Biochimica et Biophysica Acta, 692 (1982) 101–108
Elsevier Biomedical Press

BBA 71378

ANTIBODY-DEPENDENT PHAGOCYTOSIS OF HAPTENATED LIPOSOMES BY HUMAN NEUTROPHILS IS DEPENDENT ON THE PHYSICAL STATE OF THE LIPOSOMAL MEMBRANE

MALCOLM W. MUNN and J. WALLACE PARCE *

Department of Biochemistry, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103 (U.S.A.)

(Received March 30th, 1982)

Key words: Liposome; Phagocytosis; Antibody dependence; Membrane fluidity; (Human neutrophil)

In the present study we have examined the response of human neutrophils to specific antibody-dependent stimulation by spin-label haptenated fluid phase and solid phase liposomes. Both fluid and solid liposomal antigens are shown to stimulate the neutrophil respiratory burst to approximately equivalent degrees as assessed by measurement of oxygen consumption or oxidation of [1−^{14}C]glucose to $^{14}CO_2$. In contrast, release of superoxide and hydrogen peroxide from the neutrophils is stimulated to a significantly greater degree by fluid-phase liposomes than by the equivalent solid-phase liposomes. This apparent discrepancy is shown to be due to an inability of the neutrophils to phagocytose fluid-phase liposomes under conditions in which solid-phase liposomes are readily phagocytosed. A fluorescence assay, which does not depend upon binding measurements, has been developed in order to quantitate liposomal phagocytosis.

Introduction

Synthetic lipid membranes have been used extensively over the past several years to study the fundamental molecular processes involved in various aspects of immune recognition (for a thorough review of this area see Ref. 1). The use of synthetic lipid membranes as antigens is advantageous in that these membranes serve as simplified models for cell membranes in which the chemical and physical properties can be rigorously defined and manipulated. The specific antibody-dependent activation of complement by liposomal antigens has been studied in considerable detail and found to be dependent on the physical state of the liposomal membrane [2–6]. In this system it was shown that the rate of activation of the first component of complement C1 is determined by the 'fluidity' of the liposomal membrane. The rate constant for activation of C1 was found to be roughly 4-times larger for activation by fluid-phase liposomes than for activation by liposomes in the gel phase [6]. These studies generated an interest in using these same liposomes to study the antibody-dependent stimulation of human polymorphonuclear neutrophils. When neutrophils were challenged with these liposomes, it was found that fluid-phase liposomes wee much more efficient at causing the release of superoxide from the neutrophils than were similar solid-phase liposomes [7]. The interpretation of these results was that fluid liposomal antigens activated neutrophils more efficiently than solid liposomal antigens. In the present study we examined other parameters commonly used to measure the respiratory burst upon activation of neutrophils. We have found that activation of neu-

* To whom correspondence should be addressed

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; NBD-PE, *N*-4-nitrobenzo-2-oxa-1,3-diazole phosphatidylethanolamine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

trophils by fluid and solid liposomal antigens is approximately equivalent. In addition the differential release of superoxide in response to stimulation by these two types of liposome is due to a difference in the ability of the neutrophils to phagocytose these two types of antigen.

Materials and Methods

Materials

Cytochrome *c* (type VI), bovine serum albumin (essentially fatty acid free), catalase, superoxide dismutase, scopoletin, and phorbol myristate acetate were all obtained from Sigma Chemical Co., St. Louis, MO. Dipalmitoylphosphatidylcholine and dimyristoylphosphatidylcholine were obtained from either Sigma Chemical Co., St. Louis, MO, or Avanti Polar Lipids, Birmingham, AL. *N*-4-Nitrobenzo-2-oxa-1,3-diazole phosphatidylethanolamine (NBD-PE) was purchased from Avanti Polar Lipids, Hepes was purchased from Eastman Kodak Co., Rochester, NY. Freund's Complete Adjucant and Dulbecco's phosphate-buffered saline were obtained from Grand Island Biological Co., Grand Island, NY. Keyhole limpet hemocyanin was purchased from Polysciences, Inc., Warrington, PA. Isolymph was obtained through Gallard-Schlesinger Chemical Manufacturing Corp., Carle Place, NY. Gelatin was obtained through Difco Laboratories, Detroit, MI. Protein A Sepharose was obtained through Pharmacia Fine Chemicals, Inc., Piscataway, NJ. Cytochalasin B was purchased from Aldrich Chemical Co., Milwaukee, WI. All other biochemicals were obtained from Sigma Chemicals Co., St. Louis, MO. All other chemicals were of the highest grade obtainable and were purchased from Fisher Scientific Co., Fairlawn, NJ.

Preparation of neutrophils

Neutrophils were isolated from venous blood drawn from healthy human volunteers. Fibrin and the majority of platelets were removed by swirling the blood in an Erlenmeyer flask which contained 10–15 glass beads (3 mm diameter) for approx. 10 min. The defibrinated blood was mixed with plasmagel (30 g/l gelatin/7 g/l NaCl/2 g/l $CaCl_2 \cdot 2H_2O$) in a 3:1 (v/v) ratio and the red cells allowed to settle at room temperature for 30–40 min. The granulocyte-rich supernatant was centrifuged at $300 \times g$ for 10 min, the pellet was resuspended in Earle's balanced salt solution (6.8 g/l NaCl/5.95 g/l Hepes/1.0 g/l glucose/2.2 g/l $NaHCO_3$/140 mg/l $NaH_2PO_4 \cdot H_2O$/400 mg/l KCl, pH 7.4) and the cells washed at $300 \times g$ for 10 min. The cell pellet was resuspended in 3 ml of Hepes cell buffer (294 mg/l $CaCl_2 \cdot 2H_2O$/300 mg/l $MgCl \cdot 6H_2O$/400 mg/l KCl/140 mg/l $NaH_2PO_2 \cdot H_2O$/6.8 g/l NaCl/5.95 g/l Hepes/1.0 g/l glucose/2.0 g/l bovine serum albumin, pH 7.4), and the majority of contaminating red cells were removed by hypotonic lysis with the addition of 9 ml deionized water. Isotonicity was restored after 20 s by the addition of 3 ml 3.5% NaCl, and the cells were pelleted by centrifuging again. The granulocytes were separated from mononuclear leukocytes and remaining erythrocytes by centrifugation through Ficoll-hypaque. This was done by resuspending the cell pellet in 5 ml Hepes cell buffer and layering onto 4 ml Isolymph. Centrifugation at $400 \times g$ for 25 min resulted in a pellet containing 90–98% granulocytes. The pellet was resuspended to 2 ml in cell buffer, and the total cell count was determined on a hemocytometer. The cells were ususally diluted to $5 \cdot 10^6$ neutrophils/ml and placed on ice until use.

Liposome preparation

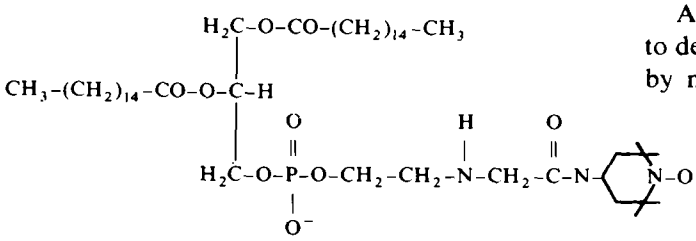
Required amounts of lipid, dimyristoylphosphatidylcholine (DMPC) or dipalmitoylphosphatidylcholine (DPPC), from 10 mM stock lipids dissolved in methanol were mixed with 1 mol% nitroxide spin-label phospholipid (Structure I). The solutions were evaporated to dryness in 10 ml round-bottom flasks. Sufficient cell buffer was added to the flasks to give a final lipid concentration of 10 mM. The lipid was allowed to hydrate for 15 min, 15°C above the phase transition of each particular phospholipid, and liposomes were formed by vigorous vortexing after hydration. This procedure forms multilamellar liposomes which are stable for months and have a size range of apporox. 0.1–50 μm with 90% of the liposomes between 1 and 10 μm [8]. Liposomes produced in this manner have been shown to have approx. 5% of the lipid exposed to the external aqueous compartment [9]. These parame-

ters are essentially the same for both DMPC and DPPC liposomes.

Antibody and hapten preparation

Antinitroxide IgG fractions were prepared as previously described [7] by protein A Sepharose purification from sera from rabbits immunized with nitroxide-labeled keyhole limpet hemocyanin. The concentration of specific antinitroxide antibody was determined to be approx. $2 \cdot 10^{-6}$ M by binding measurements with free spin labels in solution.

The phospholipid nitroxide spin-label hapten I was synthesized by the procedure of Brûlet and McConnell [3].



Structure I

Measurement of neutrophil stimulation

Superoxide release from neutrophils was determined from the superoxide dismutase-sensitive reduction of oxidized cytochrome *c* by a modification of the method of Babior et al. [10] as previously described [7]. Briefly, the assay mixture contained 0.2 ml liposomes (1.0 μmol/ml), 0.2 ml of a 40-fold dilution of the stock antibody solution, and 0.2 ml of cytochrome *c* (0.25 mM). Liposomes and antibody were incubated together for 2 h or more (23°C) prior to initiating the assay with 0.4 ml of neutrophils in cell buffer ($5 \cdot 10^6$ neutrophils/ml). The assay mixture in 13×100 mm culture tubes were kept in ice water while the neutrophil suspension was added. The assay was initiated by placing the culture tubes in a shaker bath at 37°C. The reaction was terminated by putting the culture tubes on ice again. The neutrophils and liposomes were separated from the medium by centrifuging at $7000 \times g$ for 5 min at 4°C. Cytochrome *c* reduction was calculated from the increase in absorbance at 550 nm, using an ΔA_{550} reduced minus oxidized cytochrome *c* of $2.11 \cdot 10^4 \, M^{-1} \cdot cm^{-1}$ [11].

Hydrogen peroxide release was determined by the horseradish peroxidase-catalyzed loss of scopoletin fluorescence as described by Root et al. [12].

Oxidation of [1-^{14}C]glucose was determined as described by Hafeman et al. [13].

Oxygen consumption by neutrophils was measured polarigraphically with a Clark type electrode [14]. In a typical experiment the sample chamber contained $4 \cdot 10^6$ neutrophils in 1.75 ml. The cells were stimulated by the addition of liposomes which had been preincubated with antinitroxide antibody.

Fluorescence assay for phagocytosis

A fluorescence method was developed in order to determine the fraction of liposomes internalized by neutrophils. Liposomes were prepared as described above except that 1.0% NBD-PE was added to the lipid and nitroxide spin-label PE preceding rotoevaporation. The resulting fluorescent liposomes (10 mM) were incubated with antinitroxide antibody (1:9 dilution) at least 2 h prior to the assay. Neutrophils ($5 \cdot 10^6$ in 1.9 ml cell buffer) were placed in a 3 ml fluorescence cuvette in a fluorometer (Fluorolog, Spex Industries) equipped with sample stirring and temperature control. Neutrophil activation was initiated by addition of 0.1 ml of the fluorescent liposome antibody mixture. After 15 min at 37°C, 50 μl 2.0 M acetate buffer, pH 4.0, was added to the cuvette and the NBD-PE fluorescence intensity was determined (excitation 470 nm, emission 550 nm). Immediately following, 20 μl 1.0 M $CuCl_2$ was added to the cuvette, and the fluorescence intensity was determined again. As a control to determine the total amount of liposomal fluorescence which could be quenched by the $CuCl_2$ in the absence of phagocytosis, fluorescence measurements in the presence of acetate buffer and $CuCl_2$ were performed immediately upon addition of liposomes to neutrophils. The percent of liposomal fluorescence which was protected from quenching by phagocytosis was defined as $(\Delta I_{15}/\Delta I_0)100$.

Where ΔI_0 is the difference in fluorescence intensity before and after addition of $CuCl_2$ at zero time (immediately after addition of liposomes to cells). ΔI_{15} is the same parameter determined after 15 min of incubation of cells with liposomes.

Results

Table I is a compilation of data from a number of experiments in which various parameters of the respiratory burst were determined. In each experiment the particular parameter was measured as a function of stimulation of the neutrophils by both specific antibody-bound haptenated fluid liposomes (DMPC) and equivalent solid liposomes (DPPC). The antibody and liposome concentrations were chosen from an antibody titration curve similar to that previously reported [7] for the release of O_2^- from neutrophils. The antibody-to-liposome ratio which gave a maximal difference between stimulation by DMPC and DPPC liposomes was used in all of the following experiments. Although the absolute magnitude of each measured parameter varied considerably from one cell preparation to the next, the relative magnitudes of each response when comparing stimulation by fluid and solid liposomes were quite consistent. specifically, stimulation by fluid liposomes resulted in 2.5 to 3.0-times as much O_2^- and H_2O_2 release as did stimulation by equivalent solid liposomes. It should be noted that both of those assays require the addition of extracellular enzymes. Since these enzymes cannot penetrate the cell membrane, they measure only that O_2^- or H_2O_2 which is released to the extracellular medium. In contrast to the release of O_2^- and H_2O_2, consumption of oxygen and oxidation of glucose by the hexose monophosphate shunt were stimulated to roughly equal degrees by both fluid and solid liposomes. Our results for glucose oxidation are in substantial agreement with those of Hafeman et al. [13] where stimulation of glucose oxidation in neutrophils was measured using unilamellar vesicles as antigens. Since glucose oxidation by the hexose monophosphate shunt and H_2O_2 production are less direct indicators of the respiratory burst, additional experiments focused on oxygen consumption and O_2^- production. Fig. 1 demonstrates the kinetics of oxygen consumption by neutrophils stimulated by both fluid and solid liposomes in the presence and absence of cytochrome *c*. These data demonstrate that the rates of oxygen consumption and O_2^- production are linear throughout the period of the experiment (20 min). In addition it can be seen that fluid liposomes stimulate a much greater release of O_2^- to the extracellular medium than do solid liposomes. This is demonstrated as a decrease in apparent oxygen consumption in the presence of cytochrome *c*, since cytochrome *c* oxidizes extracellular O_2^- back to molecular oxygen. A possible explanation for the data presented thus far is that solid liposomes may be readily phagocytosed whereas phatocytosis may occur slowly or not at all for fluid liposomes. If the respiratory burst was stimulated by the fluid liposome, but the neutrophil could not phagocytose that liposome, then it might be expected that much of the O_2^- would be directed extracellularly. In the case of the solid liposome, phagocytosis would result in the formation of a phagocytic vacuole and the subsequent release of O_2^- would be principally intracullular. Due to rapid dismuta-

TABLE I

AVERAGE RATES OF O_2 CONSUMPTION, O_2^- RELEASE, H_2O_2 RELEASE, AND GLUCOSE OXIDIZED BY NEUTROPHILS STIMULATED WITH DMPC (FLUID PHASE) AND DPPC (SOLID PHASE) LIPOSOMES

	Number of experiments	nmol/min per 10^6 neutrophils		
		DMPC	DPPC	DMPC/DPPC
O_2 consumption	7	3.83	3.43	1.12
O_2^- release	6	1.56	0.56	2.79
H_2O_2 release	2	0.65	0.22	2.95
Glucose oxidation	3	0.23	0.20	1.15

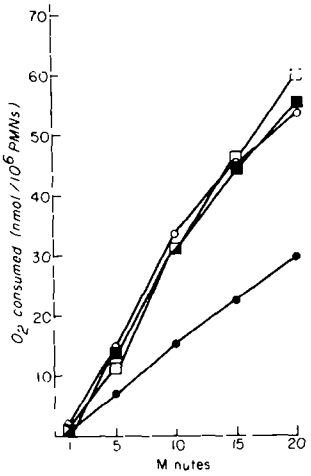


Fig. 1. Kinetics of oxygen consumption by neutrophils stimulated by fluid phase (circles) and solid phase (squares) haptenated liposomes. The oximeter chamber contained $4 \cdot 10^6$ neutrophils, 800 nmol of liposomal lipid, and approx. $1 \cdot 10^{-8}$ M specific antinitroxide antibody in 1.85 ml cell buffer. Measurements were made in the absence (open symbols) or presence (closed symbols) of 50 μM cytochrome *c*. PMN, polymorphonuclear neutrophil.

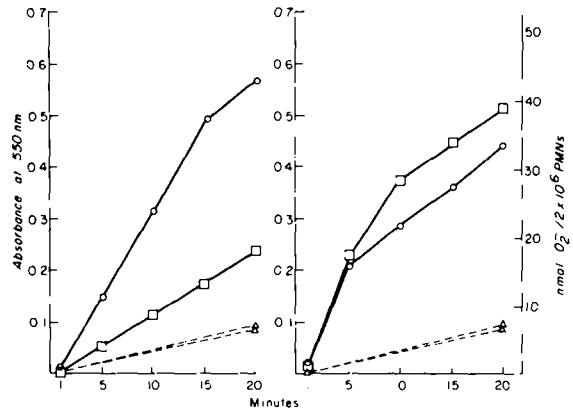


Fig. 2. Kinetics of superoxide release by neutrophils (PMN) stimulated by fluid phase (circles) and solid phase (squares) liposomes. Assay tubes contained $2 \cdot 10^6$ neutrophils, 200 nmol of liposomal lipid, approx. $1 \cdot 10^{-8}$ M specific antinitroxide antibody, and 50 μM cytochrome *c* in 1.0 ml cell buffer. Cells were preincubated for 15 min at 37°C in cell buffer in the absence (left panel) or presence (right panel) of cytochalasin B (7.33 μg/ml). Triangles represent valeus for unstimulated cells.

tion and reactions with other cellular consituents this latter case would be expected to result in little extracellular release of O_2^-.

In order to test this hypothesis, neutrophils were treated with cytochalasin B, to block phagocytosis, prior to stimulation with fluid and solid liposomes. The results of this experiment in which extracellular release of O_2^- was determined by reduction of oxidized cytochrome *c* are shown in Fig. 2. The left panel shows the results for the control experiment in which untreated neutrophils are stimulated with fluid and solid liposomes. As expected, the fluid liposomes cause the release of considerably more extracellular O_2^- than do solid liposomes. When cells from the same preparation are treated with cytochalasin B prior to stimulation with liposomes, the resultant O_2^- release is altered considerably. O_2^- release from these cells stimulated by solid liposomes is significantly increased. There is also a decrease in O_2^- released from those cells stimulated by fluid liposomes at later time points. This may be due to the inhibitory effect of cytochalasin B on glucose uptake. Consistent with this hypothesis is the fact that cyctochalasin B causes an equivalent decrease (approx. 30%) in the rate of oxygen consumption by cells stimulated with both fluid and solid liposomes (data not shown).

In order to obtain a more quantitative estimate of the amount of phagocytosis of fluid and solid liposomes, the following experiment was performed. Both types of liposomes were prepared as described in Methods, except that 1 mol% NBD-PE, a phospholipid containing a fluorescent headgroup, was incorporated into the liposomal membranes. The liposomes and antinitroxide antibodies were mixed and added to the neutrophils. The mixture was then acidified and the fluorescence intensity of the NBD-PE was determined before and after addition of $CuCl_2$ as described in the Materials and Methods section. In control experiments the acidification and measurement of fluorescence were performed immediately after addition of the liposomes to the neutrophils (zero time) in order to determine the total amount of liposomal fluorescence that could be quenched by the $CuCl_2$. The experimental values were determined by the same procedure after the liposomes and cells were incubated together for 15 min. The fractional decrease in the amount of fluorescence which could be quenched was interpreted as the fraction of liposomes which had been internalized

TABLE II

FLUORESCENCE QUENCHING ASSAY FOR DETERMINING THE DEGREE OF PHAGOCYTOSIS OF LIPOSOMES BY NEUTROPHILS

Each experiment represents a different preparation of neutrophils and liposomes. ΔI_0 is for controls, liposomes plus neutrophils quenched at zero time; ΔI_{15} is for liposomes plus neutrophils quenched at 15 min.

	ΔI_0 (% fluorescence loss)	ΔI_{15} (% fluorescence loss)	$(\Delta I_{15}/\Delta I_0)\times 100\%$	% of protected fluorescence via internalization by neutrophils
Expt. 1				
DMPC	7.80	7.60 ± 0.20	97	3.0
DPPC	9.90	7.60 ± 0.30	77	23.0
Expt. 2				
DMPC	7.50	7.05 ± 0.05	94	6.0
DPPC	7.23	4.83 ± 0.28	67	33.0

by the neutrophils and were thus protected from the fluorescence quenching effects of the $CuCl_2$. The results of two experiments, each with different cell and liposome preparations, are summarized in Table II. In the control experiments, 7–9% of the fluorescence was quenched upon addition of $CuCl_2$. This is in good agreement with other measurements of the fraction of total lipid which is exposed to the bulk aqueous phase using similar methods of liposomal preparation [9]. In addition, the fluorescence intensity of the liposomal preparation did not change over a 20 min period after addition of $CuCl_2$, indicating a very low permeability of Cu^{2+} through the liposomal membrane. Significantly less fluorescence associated with solid liposomes could be quenched after the cells and liposomes were incubated together for 15 min. This was not the case when fluid fluorescent liposomes were used as the stimulus. Measurement of O_2^- release and oxygen consumption by neutrophils stimulated with these fluid and solid fluorescent liposomes demonstrated that the presence of 1 mol% NBD-PE in the liposomal membranes had no measurable effect on the interaction of the liposomes with the neutrophil.

Discussion

The present study demonstrates that specific antibody-dependent phagocytosis of liposomes containing spin-label haptens is dependent on the physical state of the liposomal membrane. Specifically, liposomes comprised of lipids having a chain melting transition temperature above 37°C, solid liposomes (DPPC), are readily phagocytosed whereas similar liposomes having a chain melting transition temperature below 37°C, fluid liposomes (DMPC), are not detecably phagocytosed. Evidence to support this conclusion is 2-fold. First, studies in which the fluorescence of liposomes containing the fluorescent phospholipid NBD-PE was quenched by Cu^{2+} demonstrate that in a 15 min incubation with neutrophils up to 30% of the solid liposomes were phagocytosed as opposed to less than 5% phagocytosis of fluid liposomes under the same conditions. Secondly, we have examined mixtures of neutrophils and these fluorescent liposomes with a fluorescence microscope. When solid liposomes are incubated with neutrophils, many neutrophils can be identified with large numbers of internalized liposomes. Very few of these cells are found to have liposomes bound externally to the plasma membrane. In contrast, incubation of fluid liposomes with neutrophils shows most of the cells to have large numbers of externally bound liposomes, but no intracellular fluorescence is observed.

There are several possible reasons for this phenomenon. One possibiliy is that due to the aggregation of fluid liposomes in the presence of specific antinitroxide antibodies they might present themselves as a particle too large to the phagocytized.

Indeed, we have observed aggregates of fluid liposomes bound to neutrophils. On the other hand, the physical forces which hold fluid liposomes together in the presence of antibody are probalbly significantly weaker than the forces generated by the neutrophil's pseudopodia when surrounding an opsonized particle. This is supported by the observation that gentle vortexing of fluid liposomes in the presence of specific antibody disaggregates them. The fact that neutrophils can break cell-cell interactions when squeezing through vascular endothelium by extending pseudopodia between the endothelial cells argues that neutrophils should be capable of overcoming this aggregation phenomenon.

Studies on the phagocytosis of sheep erythrocytes by macrophages [15,16] demonstrated that a particle must be uniformly coated with antibody in order for phagocytosis to occur. In these studies it was concluded that phagocytosis is a sequential process (zipper mechanism) and not due to initial binding and triggering of the phagocytic cell to engulf the particle. In all probability the mechanism of phagocytosis by neutrophils and macrophages is quite similar. Presuming then that this process (zipper mechanism) occurs with neutrophils also, the possibility exists that the lateral diffusion of antibodies bound to fluid liposomes is sufficiently rapid to prevent the neutrophils' pseudopodia from surrounding the liposome. In the sequential process of binding the Fc portion of the antibody, the pseudopodia would not be able to anchor themselves before reaching toward the next Fc portion of a bound antibody. Any outward force on the liposome (e.g., due to cytoplasmic displacement by the liposome) would tend to result in a clustering of the antibody at the initial point of attachment between the neutrophil and the liposome. For a fluid liposome with a lateral diffusion constant of approx. $6 \cdot 10^{-8}$ cm^2/s [17], the average bound antibody would diffuse 2 μm in approx. 0.2 s. The time required for a neutrophil to entirely engulf a particle is approx. 30 s [18]; therefore, it is not unreasonable to presume that upon binding of a fluid liposome to the neutrophil, capping of antibody on the liposome would occur at the initial point of attachment. The phagocytosis of solid liposomes by neutrophils would not present this problem. On a solid liposome, with a lateral diffusion rate of $1 \cdot 10^{-11}$ cm^2/s [17], an antibody would require approx. 17 min to diffuse 2 μm. In comparison, then, to the phagocytic rate, the Fc portions of the bound antibodies would appear as stationary points of attachment to the neutrophil. This proposed mechanism of differential phagocytosis is illustrated in Fig. 3.

Finally, two additional points warrant discussion. First, the mechanism of fluorescence quenching of NBD-PE by Cu^{2+} is uncertain. Fluorescence inner filter effects and resonance energy transfer can be ruled out, since the absorbance of these solutions of Cu^{2+} in the region of NBD-PE absorption and emission are both below 0.004. More likely the Cu^{2+} ion is acting in a manner similar to other paramagnetic species such as niroxide spin labels, and results in fluorescence quenching by increasing the rate of intersystem crossing in the excited fluorphore [19]. This type of quenching interaction is very short-ranged, and can be abolished by separating the fluorophore from the Cu^{2+} by a distance as small as the thickness of a phospholipid bilayer.

Second, it is commonly assumed that O_2^- pro-

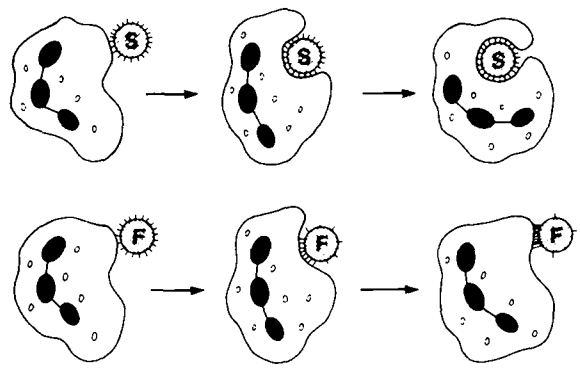


Fig. 3. Schematic illustration of proposed mechanism of differential phagocytosis of fluid (F) and solid (S) liposomes by neutrophils. In the top row, a neutrophil encounters an antibody-coated solid phase liposome and Fc receptor-antibody binding occurs. With time the neutrophil Fc receptors bind sequentially to the relatively immobile antibodies and the liposome is eventually engulfed. The bottom row depicts binding of the neutrophil to a fluid liposome. Initital attachment is similar to that of the solid liposome. Sequential binding of Fc receptors to antibodies which are very mobile in the plane of the liposomal membrane results in a clustering of antibodies at the point of attachment and phagocytosis does not occur.

duction by neutrophils, determined by cytochrome *c* reduction, yields a relatively direct measurement of the magnitude of the respiratory burst, and that this measurement is largely independent of the mode of stimulation (e.g., membrane perturbants versus phagocytic stimuli) [20]. The present study demonstrates, however, that the amount of O_2^- detected by this technique is strongly dependent on the degree of phagocytosis of the stimulus. Therefore great care should be exercised in using this assay to measure the relative ability of various stimuli to activate the respiratory burst.

Acknowledgements

This work was supported by NSF grant PCM 7922937 and NIH grant AI16810. We thank Dr. L. DeChatelet and Dr. D. Lyles for helpful discussion. Portions of this work were taken from a thesis submitted by M.M. in partial fulfillment of the requirements for the master's degree, Wake Forest University, Winston-Salem, NC.

References

1 Alving, C.R. and Richards, R.L. (1982) in The Liposomes (Olstro, M., ed.), Marcel Dekker, Inc., New York, in the press
2 Brûlet, P. and McConnell, H.M. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 2977–2981
3 Brûlet, P. and McConnell, H.M. (1977) Biochemistry 16, 1209–1217
4 Humphries, G.M.K. and McConnell, H.M. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 3537–3541
5 Esser, A.F., Bartholomew, R.M., Parce, J.W. and McConnell, H.M. (1979) J. Biol. Chem. 254, 1768–1770
6 Parce, J.W., McConnell, H.M., Bartholomew, R.M. and Esser, A.F. (1980) Biochem. Biophys. Res. Commun. 93, 235–242
7 Hafeman, D.G., Parce, J.W. and McConnell, H.M. (1979) Biochem. Biophys. Res. Commun. 86, 522–528
8 Cooper, A.D., Balakrishnan, K. and McConnell, H.M. (1981) J. Biol. Chem. 256, 9379–9381
9 Schwartz, M.A. and McConnell, H.M. (1978) Biochemistry 17, 837–840
10 Babior, B.M., Kipnes, R.S. and Curnutte, J.T. (1973) J. Clin. Invest. 52, 741–744
11 Van Gelder, B.F. and Slater, E.C. (1962) Biochim. Biophys. Acta 58, 593–595
12 Root, R.K., Metcalf, J., Oshino, N. and Chance, B. (1975) J. Clin. Invest. 55, 945–955
13 Hafeman, D.G., Lewis, J.T. and McConnell, H.M. (1980) Biochemistry 19, 5387–5394
14 DeChatelet, L.R. and Parce, J.W. (1981) in Methods for Studying Mononuclear Phagocytes (Adams, D.O., Edelson, P.J. and Koren, H., eds.), pp. 483–487, Academic Press, New York
15 Griffin, F.M., Griffin, J.A., Leider, J.E. and Silverstein, S.C. (1975) J. Exp. Med. 142, 1263–1282
16 Griffin, F.M., Griffin, J.A. and Silverstein, S.C. (1976) J. Exp. Med. 144, 788–809
17 Smith, L.M., Parce, J.W., Smith, B.A. and McConnell, H.M. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 4177–4179
18 Stossel, T.P., Mason, R.J., Hartwig, J. and Vaughan, M. (1972) J. Clin. Invest. 51, 615–624
19 Green, J.A., Singer, L.A. and Parks, J.H. (1973) J. Phys. Chem. 58, 2690–2695
20 Johnston, R.B. (1981) in Methods for Studying Mononuclear Phagocytes (Adams, D.O., Edelson, P.J. and Koren, H., eds.), p. 490, Academic Press, New York