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IgA- and secretory IgA-opsonized *S. aureus* induce a respiratory burst and phagocytosis by polymorphonuclear leucocytes

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SUMMARY

The aim of the present study was to investigate whether corpuscular immune complexes containing human IgA were able to interact with human polymorphonuclear leucocytes (PMN). As a model for corpuscular IgA immune complexes (IgA IC), heat-killed *Staphylococcus aureus* (*S. aureus*) opsonized with either purified human serum IgA or purified secretory IgA (sIgA) isolated from human colostrum was used. In order to determine the capacity of IgA and sIgA to opsonize *S. aureus* the phagocytosis of these IgA IC by PMN was measured. *S. aureus* opsonized with IgA, sIgA, IgG, heat-inactivated serum or fresh serum was ingested by $23 \pm 8\%$; $28 \pm 9\%$; $39 \pm 7\%$; $31 \pm 10\%$ and $78 \pm 10\%$ of the PMN (*S. aureus*:PMN = 10:1, $n = 4$), respectively. These results were significantly different ($P < 0{\cdot}05$) from the percentage obtained with unopsonized *S. aureus* ($9 \pm 3\%$), indicating that IgA and sIgA induce ingestion of *S. aureus*. The phagocytic index for PMN incubated with *S. aureus* opsonized with sIgA (231) was higher than for *S. aureus* opsonized with IgA (119), indicating a better uptake of *S. aureus* opsonized with sIgA in our system. Bacteria opsonized with either IgA or sIgA were also capable of triggering H_2O_2 release of PMN in a dose-dependent manner. The H_2O_2 release by PMN triggered with *S. aureus* opsonized with IgA could not be inhibited with a $F(ab')_2$ anti-Fcγ receptor monoclonal antibody, whereas the H_2O_2release triggered with *S. aureus* opsonized with IgG was fully inhibited. Soluble heat-aggregated IgA (AIgA) also induced H_2O_2 release of PMN, suggesting that the IgA itself is essential for the induction of a respiratory burst.

INTRODUCTION

IgA-containing immune complexes (IC) have been shown to occur in the circulation of patients with primary IgA nephropathy (Doi *et al.*, 1982; Lesavre, Digeon & Bach, 1982) and of patients with Henoch Schönlein purpura (Kauffmann *et al.*, 1980; Coppo *et al.*, 1984). It is not clear whether the presence of IgA IC is due to an overproduction of IgA, to a decreased clearance of IgA IC, or to a combination of both. Clearance studies in humans using radiolabelled monomeric or dimeric IgA have shown that dimeric IgA was cleared more rapidly than monomeric IgA (Delacroix *et al.*, 1982b).

The mechanism by which IgA is cleared from the circulation is not known. In rodents it has been shown that polymeric IgA (pIgA) or IgA IC are rapidly cleared from the circulation (Jackson *et al.*, 1978; Peppard *et al.*, 1981). Both pIgA and IgA IC are recognized by secretory component (SC) expressed on hepatocytes (Socken *et al.*, 1979; Orlans *et al.*, 1979) and subsequently internalized and transported to the bile as intact IgA. However, depending on the source of IgA and its SC-binding capacity, IgA can also bind to hepatocytes via asialoglycoprotein receptors, after which internalization and degradation of IgA in hepatic lysosomes can occur (Schiff *et al.*, 1986). In humans no SC can be demonstrated on hepatocytes (Hopf *et al.*, 1978; Nagura *et al.*, 1981; Delacroix & Vaerman, 1983).

Recently Sato *et al.* (1983) have demonstrated that the polymorphonuclear leucocytes (PMN) of patients with IgA nephropathy frequently contain inclusions of IgA. Roccatello, Coppo & Piccoli (1984) showed similar inclusions in monocytes from these patients. Kauffmann *et al.* (1983) could demonstrate cytoplasmic inclusions of IgA in PMN from healthy donors upon incubation with serum from patients with Henoch Schönlein purpura *in vitro*. Other investigators have shown that ox erythrocytes sensitized with rabbit secretory IgA or human myeloma IgA formed rosettes with both PMN and monocytes (Fanger *et al.*, 1980; Walsh & Kay, 1986). The formation of these rosettes could be fully inhibited by IgA but not by IgG, suggesting that PMN and monocytes have specific receptors for IgA (Fanger, Pugh & Bernier, 1981; Fanger, Goldstine & Shen, 1983). More recent investigations using calcitriol-treated HL-60 cells also indicate the presence of IgA Fc receptors (FcRα) on phagocytes (Maliszewski, Shen & Fanger, 1985).

Abbreviations: BSA, bovine serum albumin; FcRα, IgA Fc receptor; IC, immune complexes; pIgA, polymeric IgA; PMN, polymorphonuclear leucocytes; sIgA, secretory IgA.

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The present study was designed to investigated the interaction of corpuscular IC containing purified human IgA with human PMN.

MATERIALS AND METHODS

Bovine serum albumin (BSA), horseradish peroxidase (type II), lysostaphin diethylaminoethyl (DEAE)-Sephacel, (Sigma Chemical Co., St Louis, MO), carboxymethyl (CM)-Sephadex C-50, Sephacryl S-300, Sepharose 4B (Pharmacia, Woerden, The Netherlands), nutrient broth no. 2, Hanks' balanced salt solution (HBSS) (Oxoid Ltd, London, U.K.), Dowex 1×8 (Serva, Heidelberg, FRG), homovanillic acid (Fluka AG, Buchs, Switzerland), hydrogen peroxide (Merck, Darmstadt, FRG), insolubilized lactoperoxidase (Worthington, Freeholt, NJ), Na^{125}Iodine (Radiochemical Centre, Amersham, Bucks, U.K.) and gelatin (Difco, Detroit, MI) were purchased as indicated. Ficoll–Isopaque and dextran solutions (analytical grade) were prepared in the hospital's pharmacy. Normal human serum from healthy donors was divided into aliquots and stored at −70°. The mouse monoclonal antibody gran 1 (anti-PMN-Fcγ receptor) was a gift from Dr P. A. T. Tetteroo from the Central Laboratory of The Netherlands Red Cross Blood Transfusion Service in Amsterdam (Miedema *et al.*, 1984).

Isolation of PMN

Human PMN were isolated from heparinized venous blood obtained from healthy donors. PMN were isolated by gradient centrifugation on Ficoll–Isopaque according to Böyum (1968) followed by sedimentation in dextran (Leijh, van den Barselaar & van Furth, 1981). Remaining erythrocytes were lysed by hypotonic shock and the purified PMN were washed with PBS and resuspended in PBS/glucose (1 g/l) or HBSS + 0·1% (w/v) gelatin. The preparations contained at least 95% PMN and the viability was always greater than 98% as assessed by trypan blue exclusion.

Microorganisms

Staphylococcus aureus (*S. aureus*, type 42D) was cultured overnight in nutrient broth no. 2, harvested by centrifugation for 10 min at 1500 ***g*** and washed twice in PBS. The bacteria were resuspended in PBS, heat-killed (30 min, 100), centrifuged for 10 min at 1500 ***g*** and finally resuspended in HBSS.

Isolation of IgA

Serum IgA was isolated from a pool of human serum as previously described by Hiemstra *et al.* (1987). The IgA preparations were concentrated to 5–10 mg/ml and purity was assessed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) according to the method described by Laemmli (1970). The IgA preparations were shown by ELISA methods to contain less than 0·1% IgG and 0·3% IgM on a weight basis.

Isolation of secretory IgA

Secretory IgA (sIgA) was isolated from pooled human colostrum. Fat was removed by centrifugation (60 min, 49,500 ***g***) and sIgA was further purified by 50% $(NH_4)_2SO_4$ precipitation followed by anion exchange chromatography on DEAE–Sephacel and gel filtration on a Sephacryl S-300 column under conditions described for the isolation of IgA. The sIgA-containing fractions were concentrated to 3–6 mg/ml and assessed for purity by SDS–PAGE (Laemmli, 1970). The sIgA preparations were shown by ELISA methods to contain less than 0·08% IgG and 0·3% IgM on a weight basis.

Isolation of IgG

Human IgG was isolated from normal human serum by 33% ammonium sulphate precipitation followed by anion exchange chromatography on DEAE-Sephacel and rechromatography on DEAE-Sephacel to remove further impurities. The IgG preparations were shown by ELISA methods to contain less than 0·01% IgA and 0·03% IgM on a weight basis.

^{125}I labelling

Purified IgA, sIgA and IgG were each radiolabelled with ^{125}Iodine by the lactoperoxidase method (Thorell & Larsson, 1974) to a specific activity of approximately 0·1 mCi/mg protein. Radiolabelled preparations were chromatographed on Dowex 1×8 to separate protein-bound from free iodine.

Preparation of soluble aggregates of IgA and IgG

Aggregates were obtained hy heating 10 mg/ml myeloma IgA or IgG in PBS for 90 min or 20 min, respectively, at 63°. After centrifugation for 20 min at 2000 ***g*** to remove insoluble aggregates, the soluble aggregates were separated from non-aggregated immunoglobulin by gel filtration on a Sepharose 4B column that was equilibrated with veronal-buffered saline containing 0·15 M NaCl, 2 mM EDTA, pH 7·5.

Opsonization of heat-killed S. aureus

Opsonization of *S. aureus* was performed by incubating 5×10^9 bacteria in 0·5 ml PBS containing 20% (v/v) fresh donor serum, 20% (v/v) heat-inactivated serum or 500 μg purified IgG, IgA or sIgA for 30 min at 37°. For dose–response experiments the mount of immunoglobulins was varied. Excess serum or immunoglobulin was removed by centrifugation for 10 min at 1500 ***g*** and repeated washing with PBS. Finally the bacteria were resuspended to a final concentration of 1×10^{10} bacteria/ml in PBS/glucose or HBSS containing 0·1% gelatin (gelatin-HBSS).

Phagocytosis

In order to measure the capacity of PMN to ingest *S. aureus*, 1×10^7 PMN/ml and 1×10^8 heat-killed preopsonized *S. aureus*/ml were incubated in gelatin-HBSS under slow rotation (4 r.p.m.) for 60 min at 37°, after which the suspension was cooled to 4 and centrifuged for 4 min at 110 ***g*** to separate PMN from non-ingested bacteria. Next, PMN were incubated with 1 U lysostaphin/ml for 5 min at 4° to lyse all extracellular *S. aureus* and washed once with gelatin-HBSS before cytopreparations were made. After fixation and staining with Giemsa stain, the percentage of PMN with ingested bacteria and the number of bacteria per phagocytosing PMN were determined microscopically. The phagocytic index was defined as the percentage of PMN with ingested bacteria × bacteria of *S. aureus* per phagocytosing PMN.

H_2O_2 measurement

The production of H_2O_2 in response to soluble or corpuscular IgA IC was assayed by horseradish peroxidase-mediated H_2O_2-dependent oxidation of homovanillic acid as described by Ruch,

Cooper & Baggiolini (1983). Briefly, 1×10^6 PMN were suspended in 2 ml solution containing 200 μM homovanillic acid and 5 U/ml horseradish peroxidase in the presence or absence of soluble or corpuscular IgA IC and incubated for 60 min under slow rotation (4 r.p.m.) at 37°. After this incubation period 0·25 ml of 25 mM EDTA in 0·1 M glycine NaOH, pH 12, was added and the incubation mixture was centrifuged for 10 min at 1500 *g*. The homovanillic acid oxidation product was measured on a Perkin-Elmer model 3000 fluorescence spectrofluorometer. Excitation and emission were measured at 312 and 420 nm, respectively. A 30% stock solution was used to prepare H_2O_2 standards.

Statistical analysis

Results are expressed as the mean ± standard deviation of (*n*) experiments. *P* values were calculated using the Student's *t*-test.

RESULTS

Binding of IgA, sIgA and IgG to *S. aureus*

In order to detect whether IgA, sIgA and IgG were bound to *S. aureus* upon incubation with the various opsonins used, batches of 5×10^9 bacteria were incubated with either fresh serum (20%), heat-inactivated serum (20%), IgG (1 mg/ml), IgA (1 mg/ml) or sIgA (1 mg/ml) for 30 min at 37°, in a final volume of 0·5 ml PBS, containing known trace amounts of ^{125}I-labelled IgG, IgA or sIgA and subsequently assessed for the number of immunoglobulin molecules bound to *S. aureus*. The results, summarized in Table 1, reveal that opsonization of *S. aureus* with serum or heat-inactivated serum resulted in the binding of both IgG and IgA to *S. aureus*.

Phagocytosis of *S. aureus* by PMN

In order to investigate whether IgA or sIgA served as opsonins, the phagocytosis of *S. aureus* opsonized with either IgA or sIgA was compared with the ingestion of bacteria opsonized with serum, heat-inactivated serum or IgG. As shown in Table 2, *S. aureus* organisms treated with buffer were ingested by 9 ± 3% of the PMN, whereas bacteria opsonized with fresh serum were phagocytozed by 78 ± 10% of the PMN. Opsonization with heat-inactivated serum resulted in phagocytosis by 31 ± 10% of the PMN, which was similar to the degree of phagocytosis of IgG-opsonized *S. aureus* (39 ± 7%). The opsonization of bacteria with IgA or sIgA resulted in phagocytosis by 23 ± 8% and 28 ± 9% of the PMN, respectively, which were both statistically significantly different ($P < 0.05$) from the phagocytosis observed in the absence of opsonins. In order to determine whether the various reagents used for the opsonization of bacteria had an effect on the number of bacteria ingested per PMN, the number of bacteria per phagocytosing PMN was determined and the phagocytic index was calculated (Table 2). Opsonization of *S. aureus* with IgA resulted in lesser numbers of bacteria per phagocytosing PMN as compared with bacteria opsonized with IgG. On the other hand, bacteria opsonized with sIgA were ingested to the same extent as *S. aureus* opsonized with IgG. The difference in uptake of *S. aureus* opsonized with IgA and *S. aureus* opsonized with sIgA was statistically significant ($P < 0.05$).

Table 1. Binding of immunoglobulin to *S. aureus*

Opsonizing material	Number of molecules bound/*S. aureus*		
	IgG	IgA	sIgA
Serum	59,000 ± 700*	2500 ± 300	ND†
Heat-inactivated serum	84,000 ± 4000	3500 ± 700	ND
IgG	67,000 ± 400	ND	ND
IgA	ND	16,000 ± 600	ND
sIgA	ND	ND	9300 ± 500

5×10^9 heat-killed *S. aureus* were incubated for 30 min at 37° with 500 μg of purified immunoglobulin in the presence of a trace ^{125}I immunoglobulin. Excess immunoglobulin was removed by washing and the number of immunoglobulin molecules bound per *S. aureus* was calculated.

* Mean ± standard deviation.

† ND, not done.

Effect of the organism to cell ratio on the phagocytosis of IgA- or sIgA-opsonized *S. aureus*

In order to investigate whether the degree of phagocytosis of either IgA- or sIgA-opsonized *S. aureus* was dependent on the organism to cell ratio used, phagocytosis experiments were performed using various ratios. A ratio of 1 to 1 did not induce significant phagocytosis of either IgA- or sIgA-opsonized *S. aureus* (Table 3), whereas when a ratio of 100 to 1 was used almost all PMN had ingested the opsonized bacteria. However, at this ratio the number of PMN that had ingested unopsonized *S. aureus* was also increased.

Induction of H_2O_2 release by opsonized *S. aureus*

In order to determine whether interaction of PMN with *S. aureus* opsonized with IgA or sIgA also resulted in activation of the oxygen metabolism of the PMN, the H_2O_2 release of PMN was measured. The results presented in Fig. 1 show that incubation of PMN with *S. aureus* opsonized with serum resulted in the release of 13 nmol H_2O_2, whereas incubation of PMN with unopsonized *S. aureus* resulted in a background H_2O_2 release of 1·6 nmol. Also, incubation of *S. aureus* with an irrelevant protein (BSA) did not induce H_2O_2 release of PMN above this background level. Opsonization with either IgA or sIgA resulted in a statistically significant increase ($P < 0.001$) in H_2O_2 release to about 4·4 nmol. However, the degree of release was suboptimal as compared with the 7·1 nmol H_2O_2 released in the presence of *S. aureus* opsonized with IgG.

In order to exclude the possibility that the H_2O_2 release was caused by minute amounts of IgG or IgM present in the IgA preparations, the effect of opsonization with various concentrations of immunoglobulin on the H_2O_2 release was investigated. Incubation of PMN with bacteria opsonized with various concentrations of IgG, IgA or sIgA demonstrated that the amount of H_2O_2 released by PMN was dependent on the amount of immunoglobulin used to opsonize the bacteria (Fig. 2). Since opsonization of bacteria with IgM or with up to 100 μg IgG did not induce detectable release of H_2O_2 it can be concluded that the IgG and IgM present in the IgA preparations were not responsible for the H_2O_2 release by PMN.

Table 2. Phagocytosis of opsonized *S. aureus* by PMN

Opsonization of *S. aureus* with:	Concentration	Percentage of PMN with bacteria (*A*)	Mean number of ingested bacteria per phagocytosing PMN (*B*)	Phagocytic index (*A* × *B*)
Serum	20% v/v	78±10	11±1	866
Heat-inactivated serum	20% v/v	31±10	8±2	236
IgG	1 mg/ml	39±7	11±2	423
IgA	1 mg/ml	23±8*	5±1*	119
sIgA	1 mg/ml	28±9	8±2*	231
Buffer	—	9±3*	4±1	31

PMN and opsonized *S. aureus* (*S. aureus*:PMN = 10:1) were incubated for 60 min at 37° and the percentage PMN with ingested bacteria, the number of *S. aureus* per phagocytosing PMN and the phagocytic index were determined as described in the Materials and Methods. The values represent the mean of four experiments ±standard deviation.

*$P < 0.05$.

Table 3. Effect of the organism:cell ratio on phagocytosis

Opsonization of *S. aureus* with:	Organism: PMN ratio	Percentage of PMN with bacteria (*A*)	Number of ingested bacteria per phagocytosing PMN (*b*)	Phagocytic index (*A* × *B*)
IgA	1:1	5	3	13
	10:1	29	5	140
	100:1	89	19	1682
sIgA	1:1	6	4	29
	10:1	29	8	238
	100:1	97	34	3293
Buffer	1:1	4	3	12
	10:1	9	3	30
	100:1	46	7	337

PMN and bacteria were incubated for 60 min at 37° and the percentage of PMN with ingested bacteria, the number of *S. aureus* per phagocytosing PMN and the phagocytic index were determined as described in the Materials and Methods. The values represent the mean of two experiments.

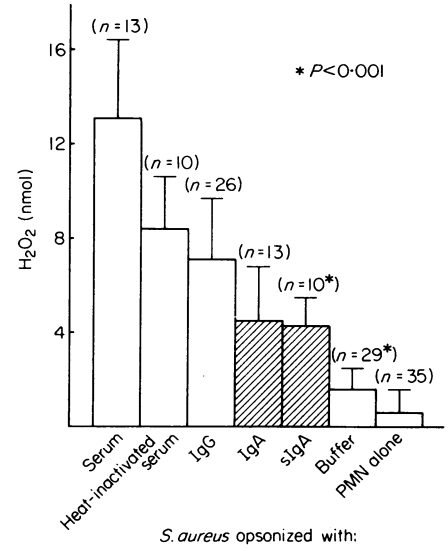

Figure 1. The effect of opsonized *S. aureus* on the H_2O_2 release of PMN. 1×10^6 PMN were incubated with 2×10^9 *S. aureus* opsonized either with purified immunoglobulin (1 mg/ml) or serum 20% (v/v) for 60 min at 37°. H_2O_2 was measured as described in the Materials and Methods.

In order to exclude the possibility that IgA was interacting with Fcγ receptors, PMN were pretreated with $F(ab')_2$ anti-Fcγ receptor antibody or buffer and subsequently incubated with either IgA- or IgG-opsonized *S. aureus*. The $F(ab')_2$ anti-Fcγ receptor antibody inhibited the H_2O_2 release in the presence of *S. aureus* opsonized with IgG by 93% whereas no significant inhibition of the H_2O_2 release (14%) in the presence of *S. aureus* opsonized with IgA was observed. Incubation of PMN with control antibody had no effect of the H_2O_2 release.

Induction of H_2O_2 release by soluble IgA IC

In order to determine whether complexes containing only IgA were also able to activate the oxygen metabolism of PMN, various concentrations of soluble aggregates of serum myeloma IgA were incubated with PMN for 60 min at 37° and next assessed for the amount of H_2O_2 released. The results revealed a concentration-dependent H_2O_2 release that was similar to the degree of H_2O_2 release observed with aggregated IgG (Fig. 3). On the contrary, monomeric myeloma IgA at all the concentrations used induced a release of approximately 1 nmol of H_2O_2, which was not significantly different from the H_2O_2 release by PMN in the presence of buffer (Fig. 1). Also, glutaraldehyde-aggregated BSA did not induce H_2O_2 release from PMN.

DISCUSSION

In the present study we have investigated whether corpuscular IC containing human IgA were able to interact with human

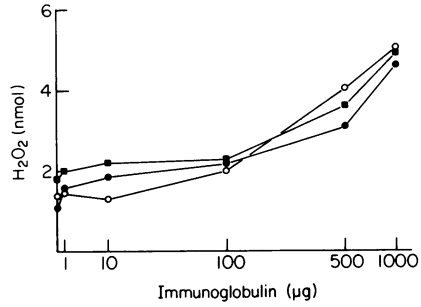

Figure 2. The effect of *S. aureus* opsonized with various doses of immunoglobulin on the H_2O_2 release by PMN. 1×10^6 PMN were incubated for 60 min at 37° with *S. aureus* opsonized with IgA (●), sIgA (○) or IgG (■). H_2O_2 was measured as described in the Materials and Methods.

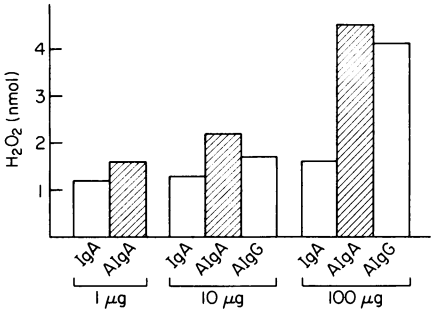

Figure 3. The effect of aggregated IgA (AIgA) on the release of H_2O_2 by PMN. 1×10^6 PMN were incubated with various amounts of aggregated immunoglobulin (AIgA or AIgG) for 60 min at 37°. H_2O_2 was measured as described in the Materials and Methods.

PMN. For this purpose the phagocytosis and H_2O_2 release upon incubation of PMN with heat-killed *S. aureus* opsonized with either purified serum IgA or sIgA isolated from human colostrum was determined.

Several studies on the opsonic activity of sIgA (Kaplan, Dalmasso & Woodson, 1972; Wilson, 1972; Zipursky, Brown & Bienenstock, 1973) have been reported. In contrast, there are only a few studies on the opsonic activity of myeloma IgA (Cheson, 1981) and human serum IgA (Hällgren & Stålenheim, 1978). From these studies it was not possible to decide whether or not serum IgA or sIgA has opsonic activity, and therefore in the present study the binding of IgA to *S. aureus* was first determined before the opsonic activity of these immunoglobulins was investigated. The results revealed that both serum IgA and sIgA bound to *S. aureus*.

Serum IgA and sIgA probably bind to protein A of this *S. aureus* strain. Binding of IgA or sIgA to *S. aureus* could either be due to antibodies directed against protein A or to the non-specific binding of IgA to protein A, for it has been demonstrated that IgA1 and IgA2 myeloma proteins are able to bind to protein A (Patrick *et al.*, 1977; Iganäs, 1981; Bruin, Faber & Biewenga, 1985). The binding of IgA to protein A seems to reside to the F(ab) portion of the IgA molecule (Iganäs, 1981; Bruin *et al.*, 1985). At the present time it is not clear whether the first, the second, or both of the above-mentioned points are applicable to our experiments.

Incubation of *S. aureus* opsonized with IgA or sIgA with PMN resulted in a significant ($P < 0{\cdot}05$) ingestion of these bacteria compared with the ingestion of unopsonized bacteria. These observations are in accordance with those of Fanger *et al* (1983), who previously observed phagocytosis of ox erythrocytosis opsonized with rabbit sIgA by oral PMN, although in their experiments peripheral blood PMN were not capable of ingesting these erythrocytes.

In contrast with these observations are the findings of Wilson (1972), who showed that sIgA-opsonized *S. aureus* was not ingested by peripheral blood PMN. The discrepancy with the present results may be explained by the use of the different *S. aureus* strains or by the different methods used to measure phagocytosis. It has been reported that different *S. aureus* strains have different opsonic requirements (Verhoef *et al.*, 1977).

Receptors specific for IgA have been demonstrated on human PMN and monocytes (Fanger *et al.*, 1981; Fanger *et al.*, 1983; Maliszewski *et al.*, 1985). However, the number of PMN or monocytes expressing FcRα is higher than the number of PMN or monocytes that ingest IgA-coated particles (Fanger *et al.*, 1983; Maliszewski *et al.*, 1985; Lowell *et al.*, 1980). One explanation for this discrepancy might be that a critical number of FcRα is required for ingestion of IgA-coated particles, and that this critical density is only present on a proportion of the FcRα-positive PMN population. Alternatively, there might be a difference in affinity for the FcRα for human IgA used in this study and rabbit sIgA used to quantify the number of PMN expressing FcRα in the studies of Fanger *et al.* (1980, 1981, 1983). Also, the density of IgA molecules on a particle and the size of the particle might influence the results. The latter hypothesis is supported by the observation that latex beads of increasing size opsonized with IgG induced an increase in β-glucuronidase release by PMN, although the total particle surface area (thus the amount of IgG) was kept constant (Henson, 1971). Interestingly, a statistically significant difference in the number of bacteria per PMN was observed after ingestion of bacteria coated with sIgA compared with bacteria coated with IgA. This is probably not due to the difference in subclass composition of serum IgA (IgA1 : IgA2 = 8 : 2) and sIgA (IgA1 : IgA2 = 13 : 7) (Delacroix *et al.*, 1982a) because Fanger *et al.* (1981) could not demonstrate a difference in the ability of myeloma IgA1 and IgA2 to block the rosette formation of rabbit sIgA-sensitized ox erythrocytes. The more efficient uptake of sIgA-coated bacteria in our system might be explained by the observation that pIgA is, on a molar basis, a more potent inhibitor of FcRα interactions (Fanger *et al.*, 1981), and thus seems to have a higher affinity for the FcRα than monomeric IgA.

In addition to phagocytosis of bacteria opsonized with IgA or sIgA, *S. aureus* organisms opsonized with IgA or sIgA are able to induce an activation of the NADPH-oxidase of PMN as judged by an increase in H_2O_2 release. These findings are in agreement with the observations of other investigators who have shown that insoluble aggregated myeloma IgA or myeloma IgA bound to non-phagocytosable surfaces are able to induce degranulation of primary and secondary granules and superoxide production by PMN (Henson, 1971; Henson, Johnson & Spiegelberg, 1972; Kiyotaki *et al.*, 1978). The amount of H_2O_2 released by the PMN was dependent on the dose of immunoglobulin used to opsonize the bacteria and seems to correlate with the number of ingested *S. aureus*. In order to exclude the possibility that the H_2O_2 release by *S. aureus*

opsonized with IgA is due to an interaction with the few IgG molecules present on the bacteria the Fcγ receptor on the PMN was blocked with a $F(ab')_2$ anti-Fcγ receptor monoclonal antibody. The results of these experiments revealed that preincubation of PMN with $F(ab')_2$ anti-Fcγ completely blocked the H_2O_2 release observed after stimulation with *S. aureus* opsonized with IgG, whereas virtually no inhibition was observed after stimulation with *S. aureus* opsonized with IgA, thus indicating that activation by *S. aureus* opsonized with IgA probably occurs via the FcRα. Furthermore, not only corpuscular IgA IC but also soluble AIgA was able to induce a respiratory burst, indicating that the IgA molecules are essential for the induction of phagocytosis and the activation of the respiratory burst. Taken together, these results indicate that human serum IgA and sIgA have opsonic activity and are able to induce a respiratory burst and phagocytosis by PMN.

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