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Effect of concanavalin A on intracellular killing of *Staphylococcus aureus* by human phagocytes

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SUMMARY

This study concerns the influence of concanavalin A (Con A) on phagocytosis and intracellular killing of *Staphyloccus aureus* by human monocytes and granulocytes. Con A binds to *S. aureus*, monocytes, and granulocytes, and is not opsonic. Con A stimulates the killing of intracellular serum opsonized *S. aureus* by monocytes, but not by granulocytes. This stimulation of intracellular killing was inhibited by α-methyl-mannoside, indicating that the process occurs via Con A specific membrane binding sites. Unlike (tetravalent) Con A, divalent succinyl-Con A does not stimulate intracellular killing, indicating that the lectin valency is important for this stimulation. Con A bound to Sephadex particles, that can not be ingested by monocytes, does not stimulate intracellular killing of *S. aureus* either, although it, like free Con A, stimulates H_2O_2 production. Pre-incubation of monocytes with Con A inhibited Fcγ and C3b-mediated ingestion of *S. aureus* as well as stimulation of the killing by serum. Divalent Con A had no effect on these functions. This inhibition by Con A is in all probability due to a steric impedance of Con A with respect to the interaction of IgG and C3b with their membrane receptors. Fluorescence techniques showed that Con A was localized on the membrane and in the cytoplasm of the monocytes, whereas granulocytes had only membrane bound lectin. Taken together, these findings suggest that cell penetration by the lectin is obligatory for the stimulation of intracellular killing.

Keywords intracellular killing membrane stimulation concanavalin A monocytes granulocytes

INTRODUCTION

As shown previously, interaction of IgG, C3/C3b and B/Bb with specific membrane receptors is required for maximal intracellular killing of micro-organisms by granulocytes and monocytes (Leijh *et al.*, 1979; Leijh, van Zwet & van Furth, 1980; Leijh, van den Barselaar & van Furth, 1981; Leijh *et al.*, 1982). These studies were performed with an assay by which the rate of intracellular killing of micro-organisms can be measured independently of the rate of phagocytosis and in which at least 80% of the viable bacteria are intracellular at the start (Leijh *et al.*, 1979, 1981).

Since concanavalin A (Con A) binds to phagocytes, but does not induce ingestion of particles (Berlin, 1972; Edelson & Cohn, 1974; Goldman, Sharon & Lotan, 1976; Cohen, Metcalf & Root, 1980), this component is a useful tool which may help answer two questions: whether extracellular stimulation of monocytes can induce the process of intracellular killing, and whether extracellular stimulation is a short trigger or a prolonged process.

The present paper reports on the effect of Con A on phagocytosis and intracellular killing of

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non- and serum opsonized *Staphyloccus aureus* by granulocytes and monocytes and the effects of α-methyl-mannoside on these monocyte functions.

MATERIALS AND METHODS

Leucocytes. Monocytes were collected by differential centrifugation of blood of healthy donors on Ficoll-Hypaque as described elsewhere (Leijh *et al.*, 1979), and suspended in Hanks' balanced salt solution (HBSS) with 0·1% (wt/vol.) gelatin (gelatin-HBSS) to a concentration of 1×10^7 monocytes/ml.

Granulocytes were obtained by dextran sedimentation of the Ficoll-Hypaque pellet to remove erythrocytes (Leijh *et al.*, 1981) and resuspension in gelatin HBSS to a concentration of 10^7 cells/ml.

Serum. Serum was prepared from blood of blood group AB donors as described elsewhere (van Furth, van Zwet & Leijh, 1978), and stored in 2 ml aliquots at −70°C.

Reagents. Con A (Miles-Yeda, Israel) and succinyl-Con A (Insdustrie Biologique Francaise, Clichy, France) were dissolved in HBSS. FITC labelled Con A (Industrie Biologique) was dissolved in phosphate-buffered saline (PBS) before use. Con A and succinyl-Con A were used in the highest concentration (150 μg/ml and 300 μg/ml, respectively) that did not give agglutination and was not toxic for monocytes, granulocytes or *S. aureus*. Sephadex G25-Con A particles were prepared by coupling Con A to Sephadex G25 particles (Pharmacia, Fine Chemicals, Uppsala, Sweden) after activation with cyanogen bromide as described elsewhere (Marck, Parikh & Creatrecases, 1974). α-methyl-mannoside (αMM) and lysostaphin were obtained from Sigma Chemical Co. (St Louis, Missouri, USA).

Micro-organisms. S. aureus (type 42D) was cultured overnight in Nutrient Broth No. 2 (Oxoid Ltd, London, UK), harvested by centrifugation at 1,500*g*, washed twice with PBS, and suspended in gelatin HBSS to a concentration of 10^7 bacteria/ml. *S. aureus* was opsonized by incubation of 10^7 bacteria/ml with 10% (vol./vol.) serum for 25 min at 37°C during rotation at 4 r/min; the excess serum was removed and the bacteria resuspended to a concentration of 10^7/ml (Leijh *et al.*, 1979).

Receptors. Fc receptors were determined with IgG coated sheep red blood cells (SRBC) as described elsewhere (Crofton, Diesselhoff-den Dulk & van Furth, 1978). Con A receptors were determined with Con A coated SRBC. A 1 ml suspension of 1% (vol./vol.) SRBC in medium 199 (Microbiological Associates, Walkersville, Maryland, USA) was incubated with 150 μg Con A for 60 min at 37°C; the cells were washed twice to remove free Con A and resuspended in medium 199 to a concentration of 0·2% (8×10^7 cells/ml) and 1 ml of this suspension was added to monocytes on cover slips. After incubation for 60 min at 37°C, the cover slips were washed with medium 199, air dried, fixed and stained with Giemsa stain.

Con A receptors on granulocytes were determined after incubation of 8×10^7/ml Con A coated SRBC cells with 2×10^6/ml granulocytes for 60 min at 4°C. The percentage of cells binding Con A coated SRBC was determined from counts made microscopically. Monocytes and granulocytes binding three or more SRBC were considered positive. Ingestion of Con A coated SRBC by monocytes was assessed after lysis of the extracellular SRBC with 0·83% (wt/vol.) NH_4Cl as described elsewhere (van Furth & Diesselhoff-den Dulk, 1980).

Assay methods to study phagocytosis of S. aureus. **Microbiological Assay** Phagocytosis of *S. aureus* by monocytes and granulocytes was measured as a decrease in the number of viable extracellular bacteria during incubation of 10^7 monocytes or granulocytes/ml with an equal volume of 10^7 bacteria/ml in the presence or absence of serum or Con A for 60 min at 37°C at 4 r/min (van Furth *et al.*, 1978). The number of viable extracellular bacteria was determined microbiologically in the supernatant after separation of monocytes or granulocytes from the bacteria by differential centrifugation for 4 min at 110*g*.

Morphological asssay Phagocytosis of *S. aureus* by monocytes was determined morphologically after 60 min incubation of bacteria and monocytes as described above. After incubation, cytocentrifuge preparations were made and the extracellular *S. aureus* were lysed with lysostaphin (1 u/ml) (van Furth & Diesellhof-den Dulk, 1980). The percentages of monocytes containing bacteria and the mean number of bacteria ingested per monocyte were calculated from microscopical counts.

Intracellular killing assay The rate of intracellular killing of *S. aureus* was determined from the decrease in the number of viable intracellular bacteria after a 3 min ingestion period (Leijh *et al.*, 1979, 1980). In short, 5×10^6 monocytes or granulocytes/ml and 5×10^6 or 5×10^7 pre-opsonized bacteria/ml were incubated for 3 min at 37°C at 4 r/min, after which phagocytosis was stopped by rapid chilling in crushed ice, and the non-ingested bacteria were removed by centrifugation for 4 min at 110*g* and two washes in HBSS at 4°C. The phagocytes containing ingested bacteria were re-incubated at 37°C at 4 r/min, samples were taken at 0, 1, and 2 h and centrifuged for 4 min at 110*g*, and then the phagocytes were suspended in distilled water containing 0·01% albumin. Lysis of granulocytes was facilitated by agitation for 1 min on a vortex mixer, and lysis of monocytes by three freeze–thaw cycles in liquid nitrogen alternated with a 37°C water bath. The number of viable micro-organisms in the suspensions was determined microbiologically.

Hydrogen peroxide (H_2O_2) production by monocytes. H_2O_2 production was measured with the scopoletin assay described by Root *et al.* (1975).

Fluorescence microscopy. Phagocytes were incubated with FITC labelled Con A for 30 or 60 min at 37°C, washed with HBSS, re-incubated with or without αMM for an additional 30 min at 37°C, and washed again. Cytocentrifuge preparations were made, fixed in 5% acetic acid (vol./vol.) and 95% ethanol for 15 min at −20°C, and washed three times with PBS. The preparations were examined with a fluorescence microscope (Leitz, Wetzlar, FRG).

General remarks and calculations. Unless otherwise stated monocytes containing *S. aureus* are to be understood as monocytes containing bacteria after 3 min phagocytosis at a bacteria-to-monocyte ratio of 1:1 at 37°C followed by differential centrifugation and two washes. In all assays in which cells were re-incubated after an initial incubation in the presence of Con A, serum or sugars, the cells were washed twice at 4°C to remove all unbound additives.

All values represent the mean and standard deviation of at least three experiments unless otherwise stated. Statistical analysis was performed with a two tailed Student's *t*-test. A *P* value of $\geqslant 0{\cdot}1$ was considered to be non-significant (NS).

RESULTS

Demonstration of Con A receptors on granulocytes and monocytes

Incubation of monocytes with Con A coated SRBC showed that $95{\cdot}5 \pm 3{\cdot}1\%$ of the monocytes form rosettes with Con A coated SRBC, but virtually no phagocytosis of lectin coated SRBC was observed (monocytes with ingested SRBC: $2{\cdot}6 \pm 1{\cdot}3\%$). Receptors for Con A were found on $89 \pm 5\%$ of the granulocytes.

Effect of Con A on the phagocytosis of S. aureus *by monocytes*

Incubation of monocytes and *S. aureus* and 150 μg Con A/ml resulted in an increase in the number of extracellular bacteria (negative value in Table 1) indicating that Con A is unable to promote ingestion of *S. aureus* (Table 1). Since free Con A may suppress phagocytosis of Con A coated bacteria by competitive inhibition, the microbiological phagocytosis assay was also performed with *S. aureus* coated with Con A. The results of these experiments too showed no phagocytosis of Con A coated *S. aureus* (Table 1). When phagocytosis was determined with a morphological assay, similar results were obtained (Table 1).

To find out whether con A can act as an additional opsonin, phagocytosis of serum opsonized *S. aureus* in the presence of Con A was measured. Phagocytosis of serum opsonized *S. aureus* occurred on the same scale in the presence and absence of Con A (phagocytic indices at 60 min: $92 \pm 4\%$ and $93 \pm 6\%$, respectively).

Effect of Con A and αMM on Fcγ- and C3b-mediated phagocytosis

After incubation of monocytes with 150 μg Con A/ml for 30 min at 37°C, phagocytosis of serum opsonized *S. aureus* and IgG coated SRBC was reduced (Table 2, line 2). Incubation of monocytes with 300 μg divalent succinyl-Con A before re-incubation with serum opsonized *S. aureus* or IgG coated SRBC, led to weaker inhibition of the phagocytic activity (Table 2, line 3).

Table 1. Phagocytosis of *S. aureus* by monocytes

	Microbiological assay*				Morphological assay‡			
Opsonin	Phagocytosis of *S. aureus* in medium containing opsonin %	(*n*)	Phagocytosis of opsonin coated *S. aureus*† %	(*n*)	Percentage monocytes containing *S. aureus* (A) %	Average number of *S. aureus* per monocyte (B) %	Phagocytic index (A × B) %	(*n*)
150 μg Con A/ml	−26±13	(3)	−53±17	(3)	9±3	0·6±9·4	5·4	(3)
10% serum	92±4	(6)	92±4	(6)	45±8	4±2	180	(3)
HBSS	−56±13	(6)	−56±13	(6)	10±2	0·5±0·5	5·0	(3)

* Phagocytosis was measured as a decrease in the number of viable extracellular *S. aureus* in the supernatant during 60 min incubation of bacteria and monocytes at a ratio of 1 : 1 under rotation at 4 r/min at 37°C. A minus sign indicates an increase in the number of viable bacteria in the supernatant.

† *S. aureus* were opsonized with serum or Con A by incubation for 30 min at 37°C, after which any excess unbound serum or lectin was removed by two washes with HBSS.

‡ Phagocytosis was performed for 60 min at 37°C at a bacteria-to-monocyte ratio of 1 : 1, after which cytocentrifuge preparations were made and extracellular *S. aureus* removed by treatment with lysostaphin (van Furth *et al.*, 1980).

Incubation of Con A pre-treated monocytes with 100 mM αMM for 30 min at 37°C partially restored the phagocytic activity of monocytes and also the percentage of cells with Fcγ receptors (Table 2, line 4).

Together, these results indicate that Con A influences Fcγ and C3b receptor-mediated phagocytosis by monocytes, that this effect is more evident with tetravalent than with divalent Con A, and that αMM reverses Con A modulation of these receptor functions.

Effect of Con A on the intracellular killing of S. aureus *by monocytes*

When monocytes containing ingested *S. aureus* were incubated in the presence of various

Table 2. Effect of Con A and αMM on the phagocytic capacity of monocytes

	Phagocytosis of								
Incubation of monocytes with*	Serum opsonized *S. aureus* %	(*n*)	P_1§	IgG coated SRBC %	(*n*)	P_2§	Percentage monocytes with Fcγ receptors‡ %	(*n*)	P_3§
HBSS	93·9±4·2	(6)		80·7±3·2	(11)		91·7±2·7	(11)	
Con A	25·9±13·2	(3)	<0·001	15·4±9·8	(6)	<0·001	64·3±13·2	(6)	<0·001
Succinyl-ConA	65·7±13·8	(3)	<0·01	60·2±11·3	(3)	<0·01	84·1±7·6	(3)	<0·01
Con A and αMM†	75·7±8·7	(3)	<0·01	60·6±6·9	(3)	<0·01	85·9±4·6	(3)	<0·01

* Monocytes were incubated with HBSS, 150 μg Con A/ml or 300 μg succinyl-Con A/ml for 30 min at 37°C and free Con A was removed by two washes.

† Con A pre-treated monocytes were incubated with 100 mM αMM for 30 min at 37°C and then washed twice.

‡ Monocytes with attached and ingested IgG coated SRBC.

§P_1, P_2, P_3: compared with corresponding values for monocytes incubated with HBSS (line 1).

(*n*) = number of experiments.

concentrations Con A a concentration dependent intracellular killing was found (Fig. 1). Exposure of monocytes containing *S. aureus* to succinyl-Con A revealed that divalent lectin does not stimulate intracellular killing (Fig. 1). Incubation of monocytes containing bacteria ingested at a bacteria-to-cell ratio of 10:1 in the presence of 150 μg Con A/ml gave a killing index of 57·6 ± 11·2% at 60 min.

The rate of intracellular killing declined in time and during the second hour of incubation there was an increase in the number of bacteria (Fig. 2a). Incubation of monocytes containing *S. aureus* with 150 μg Con A/ml at 37°C for 30 or 60 min followed by two washes and re-incubation in the presence of Con A for 60 min showed an increase instead of a decrease in the number of intracellular *S. aureus* (Fig. 2b). Incubation of monocytes containing *S. aureus* in HBSS for 30 or 60 min before addition of Con A, gave a rate of intracellular killing similar to that obtained with addition at time 0 (Fig. 2c). These results show that a continuous interaction of Con A with its receptors is obligatory for the stimulation of the intracellular killing.

Incubation of monocytes containing *S. aureus* in the presence of 150 μg Con A/ml and various concentrations of αMM revealed a concentration-dependent inhibition of Con A stimulated killing by αMM (Fig. 3).

When monocytes containing ingested *S. aureus* were incubated with Con A-Sephadex particles, no intracellular killing was observed (Table 3). However, Con A-Sephadex induced a metabolic burst, as reflected by H_2O_2 production.

Together, these results indicate that free tetravalent Con A in suspension is able to stimulate monocytes to kill ingested *S. aureus* via mannoside containing binding sites on the monocyte membrane. However, binding to the membrane is in itself not sufficient to stimulate the killing process.

Effect of Con A pre-treatment of monocytes on the stimulation of the intracellular killing by serum

When monocytes or monocytes containing ingested *S. aureus* were incubated with 150 μg Con A/ml for 30 min at 37°C or 4°C and then with serum or Con A the intracellular killing was inhibited during re-incubation (Table 4, data for incubation at 4°C not shown). When Con A treated monocytes containing *S. aureus* were re-incubated with 100 mM αMM at 37°C or 4°C, washed and next re-incubated in the presence of serum or Con A, the intracellular killing was restored to normal (Table 4).

Pre-treatment of monocytes containing *S. aureus* with 300 μg succinyl-Con A/ml for 30 min at

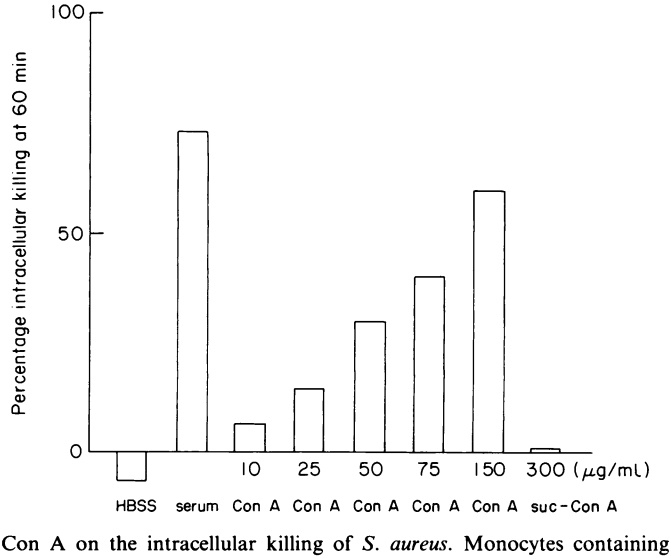

Fig. 1. Effect of Con A on the intracellular killing of *S. aureus*. Monocytes containing *S. aureus* (after phagocytosis at a bacteria-to-cell ratio of 1:1 for 3 min at 37°C) were incubated with Con A in various concentrations.

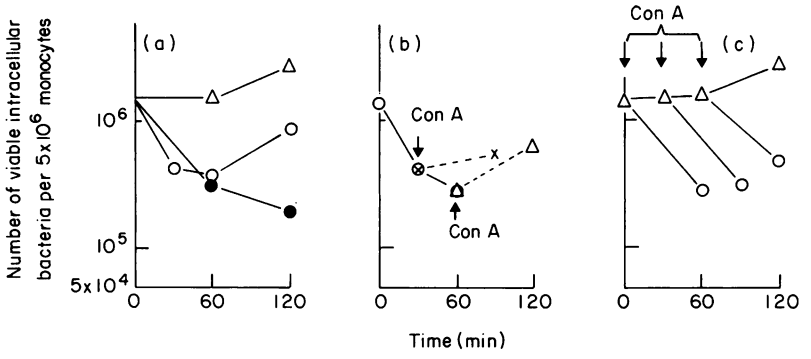

Fig. 2. (a) Kinetics of intracellular killing of *S. aureus* by human monocytes in the continuous presence of Con A (o——o). Serum = ●; HBSS = ▵. (b) Incubation of monocytes containing bacteria in presence of 150 μg Con A/ml for 30 or 60 min, followed by two washes and the re-addition of fresh Con A (x- - -x, ▵- - -▵). (c) Incubation of monocytes containing bacteria in the presence of HBSS for 30 or 60 min and next addition of 150 μg Con A/ml (o——o).

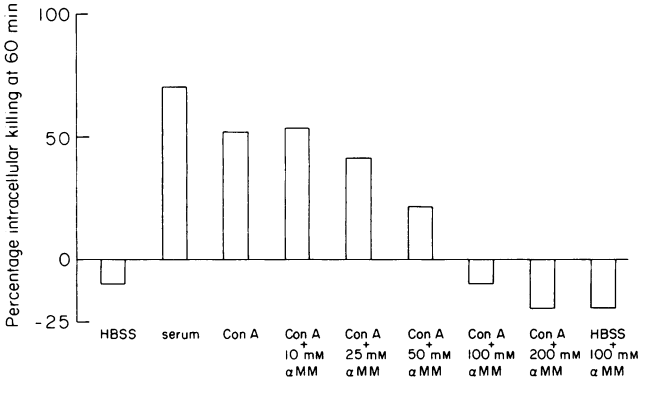

Fig. 3. Effect of αMM on the intracellular killing of micro-organisms by monocytes in the presence of 150 μg Con A/ml.

Table 3. Effect of Con A and Con A-sephadex on intracellular killing and H_2O_2 production by monocytes

Stimulus	Concentration	H_2O_2 production nmol/10^6 monocytes/ 10 min	(*n*)	Intracellular killing at 60 min* %	(*n*)
Con A	25 μg/ml	0·4 ± 0·3	(3)	27·3 ± 13·1	(3)
Con A	75 μg/ml	1·0 ± 0·4	(3)	39·6 ± 10·6	(3)
Con A	150 μg/ml	1·5 ± 0·4	(3)	55·1 ± 12·1	(3)
Con A-Sephadex	$0·5 \times 10^5$ particles/ml	0·5 ± 0·6	(3)	3·6 ± 8·0	(3)
Con A-Sephadex	$1·0 \times 10^5$ particles/ml	1·6 ± 0·6	(3)	−2·9 ± 11·2	(3)
Con A-Sephadex	$5·0 \times 10^5$ particles/ml	5·2 ± 1·4	(3)	−3·6 ± 13·2	(3)

* Measured as the decrease in the number of viable intracellular bacteria during incubation of monocytes containing ingested *S. aureus*.

Table 4. Effect of Con A–αMM interactions on the intracellular killing of *S. aureus* by monocytes

Incubation of monocytes containing bacteria with*	Intracellular killing at 60 min in the presence of 10% serum %	(*n*)	P_1‡	150 μg Con A/ml %	(*n*)	P_2‡
HBSS	73·6±12·1	(8)		51·6±13·2	(6)	
Con A	−3·2±18·1	(9)	<0·001	−15·9±13·6	(9)	<0·001
Succinyl-Con A	48·3±10·1	(3)	<0·001	12·0±13·1	(3)	<0·001
Con A and αMM†	66·9±15·2	(3)	<0·01	40·8±9·8	(3)	<0·001

* Monocytes containing *S. aureus* were incubated with HBSS, 150 μg Con A/ml, or 300 μg succinyl Con A for 30 min; free Con A was removed by two washes with HBSS.

† Con A pre-treated monocytes containing *S. aureus* were incubated with 100 mM αMM for 30 min at 37°C; free αMM was removed by two washes with HBSS.

‡P_1, P_2: compared with killing after incubation of monocytes with HBSS.

(*n*) = number of experiments.

37°C followed by re-incubation in the presence of 10% serum or 150 μg Con A/ml gave killing indices of, respectively, 48·3 and 12·0% at 60 min, which indicates that succinyl-Con A interacts with Con A receptors, thus preventing stimulation by tetravalent Con A. However, this lectin has only a slight influence on the stimulation of intracellular killing by serum.

Cellular distribution of Con A on monocytes and granulocytes during the stimulation of intracellular killing

After 30 and 60 min incubation with FITC labelled Con A, bright fluorescence was present on the cell membranes and within cytoplasmic vacuoles of monocytes. Subsequent incubation with 100 mM αMM for 30 min at 37°C led to the disappearance of the membrane bound fluorescence, whereas the vacuolar fluorescence persisted.

Incubation of granulocytes with FITC labelled Con A for 30 min at 37°C, led to a bright membrane fluorescence and a very weak cytoplasmic fluorescence. When these FITC Con A treated granulocytes were re-incubated with 100 mM αMM for 30 min the membrane fluorescence disappeared.

Effect of Con A on intracellular killing by granulocytes

Although granulocytes carry membrane receptors for Con A, Con A did not increase intracellular killing by these cells (killing index: 30±8% at 60 min) above the level obtained in the presence of HBSS (killing index 35±15% at 60 min), which is significantly lower than in the presence of 10% serum (killing index 85±11% at 60 min).

DISCUSSION

The present results show that although Con A binds to monocytes and granulocytes, this lectin is unable to promote ingestion of SRBC and *S. aureus*. Binding of Con A to monocytes proved to stimulate the intracellular killing of *S. aureus* by these cells due to an interaction with a Con A binding site. Con A binding to granulocyte membranes did not stimulate the intracellular killing.

Two findings i.e., that Con A coated SRBC bind to but are not ingested by monocytes and that *S. aureus* is not ingested in the presence of Con A demonstrate that Con A is not opsonic. This conclusion is in agreement with reports by Guerry *et al.* (1976), Allen, Cook & Poole (1972) and Baggiolini, Feigenson & Schnebli (1976); only Horwitz & Silverstein (1980) have described Con A-mediated ingestion of a special non-encapsulated strain of *E. coli* by monocytes. Since non-opsonic Con A stimulates intracellular killing of *S. aureus* by monocytes this provides

additional evidence for the reliability of the killing assay, i.e., proof that the number of viable bacteria measured represent intracellular bacteria.

Incubation of monocytes containing *S. aureus* in the presence of Con A showed a maximum killing rate during the initial 60 min period and an increase in the number of viable intracellular bacteria during the second hour. This increase is in all probability due to inhibition of intracellular killing by bound Con A. When bound Con A was removed from the monocyte membrane with αMM, interaction of Con A with its receptors started again and so did intracellular killing. These results indicate that continuous interactions of membrane receptors with its ligands is required for stimulation of the killing, a finding which is in agreement with previous observations concerning the stimulation of intracellular killing by serum factors (Leijh *et al.*, 1979).

The interaction of Con A with the membrane of monocytes also prevents functioning of Fcγ and C3b receptors, as reflected by the impaired serum stimulation of intracellular killing and the decreased capacity of Con A treated monocytes to phagocytose IgG coated SRBC and serum opsonized *S. aureus*. The latter finding is in agreement with reports of Toh, Sato & Kikucki (1979), Allen *et al.* (1972) and Berlin (1972). The inhibition of Fcγ and C3b receptor mediated monocyte functions by Con A can be explained by binding of Con A to the same membrane sites as IgG and C3b or by a steric interference of bound Con A for the binding of IgG and C3b. Binding of divalent succinyl-Con A to monocytes has only a slight effect on Fcγ and C3b receptor-mediated functions, although an effect similar to that seen for tetravalent Con A would be expected when similar binding sites are involved. This indicates that the effect of tetravalent Con A on these functions is due to steric interference; after removal of Con A from the membranes with αMM, monocyte functions mediated by Fcγ and C3b receptors were restored.

The difference between the stimulation of intracellular killing by tetravalent Con A and divalent succinyl-Con A suggests that the lectin valency is important for this stimulation. Similar observations has been reported for the metabolic stimulation of polymorphonuclear leucocytes by Con A and divalent Con A (Romeo *et al.*, 1979; Cohen *et al.*, 1980).

Binding of Con A did not stimulate killing by granulocytes, but markedly enhanced killing by monocytes. Because granulocytes neither internalized FITC labelled Con A nor enhanced killing, it seems likely that stimulation of intracellular killing by Con A is mediated via the uptake of a lectin–receptor complex. This hypothesis is supported by preliminary electron microscopical studies which showed that after incubation of monocytes containing bacteria with gold-labelled Con A for 120 min, the metal can be found in the phagosome (Leijh & de Water, unpublished observation). Furthermore the lack of killing stimulatory activity of Con A coated non-phagocytosable Sephadex particles, which bind to monocytes and evoke a metabolic burst, also indicates that uptake of the stimulus by the cell is necessary for stimulation of the killing process.

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