Vol. 113, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 31, 1983 Pages 192-198

DOES THE INDUCTION OF MACROPHAGE LYSOSOMAL ENZYME SECRETION BY ZYMOSAN INVOLVE THE MANNOSE RECEPTOR?

Judith L. Bodmer and Roger T. Dean

Cell Biology Research Group, Department of Applied Biology, Brunel University, Uxbridge, Middlesex, U.K.

Received March 28, 1983

Secretion of the lysosomal enzyme hexosaminidase induced by zymosan is inhibited by mannose and by high concentrations of mannose-6-phosphate and 2-deoxy-glucose but not by mannan. Secretion of hexosaminidase from cells storing previously endocytosed zymosan is unaffected by mannose. Exposure of the cells to mannose does not affect secretion caused by subsequent exposure to zymosan. These findings suggest a role for the mannose-glycoprotein receptor in initiation of lysosomal enzyme secretion by zymosan.

It is well known that macrophages can be induced to secrete lysosomal enzymes by a wide variety of agents (1). The close correlation between the ability of some stimuli to cause secretion of lysosomal hydrolases by macrophages *in vitro* and to cause inflammation *in vivo* suggests that macrophages may be important in chronic inflammatory lesions, and has lead to an interest in the mechanisms by which such stimuli may induce secretion. One such stimulus known to induce secretion is zymosan (2); little is known of the mechanisms by which zymosan causes secretion although it is known that macrophages continue to secrete when storing zymosan intracellularly (3) and that such cells can be induced to secrete further by an independent stimulus (4,5).

Macrophages have been shown to possess receptors for sugar termini of glycoproteins (6,7) and there is convincing evidence that macrophages interact with zymosan by the mannose/N-acetylglucosamine glycoprotein receptor and that this interaction is susceptible to competition with mannose (8,9,10).

In this study we have examined the effect of mannose and other compounds which act on the mannose/N-acetylglucosamine receptor on secretion of the lysosomal enzyme hexosaminidase induced by zymosan.

0006-291X/83 $1.50

Methods

The collection and purification of mouse peritoneal macrophages were as described previously (3). The cells were maintained in DMEM plus 10% (v/v) heat-inactivated pig serum, 100 I.U./ml penicillin and 100ug/ml streptomycin. Mannose, mannose-6-phosphate, glucose and mannan were prepared as concentrated (x100) stock solutions in phosphate buffered saline (PBS), filter sterilised and diluted into medium as required. 2-deoxy-glucose was prepared directly into the medium which was then filter sterilised. Zymosan was prepared as a 5mg/ml stock solution in PBS, sonicated briefly before each use and then diluted into culture medium. At the beginning of each experiment the cell cultures were washed four times with PBS to remove traces of serum and then fresh medium without serum applied with or without various stimuli as appropriate. At the end of the incubation the medium was collected and the cells lysed in 1.5ml PBS containing 0.1% Triton-X-100 and scraped off with a silicone rubber bung. In experiments where secretion in cells previously exposed to zymosan or mannose was to be measured the cells were first washed and then exposed to zymosan (50ug/ml) or mannose (100mM) in serum-free conditions for two hours. The cell sheets were then washed four times with PBS and fresh medium (without serum) and stimuli applied. At the end of the experiment the cells were lysed as descibed above. Triplicate cultures were used in all experiments, results are given as means $\pm$ s.d. and are representative of several experiments. Lactate dehydrogenase (E.C. 1.1.1.27) and N-acetyl-β-D-glucosaminidase (hexosaminidase, E.C. 3.2.1.30) were assayed as described elsewhere (3).

Results and Discussion

Previous reports have suggested that mannose residues are important in zymosan binding to macrophages (8), and might therefore affect secretion induced by zymosan. Such an effect of mannose on secretion of hexosaminidase induced by zymosan is shown in Figure 1. There is a dose-dependent inhibition of secretion at concentrations greater than 20mM. The total hexosaminidse activity remains constant at around 1300nmoles/culture/hour indicating that mannose is affecting distribution of the enzyme rather than its synthesis.This data provides evidence for the involvement of the mannose/N-acetylglucosamine glycoprotein receptor in initiation of secretion of lysosomal enzymes.

However, it is possible that mannose could exeert its effect from within the cell rather than by any effect on the cell surface. Figure 2 shows the effect of previous exposure to mannose on secretion induced by zymosan. The cells pre-exposed to mannose respond to zymosan in the same way as untreated cultures suggesting that mannose exerts its effect on the cell surface rather than from within the cell. Further evidence for this suggestion comes from the observation that secretion from cells storing

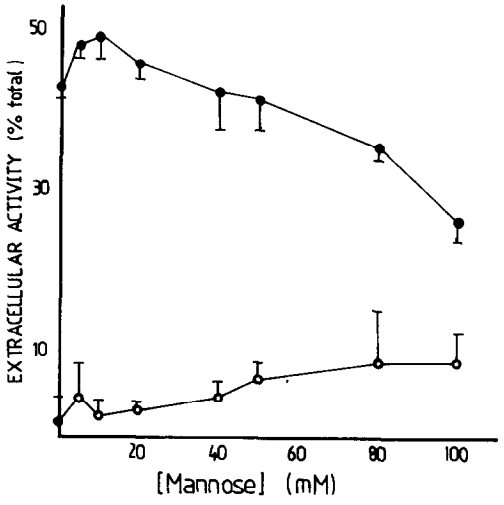

Figure 1. Release of Hexosaminidase during Exposure of Macrophages to Mannose
Mouse peritoneal macrophages were incubated for three hours in DMEM plus antibiotics in serum-free conditions. Zymosan at 50ug/ml and mannose at the concentrations indicated were also present in the medium. The cultures were incubated at 37°C and gassed with 5% Co_2 in air.
●, hexosaminidase; ○, lactate dehydrogenase.

zymosan is not affected by the addition of mannose (Figure 3). It therefore seems likely that mannose is exerting its inhibitory effect on secretion on the cell surface, probably by blocking the mannose/N-acetyl-

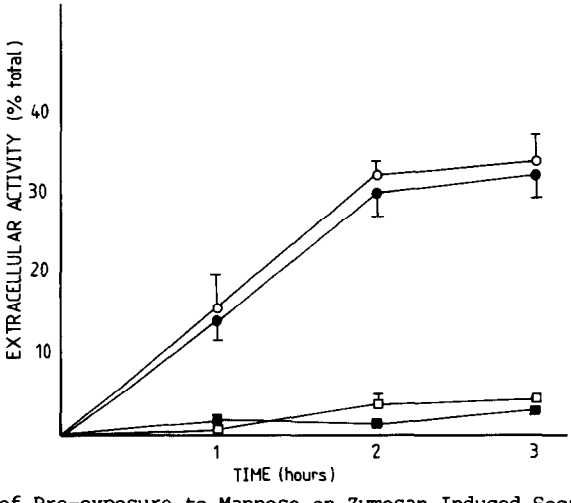

Figure 2. Effect of Pre-exposure to Mannose on Zymosan Induced Secretion
Mouse peritoneal macrophages were incubated in DMEM plus antibiotics and 100mM mannose for two hours. The cultures were then washed four times with PBS and re-incubated in fresh medium containing 50ug/ml zymosan. At each time point media and cells were harvested and assayed separately.
●, hexosaminidase
Cultures pre-exposed to mannose
■, lactate dehydrogenase
○, hexosaminidase
Control cultures
□, lactate dehyrogenase

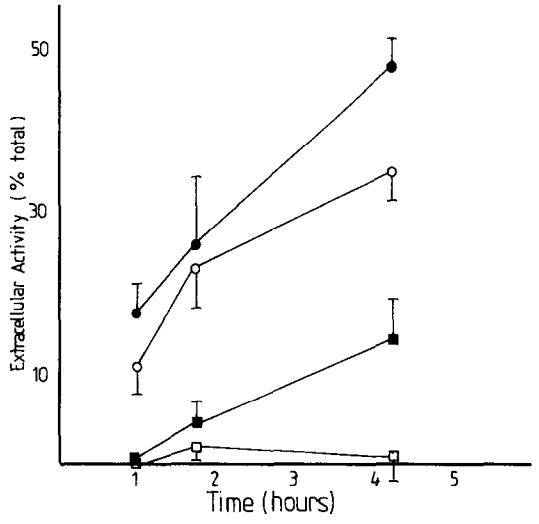

Figure 3. Effect of Mannose on Hexosaminidase Secretion From Cells Storing Zymosan
Mouse peritoneal macrophages were incubated in DMEM plus antibiotics and 50ug/ml zymosan for two hours. The cultures were then washed four times with PBS and re-incubated in fresh medium with or without 100mM mannose. At each time point cells and media were harvested and assayed separately.
●, hexosaminidase
Cultures with mannose
■, lactate dehydrogenase
○, hexosaminidase
Cultures without mannose
□, lactate dehydrogenase

glucosamine receptor. This suggests that this receptor participates in the mechanism of induction of secretion by zymosan.

We have studied the effects of several related sugars and the polysaccharide mannan on secretion (Table 1). At the concentrations used (2.5mg/ml) mannan was ineffective at inhibiting secretion; at this concentration mannan can inhibit binding of glycoproteins to macrophages (11), however higher concentrations may be needed to compete with materials such as zymosan which present a very complex array of ligands to the receptor (7). Consistent with this it has been shown that much higher concentrations of normal Saccharomyces cerevisiae mannan are required to inhibit ingestion of zymosan (9; and our unpublished observations). In view of this lack of inhibition of ingestion the observed lack of effect on secretion is to be expected.

A variety of cells possess receptors for the mannose-6-phophate termini of glycoproteins (12), therefore we tested mannose-6-phosphate to

TABLE 1

EFFECT OF VARIOUS COMPOUNDS ON LYSOSOMAL ENZYME SECRETION.

STIMULUS	EXTRACELLULAR ACTIVITY (% TOTAL)	
	HEXOSAMINIDASE	LDH
Control (zymosan only)	36.8 ± 8.0	2.1 ± 3.2
Mannan (2.5mg/ml)	38.0 ± 0.6	5.4 ± 2.5
Mannose-6-phosphate (5mM)	41.5 ± 4.1	3.7 ± 6.4
2-deoxy-glucose (100mM)	0.5 ± 0.4	3.5 ± 1.1
Glucose (100mM)	36.8 ± 1.2	5.4 ± 1.3
Control (zymosan only)	49.0 ± 1.2	0.8 ± 0.4
Mannose-6-phosphate (50mM)	37.3 ± 4.6	3.7 ± 0.9

Cultures were incubated for three hours in DMEM plus antibiotics, 50ug/ml zymosan and various compounds as indicated. (EMEM was substituted when 2-deoxy-glucose was used to maintain glucose:2-deoxy-glucose at 1:20 which is the ratio previously reported to produce maximum inhibition of phagocytosis (13)). Cells and media were harvested and assayed separately. Secretion is expressed as extracellular avctivity as a percentage of the total activity in the cultures.

see if zymosan might also interact with this receptor. Mannose-6-phosphate had no effect on secretion at concentrations (5mM) which are highly effective at preventing glycoprotein binding to the manose-6-phosphate receptor (12). At very high concentrations (50mM) some inhibition of secretion was detectable but there is no evidence to indicate that this results from an interaction with the mannose-6-phosphate glycoprotein receptor. Indeed, the evidence of Sun-Sang et al (9) makes this very unlikely and it is probable that the mannose-6-phosphate is also interacting with the mannose/N-acetylglucosamine glycoprotein receptor.

Finally we tested the effect of 2-deoxy-glucose and glucose. 2-deoxy-glucose has been reported to inhibit the phagocytosis of a whole array of particles by mouse macrophages (13). These authors have noted that 2-deoxy-glucose is more effective in inhibiting uptake of opsonised particles than unopsonised particles but even in the brief experiments they reported there is evidence of inhibition of uptake of uncoated particles. In agreement with this we found that 2-deoxy-glucose (100mM in

the presence of 5mM glucose) is the most potent inhibitor of secretion induced by zymosan. Glucose itself has no effect; this also indicates that the observed inhibitory effects are not simply due to the high sugar concentrations being used.

Because of the various inhibitory effects of 2-deoxy-glucose on matabolic processes (14) and the range of opsonised and non-opsonised particles whose uptake is inhibited it seems unlikely that 2-deoxy-glucose is acting specifically on the mannose/N-acetylglucosamine glycoprotein receptor. Thus the inhibition of phagocytosis by 2-deoxy-glucose and its inhibition of secretion (Table 1) are probably secondary consequences of some complex metabolic peterbation of the cells.

Altogether the data on the effect of mannose and related compounds on lysosomal enzyme secretion indicate that zymosan may initiate secretion via the mannose/N-acetylglucosamine glycoprotein receptor.

The intracellular signal which initiates secretion induced by zymosan is not known, nor is the biochemical sequence of events which follows. It is not clear whether zymosan initiates its effects while bound to the cell surface receptor or immediately after its endocytosis for while surface binding can be inhibited by agents such as mannose, endocytosis is only inhibited after short periods of time e.g. after 30 minutes of uptake (9); after longer periods of time (3 hours) mannose-treated cells phagocytose as much zymosan as do their untreated counterparts (unpublished observations). This work provides some evidence that the mannose/N-acetylglucosaminidase glycoprotein receptor may be important and further work will be directed towards identification of the exact role of this receptor.

Acknowledgments

JLB thanks the SERC for a CASE studentship. This work was also supported by the Arthritis and Rheumatism Research Council and the Medical Research Council.

References

1. Davies, P., and Allison, A. C. (1976) Immunobiology of The Macrophage, pp. 428-461, Academic Press, New York.

2. Davies, P., and Allison, A. C. (1976) Lysosomes in Biology and Pathology Vol. 5, pp. 61-98, North Holland Publishing Company, Amsterdam.
3. Dean, R.T., Hylton, W., and Allison, A.C. (1979) Biochim. Biophys. Acta 584, 57-65.
4. Jessup, W., Leoni,P., Bodmer, J.L., and Dean, R.T. (1982) Biochem. Pharmacol. 31, 2657-2662.
5. Riches, D.W.H., and Stanworth, D.R. (1982) Biochem. J. 202, 639-645.
6. Stahl, P.D., Rodman, J.S., Miller, M.J., and Schlesinger, P.H. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 1339-1403.
7. Lee, Y. C., and Lee, R. T. (1982) The Glycoconjugates Vol. IV, pp.57-84, Academic Press, New York.
8. Warr, G.A. (1980) Biochem. Biophys. Res. Commun. 93, 737-
9. Sun-Sang, Sung, J., Nelson, R.S., and Silverstein, S.C. (1983) J. Cell Biology 96, 160-166.
10. Bodmer, J.L., and Dean, R.T. (1983) Biochem. Soc. Trans. (in press).
11. Townsend, R., and Stahl, P. (1981) Biochem. J. 194, 209-214.
12. Sly, S. (1982) The Glycogonjugates Vol IV, pp. 3-26, Academic Press, New York.
13. Michl, J., Ohlbaum, D.J., and Silverstein, S.C. (1976) J. Exp. Med. 144, 1465-1485.
14. Michl, J., Ohlbaum, D.J., and Silverstein, S.C. (1976) J. Exp. Med. 144, 1484-1493.