Differences in the association between the oxidase-dependent activity and plasma membrane receptors for IgG, C3b and concanavalin A of human neutrophils

Jan Hed, Olle Stendahl and Tommy Sundqvist

Department of Medical Microbiology, Linköping University Medical School, S-581 85 Linköping, Sweden

Received 13 December 1982

Abstract not supplied

Neutrophil *Oxidase activity* *Phagocytosis* *Receptor*

1. INTRODUCTION

Neutrophil granulocytes (PMN) generate superoxide anions (O_2^-) as part of an important defense system [1]. The generation of O_2^- and other oxygen-derived metabolites ($OH^\cdot$, H_2O_2) are initiated at the surface level during particle–phagocyte interaction also in the absence of phagocytosis [2,3]. A metabolic burst occurs also when PMN are exposed to soluble stimuli such as concanavalin A (ConA) [2], formylated peptides [4], C5a [5,6], phorbol myristate acetate (PMA) [7] and fatty acids [8].

We have recently reported that IgG- and C3b-opsonized yeast (*Saccharomyces cerevisiae*) particles are ingested by different mechanisms [9,10]. IgG-coated yeast particles (yeast-IgG) were shown to be more potent stimuli than C3b-coated yeast particles (yeast-C3b) for inducing a metabolic activation in PMN at the initial contact with the plasma membrane. Here, we have further quantified and correlated the particle–PMN interaction to the metabolic response of the phagocytes by using 3 different particle-bound ligands (yeast-IgG, yeast-C3b and yeast-ConA).

2. MATERIALS AND METHODS

2.1. *Leukocyte preparation*

Human PMN were isolated after dextran sedimentation as in [11]. After separation, washing and hypotonic lysis of contaminating erythrocytes, the PMN were suspended in Krebs–Ringer phosphate buffer containing 10 mM glucose (KRG), pH 7.2. Trypan blue exclusion was used to assay viability.

2.2. *Preparation of yeast particles*

Heat-killed yeast cells (*S. cerevisiae*) were labelled with fluorescein isothiocyanate (FITC) (BBL, Cockeysville) as in [10,12] (0.1 mg FITC in 0.5 M carbonate buffer pH 9.5). The FITC-conjugated yeast particles (5×10^6 particles/ml) were then incubated with 25% normal human serum (NHS), rabbit anti-yeast IgG (14 μg/ml, prepared as in [10]), or ConA (250 μg/ml) (Pharmacia, Uppsala). After incubation at 37°C for 30 min the particles were washed in KRG and resuspended to the appropriate concentration in the same buffer. The particles were designated yeast-C3b, yeast-IgG, and yeast ConA according to the opsonizing ligand. The yeast-C3b particles have recently been characterized in detail [10]. All yeast preparations

Published by Elsevier Biomedical Press
00145793/83/0000–0000/$3.00

were counted and examined in a Bürker chamber immediately prior to the addition to the phagocytes. The amount of exposed IgG, C3b and ConA ligands on the surface of the yeast particles were quantified by staining with excess amount of FITC-conjugated sheep anti-rabbit IgG (F/P = 4.25) (Wellcome Lab., Beckenham), rabbit anti-human C3c (F/P = 2.2) (Behringwerke, Marburg) and rabbit anti-ConA (F/P = 3.0) (Cappel Lab., Cochranville), respectively. The fluorescence of individual yeast particles was quantified with cytofluorometry [13].

2.3. *Phagocytic assay*

5×10^6 PMN suspended in 3 ml KRG with 1% human serum albumin (AB Kabi, Stockholm) were allowed to adhere to the bottom of petri dishes (60 × 13 mm, Flow Lab., Irvine) for 45 min at 37°C. Non-adhering cells (59.7% ± 3.0 of added cells) were removed by washing, and 2 ml of KRG with 1% human serum albumin was added to the dishes containing a monolayer of over 95% PMN. One ml of prewarmed (37°C) FITC-labelled yeast particles were added to the dishes, which then were incubated on a rocking table for 30 min at 37°C. Three different concentrations (5×10^6, 2.5×10^6 and 1.25×10^6 particles/ml) of yeast particles were used to obtain different degrees of interactions to the PMN. At the end of incubation the supernatant was decanted and cold (0–4°C) KRG was added to the dishes. The recently described Fluorescence Quenching Method [12] was used to differentiate between attached and ingested particles. Immediately before microscopic examination the KRG was poured off and a few drops of crystal violet (0.8 mg/ml in 0.15 M NaCl) were added. The dye quenched the fluorescence of the extracellularly located FITC-labelled yeast particles, whereas the ingested ones remained fluorescent [12]. One hundred PMN were examined in duplicates using fluorescent and phase contrast microscopy (Zeiss incident light fluorescence microscope; Osram HBO 50; 2 × KP 470, Lp 455, Ft 510 and Lp 520 filters) and the number of attached and ingested particles were calculated.

2.4. *Superoxide (O_2^-) anion production*

Using the same monolayers of PMN as described above for the phagocytic experiments, superoxide-mediated cytochrome *c* (cyt *c*) reduction was assayed essentially as in [14]. After equilibration for 15 min at 37°C, 0.5 ml ferricytochrome *c* (final concentration 75 μM) (Sigma type IV, Sigma, St Louis) and 1 ml of yeast particles were added to parallel dishes. One set of dishes was kept on melting ice and used as blanks and the other incubated at 37°C on a rocking table. The supernatants were withdrawn after 30 min incubation and brought to 0°C, centrifuged (400 × *g*, 10 min) and assayed for the amount of reduced cyt *c* in a Beckman DU-2 spectrophotometer set at 550 nm. Specific O_2^--mediated cyt *c* reduction was monitored by assaying supernatants from reaction mixtures to which had been added 200 units/ml superoxide dismutase (SOD) (Sigma, St Louis). The amount of reduced cyt *c* was calculated using a millimolar extinction coefficient for cyt *c* of 15.5 at 550 nm [15].

2.5. *Cytochalasin B*

Cytochalasin B (Sigma, St Louis) was used in certain experiments to assay superoxide production in absence of phagocytosis [16]. Cytochalasin B was dissolved to 100 μg/ml in 2.5% dimethyl sulfoxide and used in final concentrations up to 10 μg/ml after dilution in KRG.

2.6. *Mathematical procedure*

A linear regression analysis was obtained with least-squares method [17] in an ABC 80 desk computer (Luxor AB, Motala).

3. RESULTS

3.1. *Relative quantitation of bound ligands on the yeast particle*

Relative quantitation of bound ligands on the yeast particle was assayed with fluorescent anti-IgG, anti-C3b, and anti-ConA antibodies. Table 1 shows that judging from the binding of the conjugates, ConA and C3b exposed more sites than did IgG/yeast particle. The quantitation on individual particles further shows that the number of ligands/yeast particle was homogenous.

3.2. *Localization of opsonized particles*

When phagocytic systems were exposed to a high concentration of non-opsonized yeast particles (7.5×10^6 particles/ml) the interaction was low (table 1) and no attempt was made to calculate the

Table 1

Quantitation of exposed IgG, C3c and ConA on yeast particles, using FITC-conjugated antibodies and cytofluorometry

	I[a]	SD	n[b]
Yeast-IgG	23.89	6.3	50
Yeast-C3c	54.53	13.3	50
Yeast-ConA	68.16	18.6	50

[a] Intensity expressed as uranyl units
[b] Number of particles measured

Table 2

The opsonizing effect of IgG, C3b and ConA on yeast particle[a]–PMN interaction

Particles	Association[b] (particles/ 100 PMN)	Ingestion (particles/ 100 PMN)	% Intra-cellular
Yeast	27	27	n.d.[c]
Yeast-C3b	366	256	69
Yeast-ConA	336	160	48
Yeast-IgG	200	184	92

[a] 7.5×10^6 particles/ml added
[b] Mean of 2 expt
[c] Not determined due to low interaction

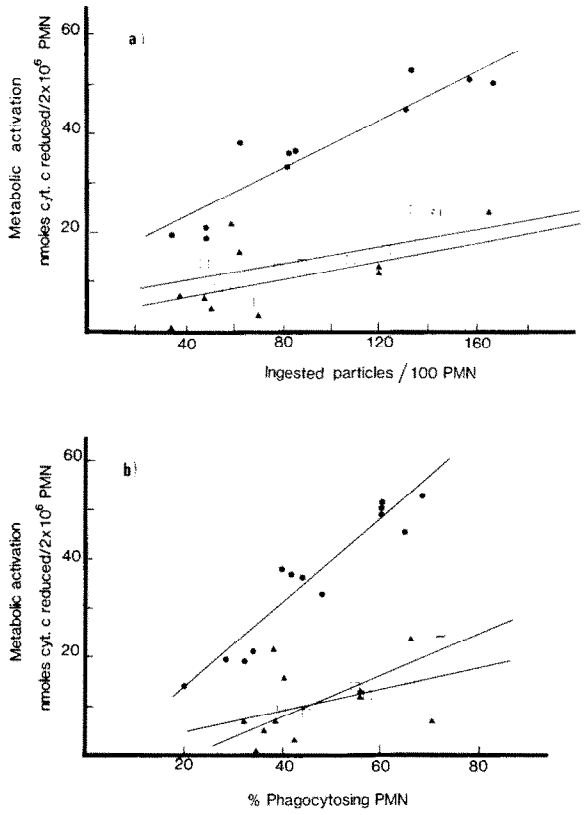

Fig.1. Correlation between metabolic activation and (a) number of ingested particles, or (b) percentage of phagocytosing PMN. (●) yeast-IgG; (▲) yeast-C3b; (□) yeast-ConA.

percentage of ingested particles. If the particles were preopsonized with anti-yeast IgG, human C3b or ConA, the interaction increased about 10-times (table 2) indicating an opsonizing effect of the bound ligands. When yeast particles interacted with monolayers of PMN only 69% of yeast-C3b and 48% of yeast-ConA were ingested as compared to 92% for yeast-IgG indicating an efficient internalization of IgG-opsonized particles.

3.3. *Correlation between interaction and metabolic response*

There was a good correlation ($r = 0.92$) between metabolic response and the number of ingested yeast-IgG, whereas no such correlation exists for ingested yeast-C3b ($r = 0.52$) and yeast-ConA ($r = 0.61$) (fig.1a). Moreover, yeast-IgG was strikingly more efficient than yeast-C3b and yeast-ConA in initiating a metabolic response. To produce the same superoxide anion release as 40 yeast-IgG/100 PMN, 160–170 yeast-C3b or yeast-ConA/100 PMN were required. A similar difference is apparent when the superoxide production is plotted against the number of phagocytosing PMN (fig.1b). 55–60% of the PMN have to ingest yeast-C3b or yeast-ConA to produce the same response as when 20% of the PMN have ingested yeast-IgG.

3.4. *Metabolic activation of cytochalasin B-treated PMN*

PMN were pretreated with cytochalasin B for 15 min before interacting with the particles to focus on the initial attachment step between the particles and PMN. A good correlation ($r = 0.84$) was observed between the number of interacting yeast-IgG and superoxide anion release. No such correlation could be seen with yeast-C3b and yeast-ConA since neither of these particles induced a metabolic

activation in cytochalasin B-treated PMN despite a good interaction with the PMN surfaces (fig.2). Only at extremely high concentrations of yeast-C3b and yeast-ConA could a metabolic response be detected. This correlation is not depicted, since quantitation of the number of particles/100 PMN is unreliable at these high concentrations.

4. DISCUSSION

Interaction of a stimulus with the PMN may induce a variety of manifestations including membrane polarisation, increased oxidative metabolism, release of platelet activating factor, granule enzymes, and arachidonic acid-derived metabolites (for review see [1] and [18]). It is, however, evident that some selectivity exists between different ligand–receptor interactions and activation [9,19].

This study confirms earlier results [10,20] showing a discriminatory effect of particles coated with IgG or C3b. Coating with C3b primarily promotes the attachment of the particles whereas IgG primarily affect the ingestion phase. Particles opsonized with ConA behave more like C3b-opsonization by being extracellularly localized to a high percentage.

We could show a correlation between the number of ingested IgG-coated yeast particles and the induced metabolic activation in both cytochalasin B and untreated PMN. These results indicate that the IgG-ligand of IgG-opsonized particles induce a metabolic activation in the PMN at the initial contact with the PMN plasma membrane. In contrast to IgG-coated particles, C3b- and ConA-coated particles showed no clear correlation between metabolic activation and the amount of interactions to PMN. Furthermore, C3b- and ConA-coated yeast particles did not induce a metabolic activation in cytochalasin B-treated PMN. This suggests that the metabolic activation induced by C3b- and ConA-coated particles is not induced at the initial contact with the PMN plasma membrane but is a phenomenon requiring involvement of a greater area of the membrane. Furthermore, it was shown that IgG-coated particles are 3–4 times as efficient as ConA- and C3b-coated particles to induce a metabolic activation, despite the fact that more C3b or ConA ligands are present on the particle (table 1). Although not determined in the present investigation, it is known from other studies that there are more Fc than C3b receptors on PMNL and macrophages [20,21]. How these receptors, determined with soluble ligands, relate to phagocytic recognition is, however, not clear.

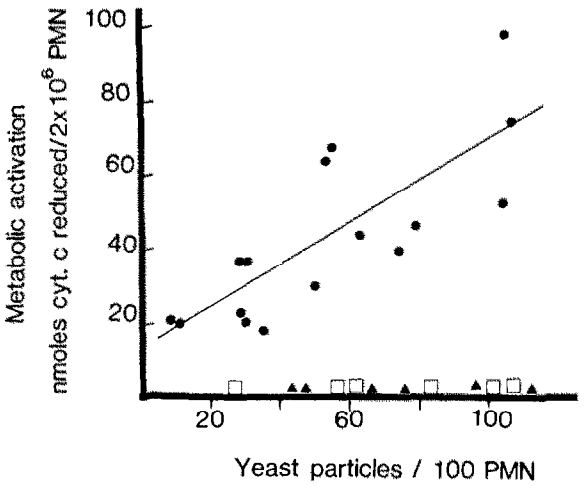

Fig.2. Correlation between metabolic activation in cytochalasin B-treated PMN and the number of interacting yeast particles. (●) yeast-IgG; (▲) yeast-C3b; (□) yeast-ConA.

These experiments rather favour the concept [9] that PMN cells possess different types of ingestion and activation mechanisms. One mechanism is represented by IgG-mediated interaction where the antibody molecules, exposing their Fc-portions, become available to interact with abundant Fc-receptors in the PMN membrane. The initial Fc-mediated interaction at the surface of the PMN is accompanied by a metabolic activation probably due to either interaction with specific Fc-receptors embedded in the lipid bilayer of the plasma membrane in close proximity with the oxidase activity [20], or the ability of the Fc-moiety to interact directly with lipid bilayers [23].

In contrast to IgG, no clear correlation was seen between C3b- and ConA-mediated attachment or ingestion and the metabolic activation. We therefore hypothesize that the receptor for C3b and the mannose residues recognized by ConA show a lower association to the oxidase activity of the plasma membrane probably due to association with more peripherally located glycoproteins.

ACKNOWLEDGEMENTS

We are grateful to Mrs Anita Lönn for skilled

technical assistance. This research was supported by grant B 81-16X-05968 from the Swedish Medical Research Council and grant 82-204 from the Swedish Society of Medical Science, which are gratefully acknowledged.

REFERENCES

[1] Klebanoff, S.J. and Clark, R.A. (1978) in: The Neutrophil: Functional and Clinical Disorders, pp.283–368, Elsevier Biomedical, Amsterdam, New York.
[2] Romeo, D.M., Jug, M., Zabucchi, G. and Rossi, F. (1974) FEBS Lett. 42, 90–93.
[3] Goldstein, I.M., Roos, F., Kaplan, H.B. and Weissmann, G. (1975) J. Clin. Invest. 53, 1662–1670.
[4] Seligmann, B., Chused, T.M. and Gallin, J.I. (1981) J. Clin. Invest. 68, 1125–1131.
[5] Goetzl, E.J. and Austen, K.F. (1974) J. Clin. Invest. 53, 591–599.
[6] Tedesco, F., Trani, S., Soranzo, M.R. and Patriarca, P. (1975) FEBS Lett. 51, 232–236.
[7] Repine, J.E., White, J.G., Clawson, C.C. and Holmes, B.M. (1974) J. Clin. Invest. 54, 83–90.
[8] Kakinuma, K. (1974) Biochim. Biophys. Acta 348, 76–85.
[9] Hed, J. (1981) Monographs Allergy 17, 92–111.
[10] Hed, J. and Stendahl, O. (1982) Immunology 45, 727–736.
[11] Bøyum, A. (1968) Scand. J. Clin. Lab. Invest. 21 (suppl. 97), 77–89.
[12] Hed, J. (1977) FEMS Microbiol. Lett. 1, 357–361.
[13] Sahlin, S., Hed, J. and Rundquist, I. (1983) Immun. Methods, in press.
[14] Curnutte, J.T. and Babior, B.M. (1974) J. Clin. Invest. 53, 1662–1672.
[15] Margoliash, E. and Frohwirth, N. (1959) Biochem. J. 71, 570–573.
[16] Davies, P. and Fox, R.I. (1973) Lab. Invest. 28, 16–22.
[17] Afifi, A.A. and Azen, S.P. (1972) in: Statistical Analysis. A Computer Oriented Approach, Academic Press, New York.
[18] Weissmann, G. (1980) The Cell Biology of Inflammation, Handbook of Inflammation 2. Elsevier Biomedical, Amsterdam, New York.
[19] Newman, S.L. and Johnston, R.B. Jr. (1979) J. Immunol. 123, 1839–1846.
[20] Yagawa, K., Onuoe, K. and Aida, Y. (1979) J. Immunol. 122, 366–373.
[21] Alexander, E.L., Titus, J.E. and Segal, D.M. (1979) J. Immunol. 123, 295–302.
[22] Arhaout, M.A., Melamed, J., Tack, B.F. and Colten, H.R. (1981) J. Immunol. 127, 1348–1354.
[23] Tagesson, C., Magnusson, K.-E. and Stendahl, O. (1977) J. Immunol. 119, 609–613.