PHAGOCYTIC INTERNALIZATION AND THE REQUIREMENT FOR MEMBRANE PERTURBATION

O. STENDAHL, J. HED, E. KIHLSTRÖM, K.-E. MAGNUSSON and C. TAGESSON
Department of Medical Microbiology, University of Linköping, S-581 85 Linköping, Sweden

Received 14 June 1977

1. Introduction

Rough (R) mutants of *Salmonella typhimurium* are more liable to associate to polymorphonuclear leukocytes (PMNL) than are the parent smooth (S) bacteria [1]. Since the difference is evident also in the absence of serum, we have suggested that the association is guided by general surface properties of the bacteria, such as negative surface charge and liability to hydrophobic interaction [2]. We have also found that physicochemical surface characteristics similar to that of R bacteria are brought about on S bacteria opsonized with IgG antibodies and complement as assessed by aqueous biphasic partitioning [3] and hydrophobic interaction chromatography (unpubl. obs.). Furthermore, *S. typhimurium* 395 MS opsonized with IgG antibodies perturb liposomal membranes, whereas non-opsonized or $F(ab')_2$-sensitized bacteria do not [4]. These and other [5,6] observations suggest that phagocytic recognition and attachment are mediated not via specific PMNL surface 'receptors' but rather via aspecific affinity for lipid surfaces.

It is not known, however, whether and how these general physicochemical surface properties determine also the eventual internalization into phagocytes. A widely accepted concept is that phagocytic ingestion requires a perturbation of the phagocytic cell membrane and a molecular mechanism providing transduction of the so generated signal to a biochemical target [7–9]. We show here that although opsonized *S. typhimurium* 395 MS and non-opsonized *S. typhimurium* 395 MR10 are internalized into PMNL to the same extent, only the opsonized bacteria perturb liposomal membranes and elicit PMNL superoxide production. We therefore suggest that although the outcome depends on general surface properties of the prey, internalization of different particles may be accomplished through different mechanisms.

2. Materials and methods

2.1. *Bacteria*

The bacteria (*S. typhimurium* 395 MS and MR10) were grown overnight, harvested, heat killed and labelled with ^{51}Cr [1]. Anti-MS IgG was separated from rabbit immune serum on diethylamino-ethyl-cellulose (DEAE) [10]. For opsonization, equal volumes of heat-killed bacteria (5×10^9/ml) and IgG (300 μg/ml) in phosphate-buffered saline (PBS), pH 7.2, were incubated at 37°C for 30 min, centrifuged and washed twice in PBS.

2.2. *Cells*

PMNL were collected from the peritoneal cavity of guinea pigs 18 h after injection of 25 ml 0.2% glycogen solution [1]. The cells were harvested, washed and suspended to the desired concentration in Krebs-Ringers phosphate buffer with 5 mM glucose (KRG), pH 7.2.

2.3. *Liposomes*

Liposomes with trapped 4-methylumbelliferylphosphate were prepared from phosphatidylcholine, cholesterol and dicethylphosphate as described elsewhere [4].

North-Holland Publishing Company – Amsterdam

2.4. *Phagocytosis and superoxide production*

The phagocytosis system with PMNL adhering to cellulose acetate filters has been described elsewhere [1]. That internalization was measured in this assay was checked either by inhibiting the association with cytochalasin B (10 μg/ml) (Sigma Chemical Co.) or by counting the number of fluorescein isothiocyanate (FITC)-labelled bacteria not extinguished by crystal violet [12]. Superoxide-mediated cytochrome *c* (cyt. *c*) reduction was assayed using a modification of the method described by Curnette and Babior [13]. Ten million PMNL were allowed to adhere to the bottom of tissue culture plastic petri dishes (50 × 13 mm) (Flow laboratories). To parallel dishes were then added 75 μM horse heart ferri-cytochrome *c* (type III, Sigma Chemical Co.) 2 × 10^9 bacteria and KRG to final vol. 4 ml. One was incubated at 37°C and the other kept on melting ice and used as a blank. After 60 min the supernatant was withdrawn, centrifuged (400 × *g*, 10 min) and assayed for reduced cyt. *c* in a Beckman DU-2 spectrophotometer set at 550 nm. Specific O_2^--mediated cyt. *c* reduction was monitored by assays of supernatants from reaction mixtures to which has been added 200 IU superoxide dismutase (SOD) (Sigma Chemical Co.). ΔA_{mM} (ferro-cytochrome *c* minus ferri-cytochrome *c*) at 550 nm was taken as 15.5 [14].

2.5. *Perturbation of liposomes*

To a pellet of 5 × 10^8 bacteria was added 0.5 ml liposome dispersion and the bacteria suspended with a Vortex mixer. After incubation at 37°C, 50 μl samples were withdrawn and 450 μl 150 mM NaCl–20 mM Tris, pH 7.5, and 500 μl 20 mM glycine NaOH, pH 9.8, with 10 μg alkaline phosphatase was added (EC 3.1.3.1, Sigma Type I). The amount of released UMP was then determined and expressed as percent of the trapped [4].

3. Results and discussion

Figure 1 shows that nonopsonized *S. typhimurium* 395 MS are not phagocytosed by guinea pig PMNL. Accordingly, no stimulation of superoxide production or perturbation of liposomes occurred. On the other hand, the physicochemically similar particles, opsonized *S. typhimurium* 395 MS and nonopsonized MR10, were equally well phagocytosed but only the IgG-coated MS bacteria elicited any detectable superoxide production or caused release of UMP from the liposomes. That the difference in O_2^--production is due to inefficient trapping of O_2^- in the presence of MR10 bacteria is less likely, since the requirement for cytochrome *c* is similar in the two systems. Furthermore, the possible influence of the soluble O_2^--stimulating factors released from bacteria incubated with serum [13] was eliminated by using preopsonized particles.

These data indicate that physicochemically similar

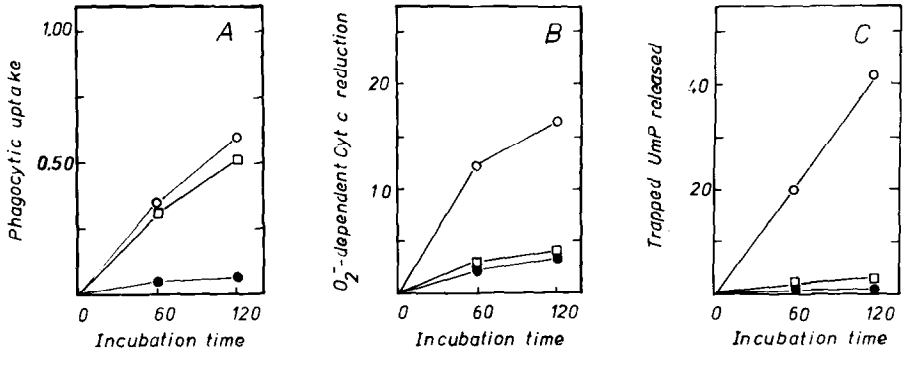

Fig.1. The effect of IgG-opsonized *S. typhimurium* 395 MS and unopsonized MR10 bacteria on the phagocytic uptake (A) and O_2^--dependent cyt. *c* reduction (B) in guinea pig PMNL and liposomal perturbation (C). Phagocytic uptake is expressed as percent of added bacteria phagocytosed; O_2^--dependent cyt. *c* reduction is expressed as nmol cyt. *c* reduced/4 × 10^6 PMNL and liposomal perturbation as percent trapped UMP released. Symbols: (○) *S. typhimurium* MS + IgG, (●) *S. typhimurium* 395 MS and (□) *S. typhimurium* 395 MR10.

particles that encounter the phagocytic cell membrane, may be internalized through different mechanisms, and may not require membrane perturbation to the same extent. The similarities between O_2^--production and release of UMP from liposomes support the hypothesis that the receptor site for the opsonized bacterial surface is the phospholipid bilayer-specific Fc and C3 activity may in fact reflect differences in binding strength, thus leading to variations in membrane perturbation, rather than different receptor sites per se.

A dissociation of phagocytosis and its associated metabolic changes has been found in other systems. Tsan and McIntyre [15] recently reported that neuraminidase-treated human PMNL did not produce O_2^- but still phagocytosed latex particles. The phagocytic uptake of opsonized *Staphylococcus aureus* bacteria was also intact [16], but the subsequent killing was impaired after neuraminidase treatment. Analogously, Weening et al. [17] reported that in a patient with recurrent infections due to ineffective bactericidal activity in his leukocytes, opsonized and nonopsonized latex particles were equally well phagocytosed, but only opsonized ones stimulated the oxidative metabolism. Colchicine [18] and phenylbutazone [19] while not inhibiting phagocytosis, depress the respiratory stimulation and hexose monophosphate activity in phagocytosing PMNL. Michl et al. [20] and Baehner et al. [21] showed that 2-deoxyglucose inhibited primarily opsonin-dependent phagocytosis, thus indicating that Fc and C3 mediated phagocytosis differ qualitatively from the ingestion of latex or zymosan. Our observation suggest that although being physicochemically similar and equally well recognized by the phagocyte, only bacteria coated with IgG molecules induce membrane perturbation measured as O_2^--production and release of UMP from liposomes. Thus, the cell surface perturbation of PMNL during phagocytosis is not an all or none phenomenon [22] – vital functions of these cells may be mediated or modulated independently by opsonized and nonopsonized particles although they are physicochemically similar. These properties would be appropriate for the influence of leucocytes in the inflammatory response.

Acknowledgements

The technical assistance of Ellinor Granström and Britinger Thorén is gratefully acknowledged. The study was supported by grant 16X-2183-11 from the Swedish Medical Research Council.

References

[1] Stendahl, O. and Edebo, L. (1972) Acta Path. Microbiol. Scand. Sec. B80, 481–488.
[2] Magnusson, K.-E., Stendahl, O., Tagesson, C., Edebo, L. and Johansson, G. (1977) Acta Path. Microbiol. Scand. In press.
[3] Stendahl, O., Tagesson, C., Magnusson, K.-E. and Edebo, L. (1977) Immunol. 32, 11–18.
[4] Tagesson, C., Magnusson, K.-E. and Stendahl, O. (1977) J. Immunol. in press.
[5] Van Oss, C. J. and Gillman, C. F. (1972) J. Reticuloendothel. Soc. 12, 497–502.
[6] Weissmann, G., Brand, A. and Franklin, E. C. (1974) J. Clin. Invest. 53, 536–543.
[7] Patriarca, P., Cramer, R., Moncalvo, S., Rossi, F. and Romeo, D. (1971) Arch. Biochem. Biophys. 145, 255–261.
[8] Rossi, F. and Zatti, M. (1968) Biochim. Biophys. Acta 153, 269–272.
[9] Romeo, P., Cramer, R. and Rossi, F. (1970) Biochem. Biophys. Res. Commun. 41, 582–588.
[10] Stendahl, O., Tagesson, C. and Edebo, L. (1974) Infect. Immun. 10, 316–319.
[11] Dahlgren, C., Kihlström, E., Magnusson, K.-E., Stendahl, O. and Tagesson, C. (1977) Exp. Cell Res. in press.
[12] Hed, J. (1977) FEMS Microbiol. Lett. 1, 357–361.
[13] Curnette, J. T. and Babior, B. M. (1974) J. Clin. Invest. 53, 1662–1672.
[14] Margoliash, E. and Frohwirt (1959) Biochem. J. 71, 570–572.
[15] Tsan, M. F. and McIntyre, P. A. (1976) J. Exp. Med. 143, 1308–1316.
[16] Tsan, M. F., Douglass, K. H. and McIntyre, P. A. (1977) Blood 49, 437–444.
[17] Weening, R. S., Roos, D., Homan-Müller, J. and Van Schaik, M. (1976) Agents Actions, 6, 281.
[18] Malawista, S. E. and Bodel, P. T. (1967) J. Clin Invest 46, 786–796.
[19] Strauss, R. R., Paul, B. B. and Sbarra, A. J. (1968) J. Bactiol. 96, 1982–1992.
[20] Michl, J., Ohlbaum, D. J. and Silverstein, S. C. (1976) J. Exp. Med. 144, 1484–1493.
[21] Baehner, R. I., Boxer, L. A. and Davis, J. (1976) J. Cell Biol. 70, 1059.
[22] Goldstein, I. M., Kaplan, H. B., Radin, A. and Frosch, M. (1976) J. Immunol. 117, 1282–1287.