Glucan receptor and zymosan-induced lysosomal enzyme secretion in macrophages

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A receptor for β -glucan was in the present study shown to mediate binding of zymosan particles to resident mouse peritoneal macrophages. Lysosomal enzyme secretion in response to zymosan was maximal at a low particle/cell ratio, continuous for at least 3 h after particle/cell contact and inhibitable by soluble glucan. Latex particles of various size caused no selective secretory response, but at high particle/cell ratios were toxic. By use of a fluorescent ligand, the macrophage β -glucan receptor

was shown to be trypsin-sensitive, $\text{Ca}^{2+}/\text{Mg}^{2+}$ -independent, recirculating and also present in an intracellular mobilizable pool. Binding of ligand to the β -glucan receptor and inhibition of the lysosomal secretory response to zymosan were both more efficient with glucans of larger size, indicating that clustering of glucan receptors at the cell surface occurs. Such clustering could stabilize ligand binding by multiple interactions and possibly trigger intracellular signalling events on binding of zymosan particles.

INTRODUCTION

The outcome of inflammatory reactions is highly dependent on several macrophage activities such as the generation of eicosanoids and cytokines, the processing and presentation of antigen and the secretion of lysosomal hydrolases [1]. The latter may act to aggravate an inflammatory condition by causing tissue damage, but may also be beneficial by contributing to tissue remodeling. In the acid environment of some inflammatory foci [2,3], secreted degradative enzymes could be operative, in particular over a short distance, in the acid milieu created near activated macrophages [4]. Macrophages have been shown to secrete lysosomal enzymes in response to various particulate stimuli, which when injected into experimental animals induce chronic inflammatory lesions [5,6]. A relationship between the pathogenesis of such lesions and lysosomal enzyme release has been hypothesized, and inert particles, such as latex beads, induce neither secretion nor inflammation.

The mechanisms for and regulation of the secretion of lysosomal contents in response to particulate agents are not known, but are considered to differ from those of the secretory response to soluble stimuli [7,8]. At an inflammatory site, causative agents and cell debris are phagocytosed by a process, the regulation of which is as incompletely understood as the control of the secretory response and the fate of the forming phagosome. Secretion induced by phagocytosable stimuli could occur after the fusion of phagosomes with the lysosomal compartment, a process known to be inhibited by some intracellular pathogens [9]. In this case, expulsion of partially degraded phagosomal contents would be likely to parallel the secretion of lysosomal enzyme, and a relationship between such a process and antigen processing and presentation would be possible. On the other hand, phagocytosis is not a necessary prerequisite for lysosomal enzyme secretion, and a secretory response may occur parallel to or totally independently of phagosome–lysosome fusion. Intracellular signalling initiated by receptor occupancy could then lead to fusion of lysosomes with the plasma membrane without a need for phagocytic uptake of the particle.

Zymosan particles are yeast cell derivatives with an average

particle diameter of 3 μm composed mainly of the carbohydrate polymers glucan and mannan [10]. Attachment of zymosan particles to mouse macrophages has been suggested to be mediated by receptors for complement components (by interaction either with zymosan-bound C3bi or by a glucan-binding site on CR3), mannan and/or β -1,3-glucan [11–14]. It is possible that several types of receptor could mediate binding [15] and different cellular responses could be triggered by binding to specific receptors. Redundancy of receptors mediating the clearance of a specific pathogen could allow regulation of host response by modulation of cell surface receptors. Cell activation is necessary for a phagocytic response to ligation of CR3 by C3bi [16]. However, such activation is not necessary when the glucan-binding site on CR3 mediates binding [12]. Characteristics of the macrophage receptor for β -glucan and the secretion of lysosomal contents in response to zymosan particles are examined in the present paper.

MATERIALS AND METHODS

Materials

4-Methylumbelliferyl *N*-acetyl- β -D-glucosaminide, NADH, BSA, mannan, glucan (from baker's yeast) and zymosan were purchased from Sigma. Polystyrene latex beads were from either Sigma (0.8, 3.0 and 11.9 μm) or Polysciences, Warrington, Cheshire, U.K. (2.0, 4.0 and 6.0 μm). Unlabelled glucan (MolPro-glucan), fluorescein-conjugated glucan (F-MolPro-glucan), dextran (Texas Red conjugate, M_r 40000) and dextran (fluorescein and tetramethylrhodamine conjugate, M_r 10000) were from Molecular Probes, Eugene, OR, U.S.A. Trypsin and all material for cell culture were purchased from Flow Laboratories.

Experimental medium

Na^+ -based solution with nominal bicarbonate (Na-medium) contained: NaCl, 127 mM; KH_2PO_4 , 1.2 mM; KCl, 5.4 mM; MgSO_4 , 0.8 mM; CaCl_2 , 1.8 mM; glucose, 5.6 mM; Hepes,

10 mM. Adjustment of pH to the indicated value was performed at 37 °C.

Macrophage culture and stimulation

Resident cells were harvested from female outbred NMRI mice (ALAB, Stockholm, Sweden or Bommice, Copenhagen, Denmark) by peritoneal lavage, and enriched for macrophages by adherence as described previously [8]. After 14–22 h culture, serum-free medium was applied 30 min before the experiments.

Experimental agents were added in water to the experimental media in a volume never exceeding 5.0 % of the total.

Enzyme assays

Measurements of *N*-acetyl- β -D-glucosaminidase (NAG) and lactate dehydrogenase (LDH) were performed as described previously [8]. Unless otherwise stated, release of LDH was negligible under all experimental conditions described, indicating preserved cellular integrity [17].

Preparation of and stimulation by particles

Zymosan and latex particles were subjected to three consecutive washes, dispersed by vortex mixing and counted in a Bürker chamber. Freshly prepared particle suspensions were always used. When chase experiments were performed, particles that were not cell-associated were removed by three consecutive washes and shaking of the tissue culture dishes. Efficient removal was always verified by phase-contrast microscopy.

Enumerating cell-associated particles from micrographs

Micrographs were taken on a Nikon inverted microscope DIAPHOT model TMD with a Nikon F-601 camera. The number of particles associated (bound or internalized) with the cells was counted. For each experimental condition more than 500 cells were examined.

Preparation of soluble glucan

Soluble glucans were prepared from glucan particles, derived from baker's yeast, essentially as described [14]. To determine the average degree of polymerization of the molecules in the fractions obtained, the phenol/H₂SO₄ and the Somogyi–Nelson methods were used [18]. The fraction used for most inhibition experiments contained an average of 74 glucose residues (74-glucan).

Fractionation of F-MolPro-glucan

Gel-permeation chromatography was performed at 4 °C on a column of Sephadex G-200 with a bed volume of 34 ml and a void volume of approx. 10 ml, as determined with Blue Dextran 2000. Fractions (1.1 ml) were collected every 60 min. The column was eluted with PBS either with or without 0.1 % (v/v) Triton X-100. In the latter case, fractions were collected in BSA-containing tubes. Dextran of known *M_r* were applied and fractionated similarly in order to calibrate the column [19].

Binding of F-MolPro-glucan to macrophages

After incubation of macrophage cultures for 60 min at 4 °C with fractionated F-MolPro-glucan in medium containing 0.5 % (w/v) BSA, unbound glucan was removed by 4 × 1 min washes at 4 °C (the last of which was without albumin). The cells were then lysed with 0.1 % Triton X-100 and collected with a Teflon 'policeman'.

Fluorescence of the lysates was measured using conditions similar to those for analysis of fluorescein-conjugated dextran employed earlier [8]. In the presence of BSA, non-specific binding was negligible.

RESULTS AND DISCUSSION

Concentration- and time-dependence of NAG secretion induced by zymosan particles

Early studies on macrophage function reported avid phagocytosis of, e.g., bacteria, dental plaque, asbestos fibres, zymosan and latex particles [20]. The two latter agents have been extensively used in model systems for the study of cellular responses to the phagocytic process in macrophages and other leucocytes [6,21,22].

As shown in Figure 1, macrophages secreted more than 20 % of their content of NAG over 60 min when exposed to more than 50 μ g/ml zymosan particles. This response represents secretion of preformed lysosomal contents, since fluorescein-conjugated dextran (preloaded into the lysosomal compartment by pinocytosis) was released in parallel to NAG (not shown). If zymosan particles not firmly bound to the macrophages were washed away after a 15 min preincubation period, slightly higher concentrations of zymosan were required for similar secretion of lysosomal enzyme to occur (Figure 1, open symbols). Under these conditions, exposure to approx. 200 μ g/ml zymosan during the preincubation period was required for secretion of 20 % NAG during the 60 min chase. The efficacy of our washing procedure was verified by phase-contrast microscopy, by which only cell-associated zymosan particles could be seen after the wash.

In Figure 2 is shown the distribution of macrophages according to number of cell-associated particles after a 15 min pulse at 37 °C in Na-medium, pH 7.2, with zymosan particles at 50, 150 and 300 μ g/ml. Distribution was assessed by ocular inspection of micrographs and was very similar when micrographs obtained after 60 and 120 min chase periods were compared. NAG secretion from these cells, assayed after a 120 min chase period, amounted to 15, 31 and 42 % for Figures 2(a), 2(b) and 2(c) respectively. The increase with increasing dose of zymosan could reflect either an increased proportion of cells interacting with particles or an increase in the number of cells interacting with several particles (compare Figures 2a–2c). It may be noted that

Figure 1 Concentration-dependence of zymosan-induced NAG secretion

Macrophage NAG secretion was assayed after a 60 min incubation either in the presence of the indicated concentrations of zymosan particles (●) or after a 15 min preincubation period, after which all non-adherent particles were removed (○). Experiments were conducted at 37 °C in 1 ml of Na-medium, pH 7.2. The data presented are compiled from more than five separate experiments.

Figure 2 Distribution of cell-associated particles after preincubation with different zymosan particle concentrations

After preincubation with zymosan particles for 15 min at 37 °C in Na-medium, pH 7.2, and a chase period of 60 or 120 min, micrographs were recorded and the number of cell-associated zymosan particles counted. In (a) 50 µg/ml, in (b) 150 µg/ml and in (c) 300 µg/ml zymosan particles were applied during the 15 min preincubation.

Figure 3 Time-dependence of zymosan-induced NAG secretion

Macrophage NAG secretion after incubation either in the continuous presence of 1000 µg/ml zymosan particles (●) or after a 15 min preincubation with 200 µg/ml zymosan particles with subsequent removal of non-adherent particles (○). Experiments were conducted at 37 °C in 1 ml of Na-medium, pH 7.2. The data presented are compiled from more than five separate experiments.

the amount of lysosomal enzyme secreted was not directly proportional to the number of cell-associated particles.

That an increase in the number of particles/cell above a certain level does not lead to a further increase in lysosomal enzyme secretion is indicated in Figure 3. Cells that were preincubated for 15 min with 200 µg/ml zymosan (open symbols) and those exposed continuously to a fivefold higher particle/cell ratio exhibited similar linear secretory responses over time. We find it highly unlikely that the phagocytic capacity of the cells would be saturated after preincubation for 15 min with 200 µg/ml zymosan particles (compare Figures 2b–2c). We suggest that the data presented argue against a 'regurgitation during feeding' hypothesis [20], according to which lysosomal enzyme release is a consequence of fusion of lysosomes with phagosomes not yet closed against the extracellular milieu.

NAG release induced by latex particles

Latex particles have been widely used in studies on phagocytosis, because they can be obtained in well-defined sizes and are avidly

Table 1 NAG secretion induced by latex particles

Macrophage cultures were incubated with latex particles in Na-medium, pH 7.2, either in their continuous presence or as a chase experiment performed in the absence of particles after a prior 15 min exposure. The data presented are compiled from two different experiments. (***) Denotes massive LDH release (> 20%).

Pulse with latex particles (min)	Chase period (min)	Diameter of latex particles (µm)	$10^{-6} \times$ Number of particles/ml	NAG secretion (%)
120		0.8	236	1.0
	120	0.8	800	1.0
	120	0.8	1600	(***)
	120	2	100	7.5
	120	2	200	(***)
120		3	8	2.0
120		3	32	24.2
	60	3	32	0.0
	120	3	32	5.2
	180	3	32	22.0
	120	3	160	(***)
	120	4	16	31.5
	120	4	32	(***)

bound and phagocytosed. In human monocytes and monocyte-derived macrophages, latex particles have been reported to induce lysosomal enzyme secretion [21,23], a response not seen in mouse macrophages [6,24], a cell type in which latex particles often have been used as a negative control in the study of responses to other particles. To our surprise, release of NAG was induced by latex particles of diameter 2, 3 or 4 µm (Table 1). It may be noted that the size of these latex beads roughly corresponds to the size of the zymosan particles (3–4 µm). However, when slightly higher concentrations of latex particles were applied, massive release of LDH occurred (Table 1). This was also the case after preincubation with latex particles. Thus the NAG release observed might well be a consequence of toxicity. When macrophages were incubated with latex particles with a diameter of 0.8 µm at a concentration of 236×10^6 /ml, no secretion was induced over 120 min. This number of particles represents seven times the

Figure 4 Distribution of cell-associated particles after preincubation with zymosan particles in the presence of 74-glucan

After preincubation with zymosan particles for 15 min at 37 °C in Na-medium, pH 7.2, in the presence of 50 µg/ml 74-glucan and a chase period of 60 or 120 min, micrographs were recorded and the number of cell-associated zymosan particles counted. In (a) 150 µg/ml and in (b) 300 µg/ml zymosan particles were applied during the preincubation.

number applied with 200 µg/ml zymosan (34×10^6 /ml). When 6.0 or 11.9 µm latex particles were applied, no secretion of NAG could be triggered without a concomitant massive release of LDH (not shown).

Inhibition of zymosan particle binding and NAG secretion by soluble glucan

Zymosan particles are mainly composed of the carbohydrate polymers glucan and mannan, and their attachment to murine macrophages has been shown to be in part mediated by receptors for β -glucan [14,15]. Such a receptor also exists in human phagocytes and has recently been characterized [25–27]. Earlier reports have claimed that the attachment is mediated by either Man/Fuc receptors [13] or receptors for C3b after local opsonization of the particles by macrophage-produced C3b [11]. Binding via a glucan-binding site on CR3 has also been considered [12]. The distribution of cell-associated zymosan particles after incubation in the presence of 74-glucan (50 µg/ml) is shown in Figure 4. Both the number of cell-associated particles and their cellular distribution were shifted to resemble the situation after exposure to a low concentration of zymosan (Figure 2a). NAG secretion by the cells counted in Figures 4(a) and 4(b) amounted to 5.0 and 12.5% respectively when assayed after a 120 min chase. Thus 74-glucan inhibited NAG secretion, probably by decreasing binding and/or uptake of zymosan particles. The size of the soluble glucan was important, because preparations of glucan with a shorter average chain length caused progressively less inhibition of the secretory response (not shown).

Further characteristics of the inhibition of zymosan-induced secretion of NAG by soluble glucan are shown in Table 2. After exposure to 200 µg/ml zymosan in the presence of as little as 25 µg/ml 74-glucan for 15 min, approx. 70% inhibition of the secretory response was seen over 3 h compared with parallel experiments performed in the absence of glucan. If the concentration of zymosan was increased, less inhibition was seen (not shown) consistent with the notion that glucan treatment reduced the binding of zymosan particles to macrophages. If the chase period was reduced to 60 min, almost total inhibition

Table 2 Inhibition of NAG secretion by 74-glucan

74-Glucan was added to the cells 15 min before zymosan (200 µg/ml) at the concentrations indicated. Experiments were conducted in Na-medium, pH 7.2, either in the continued presence of zymosan and 74-glucan or as a chase experiment performed in the absence of these agents after exposure of the cells to 74-glucan (30 min) and zymosan (15 min). The data presented are representative of seven separate experiments.

Pulse with zymosan and 74-glucan (min)	Chase period (min)	Concentration of 74-glucan (µg/ml)	Inhibition of NAG secretion (%)
180		200	45.8
180		25	20.0
	180	200	59.1
	60	200	85.1
	180	25	72.3
	180	10	57.0
	180	2.5	48.5

(85%) was observed, whereas if glucan was added after exposure to zymosan (i.e. during the chase) no inhibitory effect was observed (not shown). As can also be seen in Table 2, inhibition by 74-glucan was large even at 2.5 µg/ml. If zymosan was present continuously, less inhibition of secretion was observed than if secretion was monitored after a shorter exposure to zymosan and 74-glucan (Table 2). However, increasing the concentration of 74-glucan (200 µg/ml) or lowering that of zymosan (50 µg/ml) resulted in a greater inhibitory effect of 74-glucan even in the continued presence of zymosan. Mannan at concentrations up to 5 mg/ml had no effect on secretion (not shown). It may also be noted that the soluble 74-glucan (200 µg/ml) by itself induced no secretory response over 3 h (not shown).

Characterization of the binding of F-MolPro-glucan to macrophages

As we observed a dependence on the size of the soluble glucan for its inhibitory effect on zymosan-induced NAG secretion, size-exclusion chromatography of F-MolPro-glucan was performed in order to generate fractions for cell-binding studies. Fractionation was performed at 4 °C on a Sephadex G-200 column after calibration with dextrans of defined M_r of 40000 and 10000 (Figures 5a and 5b). These dextrans were well separated despite their broadly eluted peaks, as the result of either a heterogeneous size distribution of the dextrans or interaction with the bed material. Separation of F-MolPro-glucan yielded three major peaks (Figure 5c), the last of which probably represented free fluorescein isothiocyanate. When separation was performed in the absence of Triton X-100, in order to yield material for cell-binding studies, the first peak was eluted slightly earlier whereas the position of the second and third peaks were unchanged. When pooled fractions from these peaks were applied to macrophage cultures for 60 min at 4 °C, with 0.5% BSA present to reduce non-specific binding, approx. 30 times more F-MolPro-glucan from the first peak than the second bound, when the material added from the peaks had similar absolute fluorescence intensity (not shown). Material from the third peak did not bind to the macrophages. Separation of F-MolPro-glucans bound to cells under similar conditions, but using unfractionated F-MolPro-glucan, resulted in the elution profile shown in Figure 5(d).

Figure 5 Characterization of F-MolPro-glucan and its binding to cells by gel-permeation chromatography

Fractionation was on a column of Sephadex G-200 with a bed volume of 34 ml and a void volume of approx. 10 ml as determined with Blue Dextran 2000. Chromatography was performed at 4 °C and fractions (1.1 ml) were collected every 60 min. In (a), 10 μ g of dextran (Texas Red conjugate, M_r 40 000), in (b) 10 μ g of dextran (fluorescein and tetramethylrhodamine conjugate, M_r 10 000) and in (c) 7 μ g F-MolPro-glucan (800 μ l) was applied to the column. The column was eluted with PBS supplemented with 0.1% (v/v) Triton X-100. In (d), cells in six 35 mm culture dishes were incubated with 5 μ g/ml F-MolPro-glucan as described in the Materials and methods section. After a wash procedure, cell-bound F-MolPro-glucan was collected in 800 μ l of elution buffer and applied to the column. The solid bars in (d) represent fluorescence corrected for the autofluorescence of a similar amount of cells not treated with F-MolPro-glucan and fractionated similarly. The fluorescence intensity of the fractions is expressed as the percentage of the fraction with highest fluorescence, which in (a)–(d) represented approx. 5000, 59 000, 4000 and 300 relative units respectively. The separation of F-MolPro-glucan was performed four times and that of cell-bound F-MolPro-glucan in triplicate.

Figure 6 Concentration- and time-dependence of F-MolPro-glucan binding to macrophages

In (a), incubation of macrophage cultures with the indicated concentration of F-MolPro-glucan was for 60 min. In (b), incubation was with 250 μ l from the first peak eluted from a Sephadex G-200 fractionation performed in the absence of Triton X-100 and instead collected in BSA-containing tubes. The elution profile was similar to that shown in Figure 4(c) except for a small shift of the first peak towards earlier fractions. Removal of probe that was not cell-bound was as described in the Materials and methods section. The amount of F-MolPro-glucan bound is related to the highest binding achieved (100%) which in (a) was 590 and in (b) 320 relative fluorescence units.

Apparently, binding of larger F-MolPro-glucans was preferred, which is even more evident after correction (solid bars) for cellular autofluorescence.

The binding of F-MolPro-glucan to cells approached saturation with respect to both dose and time (Figure 6). Non-specific binding to wells without macrophages always amounted to less than 5% (not shown). When cells were incubated with peak-1 F-MolPro-glucan, saturation was evident after approx. 60 min incubation at 4 °C (Figure 6b). Such kinetics are in agreement with previous studies on other receptor–ligand interactions [28]. As the amount of fluorescence/glucan residue is not known, it was not possible to quantify the binding in terms of receptor number. Instead, further characterization of the binding of F-MolPro-glucan with respect to dependence on bivalent cations, temperature, trypsin-sensitivity and activation/inhibition was attempted.

The binding of fractionated F-MolPro-glucan to macrophages was relatively insensitive to manipulation of the Ca^{2+}/Mg^{2+} content (Table 3). Varying the temperature revealed that the F-MolPro-glucan remained bound to a large extent during a chase period of 4 °C but less so at 37 °C (Table 3), indicating that the binding is either less efficient or rapidly reversible at higher temperatures. Binding of ligands to CR3 can be enhanced by activation of protein kinase C and is believed to result from changes in receptor avidity and/or receptor number [16,29,30]. The binding of F-MolPro-glucan to macrophages, however, was not affected by protein kinase C activation, but incubation with zymosan particles at 37 °C resulted in an increase in F-MolPro-glucan binding. Unlabelled MolPro-glucan at a concentration of 25 μ g/ml had only small effects on the binding at 4 °C if applied before F-MolPro-glucan (Table 3). Possibly, the size of the unlabelled MolPro-glucan made it unsuitable to compete for binding with the fractionated F-MolPro-glucan. Binding of F-MolPro-glucan was highly sensitive to trypsin treatment (Table 3), strongly suggesting that the binding was receptor-mediated. Furthermore, recirculation of receptors at higher temperature

Table 3 Binding of F-MolPro-glucan to cells

After gel-permeation chromatography of crude F-MolPro-glucan performed in the absence of Triton X-100, macrophage cultures were incubated for 60 min with F-MolPro-glucan collected from the first peak (at 11–16 ml), which was slightly shifted to the left compared with separation in the presence of Triton X-100 (Figure 4c). BSA (0.5%, w/v) was present to minimize non-specific binding, and experimental conditions for preincubation and chase were as indicated. Incubation with F-MolPro-glucan was at 4 °C, unless otherwise specified. Specific binding of F-MolPro-glucan is expressed as the percentage of that bound to cells in the same experiment not subjected to the special conditions indicated but otherwise similarly treated.

Special condition	Relative binding (%)
Medium without $\text{Ca}^{2+}/\text{Mg}^{2+}$ containing 1 mM EDTA	85
Chase at 4 °C for 60 min	73
Chase at 37 °C for 15 min	43
Incubation performed at 16 °C	61
Incubation performed at 37 °C	41
Preincubation with 300 nM phorbol dibutyrate	91
Preincubation with 200 $\mu\text{g}/\text{ml}$ zymosan particles for 30 min at 16 °C	83
for 15 or 30 min at 37 °C	171
Preincubation with 25 $\mu\text{g}/\text{ml}$ MolPro-glucan for 60 min at 4 °C	76
for 15 min at 37 °C	100
Chase with 25 $\mu\text{g}/\text{ml}$ MolPro-glucan for 60 min at 4 °C	80
Preincubation with 0.1% trypsin at 4 °C for 30 min	10
Incubation performed at 37 °C after pre-incubation with 0.1% trypsin at 16 °C for 30 min	53

was indicated by a regained capacity of trypsin-treated cells to bind F-MolPro-glucan at 37 °C.

Concluding remarks

Zymosan particles may trigger secretion of lysosomal enzymes by either (a) intracellular signalling triggered by receptor ligation in a manner independent of particle uptake or (b) mechanisms related to the handling of the particles in the endocytic pathway. In the former currently most widely accepted mechanism, any lysosomal structure might be engaged and direct fusion of lysosomes with the plasma membrane would be feasible. In the latter case, secretion would be expected to occur subsequent to phagosome–lysosome fusion and be related to the process of antigen presentation. A maximal secretory response was obtained at a rather low zymosan particle/cell ratio and was inhibitable by soluble glucan. The parallel inhibition of the cell association of zymosan particles by glucan suggests that unopsonized zymosan binds to a glucan receptor of resident peritoneal mouse macrophages and by this interaction triggers a secretory response. The lack of effect of mannan on either binding or secretory response is in accord with the reported low expression of mannose receptors on resident macrophages [15]. Such expression is dependent on the phenotypic state of the macrophage and is subjected to immunological regulation [31]. Other, less specific, receptors are likely to interact with zymosan particles as well as with latex particles, but no secretory response was triggered by such non-specific interaction.

The receptor for β -glucan was shown to be trypsin-sensitive, $\text{Ca}^{2+}/\text{Mg}^{2+}$ -independent and not induced by protein kinase C activation. The two latter characteristics distinguish this receptor

from the mannose receptor and CR3 respectively. Local opsonization of zymosan particles by C3bi allowing zymosan–CR3 interaction [11] appears unlikely in view of our results. A role for a glucan-binding site on CR3 [12] in macrophage–zymosan interactions cannot be ruled out, however.

Binding of ligand to a β -glucan receptor depended on the size of the ligand. As the preferred unit ligand has been reported to be a heptaglucoide [26], multiple receptor–ligand interactions could strengthen the binding of larger glucan molecules or zymosan to the cell. Multivalent ligands may cause clustering of receptors and possibly thereby transmission of signals to the cytoskeleton and other cellular components. Evidence for an internal pool of glucan receptors includes mobilization after trypsin treatment and up-regulation by treatment with zymosan particles. Recirculation of receptors and regulation of receptor expression on the cell surface is analogous to the reported function of other receptors [28–31]. Trypsin-sensitivity and internalization of soluble glucan bound to mouse peritoneal macrophages were recently demonstrated [32].

A knowledge of the macrophage β -glucan receptor is crucial not only because of its role in fungal infections, but because signalling by this receptor has been shown to have immunomodulatory roles [33–36], and, furthermore, its phagocytic function is sensitive to immunomodulation [37]. Preparations of β -glucan have been used for many years in the treatment of certain forms of human cancer [12], and current clinical trials suggest that β -glucan-receptor ligands enhance non-immune resistance to infection [34].

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