and lactate dehydrogenase (LDH) as a marker for free cytoplasmic enzymes. After incubation of PMN with preopsonized or unopsonized *S. aureus* Ev as described above, the reaction was stopped after 60 min by placing the cuvettes on ice. Control reaction mixtures contained 0·1 ml of Gel-HBSS without bacteria and 0·1 ml of PMN. Reaction mixtures from four identical cuvettes were resuspended, collected, and transferred to Eppendorf vials (Eppendorf, Hamburg, West Germany), and centrifuged in the cold at 160 *g* for 10 min. Supernatants were collected. Cell pellets were resuspended in 500 μl of 0·1% Triton X-100 (BDH Chemicals Ltd, Poole, U.K.) and sonicated twice for 15 sec (Branson Sonifier B12, Branson Sonic Power Company, Danbury, CO, U.S.A.). The amount of lysozyme in the cell lysate was determined by the rate of lysis of *Micrococcus lysodeikticus* (dried cells; Sigma Chemical Company) measured by the decrease in absorption at 450 nm (Decker, 1977b). LDH was determined spectrophotometrically at 366 nm by measuring the reduction of NADH by pyruvate (Decker, 1977a). Simultaneously, total amounts of enzyme activities present in unstimulated PMN were determined in reaction mixtures to which no bacteria had been added, and which had been incubated for 0 min. The amount of enzyme release was calculated by substracting the amount of enzyme activity in the lysate of cells to which bacteria had been added (residual activity after phagocytosis) from the total amount of activity measured in the lysates of unstimulated PMN. Enzyme release was then expressed as a percentage of the total enzyme activity.

Superoxide anion generation

Generation of superoxide anion was assayed by measurement of the reduction of ferricytochrome *c* (Horse heart type VI; Sigma Chemical Company) using a modification of the method described by Johnston *et al.* (1975). Preopsonized *S. aureus* Ev (0·1 ml, 5×10^8 CFU/ml) or 0·1 ml of unopsonized *S. aureus* Ev (5×10^8 CFU/ml) were centrifuged for 5 min at 1600 *g* in glass cuvettes, as described above. To 1·6 ml of PMN (10^7/ml), 0·01 ml of ferricytochrome *c* (1·6 mM) were added. After mixing, 0·1 ml of the PMN-ferricytochrome *c* suspension was carefully added to the pelleted bacteria. The reaction mixtures were incubated stationary at 37°. Paired reactions with and without added superoxide dismutase (100 μg; E.C. 1.15.1.1.; bovine blood type I, Sigma Chemical Company) were employed. Control reaction mixtures contained 0·1 ml of Gel-HBSS without bacteria and 0·1 ml of the PMN-ferricytochrome *c* suspension. After incubation for 5, 40 and 90 min, the reaction mixtures were centrifuged in the cold at 160 *g* for 10 min, the supernatant fractions were centrifuged again at 4°, 1600 *g* for 15 min. The difference in absorption between the supernatants was determined at 550 nm in a double beam spectrophotometer (Perkin-Elmer model 124). Nanomoles of ferricytochrome *c* reduced were determined from the increase in absorption at 550 nm using the extinction coefficient $E_{550} = 2{\cdot}1 \times 10^4 M^{-1}cm^{-1}$. Superoxide generation was expressed as nanomoles superoxide per 10^7 PMN.

Statistical analysis

Results have been expressed as the mean of three or more independent observations ± standard deviation. For significance analysis Student's *t*-test was performed. *P* values exceeding 0·05 were considered not significant.

RESULTS

Phagocytosis of preopsonized and unopsonized *S. aureus* Ev

Normal PMN readily phagocytosed preopsonized and unopsonized *S. aureus* Ev, which were pelleted on a glass surface (Fig. 1). However, the rate of uptake of unopsonized bacteria was much slower than that of preopsonized *S. aureus* Ev. To determine whether the bacteria were really ingested by the PMN, and not merely attached to the leucocyte cell surface, the PMN were incubated with lysostaphin after phagocytosis, to lyse adherent, but extracellular, staphylococci. After treatment with lysostaphin, between 1 and 8% of leucocyte-associated radioactivity was lost (Table 1). This indicated that the great majority of the bacteria was ingested. When the experiments were carried out at 4°, less than 20% uptake was measured after 90 min, which provided evidence that the association of the bacteria with the PMN was a specific process.

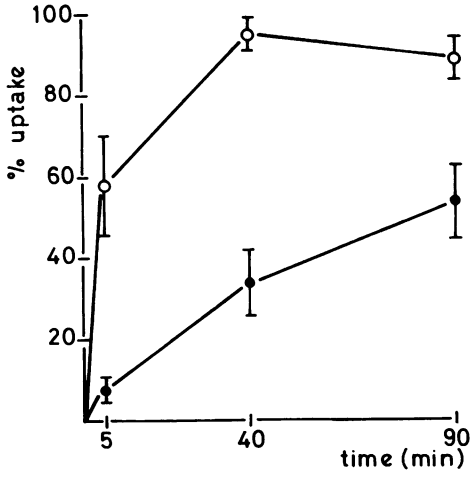

Figure 1. Phagocytosis of preopsonized (○) and unopsonized (●) *S. aureus* Ev by human PMN. 100% represents total radioactivity added. Results are means of eight independent observations ± standard deviation.

Table 1. Lysostaphin treatment of PMN after phagocytosis

	Minutes of phagocytosis	% Leucocyte-associated radioactivity* Untreated PMN	% Leucocyte-associated radioactivity* Lysostaphin-treated† PMN
Opsonized *S. aureus*	5	55 ± 15	51 ± 8
	40	96 ± 5	88 ± 6
	90	91 ± 6	85 ± 4
Unopsonized *S. aureus*	5	10 ± 3	9 ± 1
	40	33 ± 5	32 ± 2
	90	53 ± 2	50 ± 1

* Values are means ± standard deviation of three independent duplicate observations.

† Lysostaphin was added after phagocytosis (see 'Materials and Methods').

Enzyme release

During phagocytosis of opsonized bacteria, lysosomal enzymes are released in the extracellular medium. To determine if enzyme release also occurred when unopsonized bacteria were ingested, the release of the lysosomal enzyme lysozyme was measured during surface phagocytosis of both preopsonized and unopsonized *S. aureus* Ev. At 60 min, PMN phagocytosing preopsonized bacteria, released 32% (±2) of their total amount of lysozyme. PMN phagocytosing unopsonized *S. aureus* Ev released 24% (±1) of their lysozyme content. Less than 4% ($P < 0{\cdot}001$) enzyme release was noted when PMN were incubated without bacteria. The cytoplasmic enzyme LDH was not released during incubation with either preopsonized or unopsonized *S. aureus* Ev. This indicated that the release of lysozyme was due to true exocytosis, and not just to cell damage.

Superoxide anion generation

Ingestion of opsonized bacteria by PMN is accompanied by the generation of toxic oxygen products, through activation of the oxidative metabolism of the PMN (Babior, 1978). During phagocytosis of unopsonized bacteria, superoxide anion was generated at the same rate as during phagocytosis of opsonized bacteria (Table 2). Adherence of the PMN to a glass

Table 2. Superoxide anion generation by PMN during phagocytosis of opsonized and unopsonized *S. aureus* Ev

PMN phagocytosing	Nanomoles superoxide/10^7 PMN*		
	40 min	60 min	90 min
Preopsonized *S. aureus*	7·6±0·7	8·8±0·5	11·7±0·5
Unopsonized *S. aureus*	7·1±2·4	8·6±1·4	11·4±1·2
No bacteria	2·6±1·9	4·5±0·7	6·4±0·7

* Values are means of two independent duplicate observations. All values are significant ($P<0·005$) compared to unstimulated PMN (no bacteria added).

surface can activate their metabolism (Yanai & Quie, 1981). To exclude this possibility, PMN were incubated in the glass cuvettes without bacteria. Some superoxide anion production could be measured, but a significant smaller amount ($P<0·005$) than when bacteria were present.

Effect of treatment of the PMN with pronase, cytochalasin B, 2-deoxy-D-glucose and mannose

To study the nature of the interaction between PMN and unopsonized bacteria, PMN were treated with various inhibitors (Figs 2, 3). To examine whether the binding of unopsonized bacteria and the PMN was mediated via a pronase-sensitive receptor, PMN were treated with pronase. Pronase inactivates protein receptors, such as the complement receptor in the membrane of the PMN (Crofton, Diesselhof-den Dulk & van Furth, 1978). Pronase treatment of the PMN had no effect on their ability to ingest opsonized *S. aureus* Ev, whereas the phagocytosis of unopsonized bacteria was reduced by 57% (Fig. 2). Cytochalasin B, a metabolite from the mold *Helmintosporium dematioideum* interferes with the function of cytoplasmic microfilaments causing inhibition of normal cellular movements and phagocytosis (Allison, Davies & de Petris, 1971). Treatment of PMN with cytochalasin B reduced the uptake of opsonized bacteria by about 50% and almost completely inhibited the phagocytosis of unopsonized bacteria (Fig. 3). 2d-glu, an analogue of glucose, reduces adenosine triphosphate (ATP) levels in cells by inhibition of glycolysis and hexose monophosphate shunt. 2-d-glu only slightly inhibited the phagocytosis of opsonized bacteria, but markedly inhibited the uptake of unopsonized *S. aureus* Ev (Fig. 3). Preincubation of PMN with glucose had no effect.

Lectin-like receptors capable of binding various bacterial species, e.g. *S. epidermidis* and *Corynebacterium parvum* have been demonstrated on the membrane of macrophages, neutrophils and eosinophils (Glass, Stewart & Weir, 1981). These receptors recognize bacterial cell wall sugars. Binding through these receptors can be inhibited by incubating the phagocytes in various sugars. Incubation of PMN with

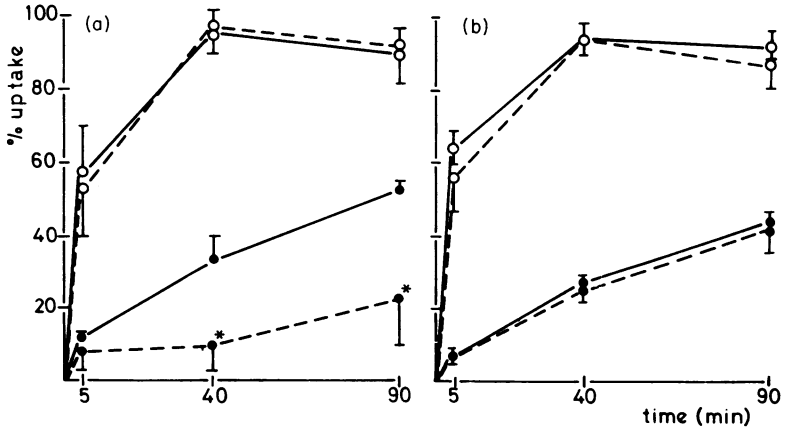

Figure 2. Phagocytosis of preopsonized (○) and unopsonized (●) *S. aureus* Ev by control PMN (——) and treated PMN (– – –). (a) Pronase treatment; (b) mannose treatment. Results are means of three independent observations ± standard deviation. *$P<0·05$.

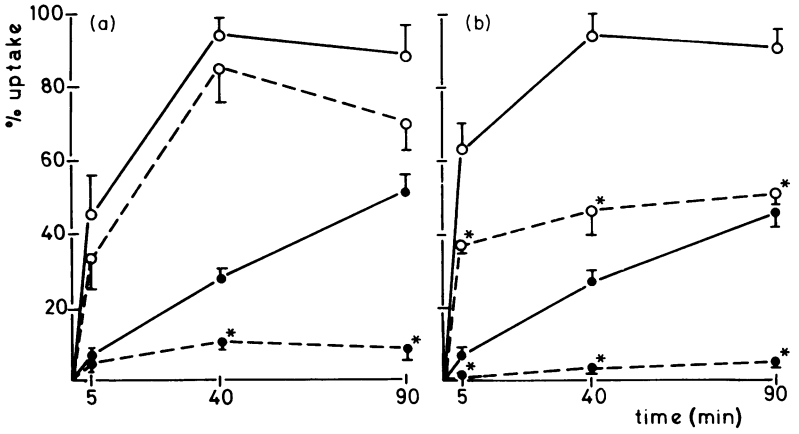

Figure 3. Phagocytosis of preopsonized (○) and unopsonized (●) *S. aureus* Ev by control PMN (——) and treated PMN (– – –). (a) 2d-glu treatment; (b) cytochalasin B treatment. Results are means of three independent observations ± standard deviation. *$P < 0.05$.

glucose or mannose had no effect on the binding of opsonized or unopsonized *S. aureus* Ev to the PMN (Fig. 2).

Role of protein A, teichoic acid and capsule in the phagocytosis of unopsonized *S. aureus*

To investigate the role of the cell wall composition of the bacteria on their uptake by the PMN in the absence of opsonins, bacteria with different cell wall composition were used. *S. aureus* Cowan I and its mutant Cowan EMS differed in their protein A content: the parent strain contained 5 ng protein A/10^6 bacteria, the mutant Cowan EMS less than 0·04 ng/10^6 bacteria (Spika, Verbrugh & Verhoef, 1981). A teichoic acid-deficient mutant (*S. aureus* 52A5) and its parent strain (*S. aureus* H) were used to evaluate the role of teichoic acid. *S. aureus* M and the unencapsulated mutant M variant were used to determine the influence of a capsule.

Phagocytosis of unopsonized staphylococci was independent of the amount of protein A in the cell wall (Fig. 4): the protein A-deficient mutant Cowan EMS was phagocytosed as well as the parent strain, Cowan I. Teichoic acid was not required for phagocytosis of unopsonized staphylococci, as *S. aureus* 52A5 was equally well phagocytosed as the parent strain H. However, the presence of a capsule interfered with the phagocytosis of unopsonized staphylococci. *S. aureus* M was not taken up by the PMN (< 5%) in contrast to the unencapsulated mutant M variant (30%).

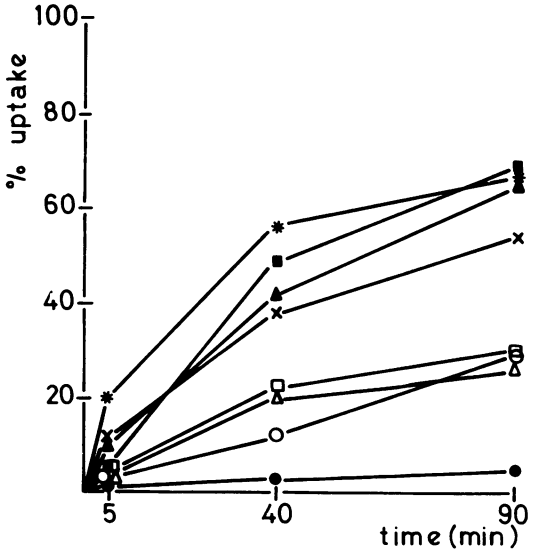

Figure 4. Phagocytosis of various *S. aureus* strains by human PMN in the absence of opsonins. (×) *S. aureus* Ev; (*) *S. aureus* Cowan I; (■) *S. aureus* Cowan EMS; (▲) *S. aureus* Stephens; (△) *S. aureus* H; (□) *S. aureus* 52A5; (●) *S. aureus* M; and (○) *S. aureus* M variant.

DISCUSSION

The question whether bacteria need opsonins for uptake by PMN and for stimulation of microbicidal mechanisms in the PMN has always been controversial. Since the description of the opsonic effect of

serum (Wright & Douglas, 1903), subsequent studies showed that specific immunoglobulins and the activated third complement component act as ligands between the bacteria and the PMN (Griffin *et al.*, 1975). Uptake of opsonized bacteria and triggering of the PMN activation proceeds via a receptor on the PMN membrane for the Fc fragment of the antibody molecule and via a receptor for the C3b molecule. In most studies on phagocytosis of staphylococci, PMN are incubated with bacteria under constant shaking. Under these conditions, virtually no uptake is measured when opsonins are absent (Verhoef *et al.*, 1977a; Verbrugh *et al.*, 1979). However, already in 1946, Wood *et al.* showed that pneumococci can be phagocytosed by the PMN in the absence of opsonins, when the bacteria are trapped against a surface. More recently, the phenomenon of phagocytosis in the absence of opsonins was extensively studied by Van Oss & Gillman (1972a, b). These authors showed that phagocytosis of unopsonized bacteria depends on the hydrophobicity of the microorganisms. Bacteria which are more hydrophilic than the PMN are only taken up when opsonized. Van Oss & Gillman measured phagocytosis microscopically by counting the number of bacteria phagocytized by the PMN, after Gram-staining of the bacteria. No distinction could be made between ingested and adherent bacteria. Wood *et al.* observed phagocytosis while it was actually occurring and saw the PMN engulf the microorganisms.

We reinvestigated the process of phagocytosis of unopsonized bacteria with an assay that uses radiolabelled bacteria to measure the association of bacteria with the PMN. Instead of using a shaking system, as described previously (Verhoef *et al.*, 1977b), the conditions of the assay were modified to allow phagocytosis to occur on a surface. Bacteria were pelleted in a glass cuvette and PMN were allowed to settle on this bacterial sediment by gravity. Opsonized and unopsonized bacteria were used. In this assay, uptake of both opsonized and unopsonized bacteria by PMN was observed. With the use of lysostaphin, which lyses the extracellular, adherent, bacterial population, we demonstrated that most of the opsonized and unopsonized staphylococci were not just attached to the cell membrane but internalized by the PMN.

The interaction between opsonins and receptors on the PMN membrane not only leads to ingestion of the microorganisms, but also induces a metabolic burst in the PMN, and causes degranulation of lysosomes into the phagocytic vacuole (Babior, 1978; Hirsch & Cohn, 1960). Activation of the PMN metabolism does not necessarily follow ingestion of particles. Fibronectin-coated particles, such as zymosan, although phagocytosed, fail to stimulate PMN metabolism (Gudewicz *et al.*, 1982). The ingestion of particles, mediated via the C3b receptor, is not efficient in inducing a metabolic burst in PMN (Hed & Stendahl, 1982). Ingestion of particles *per se* therefore appears to be insufficient for the activation of microbicidal mechanisms in PMN. Activation may require interaction with specific receptors on the PMN membrane. We therefore measured the generation of superoxide anion and the release of lysozyme by the PMN upon stimulation with opsonized and unopsonized *S. aureus*, as a measure of metabolic burst activity and degranulation. It could be shown that unopsonized bacteria were also able to stimulate degranulation of lysosomes and the metabolic burst of the PMN. Although it has been shown that contact between PMN and glass can already activate the metabolic burst of the PMN (Yanai & Quie, 1981) we showed that the superoxide production and the release of lysozyme by the PMN was significantly higher in the presence of bacteria than on bare glass. This indicated that under the conditions tested, the unopsonized bacteria were able to activate the PMN. Moreover, the stimulation of PMN by unopsonized staphylococci occurred to the same extent: no difference was found between the amount of superoxide produced upon stimulation by opsonized or unopsonized bacteria. The rate of superoxide generation was low, as compared to the superoxide generation which is measured with cell suspension systems (Henricks, van Erne-van der Tol & Verhoef, 1982). This may be explained by a less efficient recovery of superoxide anion in our assay system, where diffusion of superoxide through a layer of cells is needed, before reduction of cytochrome *c* can occur. Stimulation of oxidative metabolism and degranulation of PMN during contact with microorganisms in the absence of serum has been shown to occur with type I fimbriae-bearing strains of *E. coli* (Öhman, Hed & Stendahl, 1982). Also, pseudohyphal forms of *Candida albicans* can be damaged by neutrophils in the absence of serum, through oxidative microbicidal mechanisms and release of lysosomal enzymes (Diamond, Krzesicki & Jao, 1978; Diamond & Krzesicki, 1978). Lee *et al.* (1983) found that unopsonized bacteria elicit a chemiluminescent response in PMN.

In opsonin-mediated phagocytosis, ingestion is dependent on metabolic energy and functional microfilaments (for review, see Klebanoff & Clark, 1978). To

determine whether these same requirements apply to opsonin-independent phagocytosis, the PMN were treated with 2d-glu and cytochalasin B. 2d-glu inhibits glycolysis and hexose monophosphate shunt and therefore reduces ATP levels in the cells (Klebanoff & Clark, 1978). Treatment of PMN with 2d-glu slightly inhibited phagocytosis of opsonized *S. aureus* Ev, whereas an almost complete inhibition was seen of the uptake of unopsonized bacteria. The effect of 2d-glu on opsonin-independent phagocytosis was not due to competition with glucose residues for lectin-like receptors (Glass *et al.*, 1981), as treatment of the PMN with 50 mM glucose had no effect. The effect of 2d-glu was comparable to the effect of cytochalasin B, an agent that interferes with the function of cytoplasmic microfilaments (Allison *et al.*, 1971). It is possible that 2d-glu acts on opsonin-independent phagocytosis by the same mechanism as cytochalasin B, namely by inhibition of normal cellular movement. It has been shown that reduction of ATP levels in PMN also inhibits cell motility (Klebanoff & Clark, 1978). An intact locomotion apparatus may be an important feature for surface phagocytosis of unopsonized bacteria. Attachment and ingestion of *S. aureus* by PMN, followed by triggering of oxidative metabolism and degranulation, suggested recognition of the staphylococci by receptors on the cell surface of the PMN. To examine the possibility that the receptor involved is of protein-nature, PMN were treated with pronase. Pronase treatment of PMN resulted in a large inhibition of phagocytosis of unopsonized *S. aureus* Ev, whereas uptake of opsonized bacteria was not affected. This indicated that a protein-containing structure on the surface of the PMN was needed for interaction with unopsonized *S. aureus*. Our data also suggest that in the assay employed most of the opsonized bacteria are phagocytized via the Fc receptor, as pronase is known to affect the C3b receptor on the membrane of the PMN (Crofton *et al.*, 1978). This is in contrast with the finding using cell suspension systems, where inactivation of the C3b receptor leads to a marked reduction of the phagocytosis of staphylococci, opsonized in normal human pooled serum (Verhoef *et al.*, 1977a).

Lectin-like receptors which recognize mannose have been shown on several phagocytic cells, such as PMN, macrophages and eosinophils (Glass *et al.*, 1981). These receptors promote attachment of *S. epidermidis*, *E. coli* (Öhman *et al.*, 1982) and *Corynebacterium parvum* (Ögmundsdóttir & Weir, 1976). In our system, however, the addition of mannose did not inhibit phagocytosis of *S. aureus* Ev. Therefore, a mannose-sensitive receptor did not seem to be involved. The studies by Diamond & Krzesicki (1978) with *Candida* pseudohyphae showed that the interaction between *Candida* and PMN also is not sensitive to mannose.

To further investigate the nature of the interaction between PMN and unopsonized microorganisms, bacteria with various cell wall composition were used. These experiments showed that not all unopsonized *S. aureus* strains were phagocytosed. No relation could be found between protein A content of the staphylococcal cell wall and degree of uptake of staphylococci. The mechanism of surface phagocytosis, therefore, appears different from the mechanism of phagocytosis of unopsonized staphylococci by alveolar macrophages. This was shown to be mediated by protein A (Verbrugh *et al.*, 1982). The result with *S. aureus* H and its teichoic acid deficient mutant 52A5 indicate that the interaction between PMN and staphylococci did not require teichoic acid. The presence of a capsule totally inhibited phagocytosis. This was also demonstrated by Van Oss & Gillman (1972a), under different conditions. Our data confirm that a hydrophilic capsule influences phagocytosis of unopsonized staphylococci. It is possible, however, that a capsule also masks the surface structures on the bacteria, which are responsible for recognition of the staphylococci by the PMN and subsequent stimulation of ingestion, metabolic activity and degranulation.

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