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Induction of a β-1,3-D-glucan receptor in P388D1 cells treated with retinoic acid or 1,25-dihydroxyvitamin D_3

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SUMMARY

Retinoic acid (RA) and 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) induce the capability to phagocytose heat-killed yeast (Y) (*Saccharomyces cerevisiae*) in P388D1 cells. Y phagocytosis is specifically inhibited (100%) by particulate and soluble β-1,3-D-glucan. Other polysaccharides, such as agarose, dextran and dextran sulphate, are not inhibitory. The inhibitory capacity of mannan was totally abrogated by treatment with β-glucanase, suggesting that its activity is derived from a residual β-glucan structure. Partial hydrolysis of glucan particles with formic acid yielded soluble glucan that was fractionated according to size. Glucan[1], glucan[2] and glucan[3] had an average chain length of 34, 23·5 and 15·5 glucose units, respectively. Fifty percent inhibition of Y phagocytosis by RA-P388D1 cells was attained at $<0{\cdot}02$ μg/ml (~ 2 nM) glucan[1] and at 1·1 μg/ml glucan[3]. A further decrease in chain length ($\leqq 12{\cdot}6$) resulted in oligomers of marginal inhibitory activity. Preincubation of RA- and $1,25(OH)_2D_3$-P388D1 cells with glucan[1] for 30 seconds to 5 min, at 4° or 37°, followed by washes with buffer, sufficed to bring about 85–95% inhibition of Y phagocytosis. Recovery of the phagocytic capability was time dependent and required protein synthesis, suggesting a glucan[1]-induced removal of membrane receptors. The results suggest that recognition and ingestion of Y by RA- or $1,25(OH)_2D_3$-treated P388D1 cells depends almost exlusively on a β-glucan-specific receptor.

INTRODUCTION

Various receptors have been implicated as taking part in the recognition, attachment and ingestion of opsonized and unopsonized zymosan or heat-killed yeast particles (Y). Zymosan is a yeast (*Saccharomyces cerevisiae*) cell-wall preparation obtained by boiling baker's yeast before and after extensive treatment with trypsin. About 60% of the dry weight of zymosan consists of glucan, a β-linked branched chain polysaccharide of glucose subunits, and of mannan, an α-linked polymer of mannose subunits (DiCarlo & Fiore, 1958). Zymosan or Y are classical activators of complement via the alternative pathway; in the course of complement activation C3b and C3bi become fixed to their surface (Newman & Mikus, 1985), and the particles become opsonized and rendered recognizable by the three types of complement receptors that have been described. Recent studies give a somewhat perplexing view of the mode of recognition for phagocytosis and induction of the respiratory burst of unopsonized zymosan and Y in human and murine phagocytes. On the one hand, it is suggested that mature human monocyte-derived macrophages (Speert & Silverstein, 1985) and mouse peritoneal macrophages (Sung, Nelson & Silverstein, 1983) phagocytose unopsonized zymosan predominantly via mannose receptors. On the other hand it was suggested that zymosan ingestion by monocytes and macrophages is mediated via complement receptor type 3 (CR3), a process requiring a step of local opsonization of the particles with macrophage-secreted complement (Ezekowitz *et al.*, 1983; Johnson *et al.*, 1984). Czop & Austen (1985a) have recently designated the monocyte phagocytic receptor for particulate activators as a β-glucan receptor. Williams *et al.* (1986) have shown further that phagocytosis of zymosan by human PMN is dependent primarily on its glucan component. Ross, Cain & Lachmann (1985) provided evidence that the human neutrophil CR3 binds both zymosan and Y without a step of local opsonization and also bind via a disparate-binding site C3bi-opsonized Y. They propose that the two distinct binding sites differ in their ability to trigger the respiratory burst.

P388D1 cells, a mouse macrophage-like tumour cell line, do not ingest unopsonized Y, they secret C3 (Goodrum, 1987), express Mac-1 (CR3) and have a limited capacity for phagocytosis via complement receptors (Van Furth *et al.*, 1985). In previous studies I have shown that P388D1 cells can be induced

Abbreviations: $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; $1,25(OH)_2D_3$-P388D1, $1,25(OH)_2D_3$-treated P388D1 cells; BSA, bovine serum albumin; FA, formic acid; glucan, β-1,3,-D-glucan; glucan[1], glucan[2] and glucan[3] water-soluble 70%, 80% and 90% ethanol-precipitable glucan, respectively; glucan[P], particulate glucan; glucan[S], water-soluble glucan; HI-FCS, heat-inactivated fetal calf serum; HI-HS, heat-inactivated horse serum; PBS, Dulbecco's phosphate-buffered saline containing Ca^{2+} and Mg^{2+}; RA-P388D1, RA-treated P388D1 cells; RA, retinoic acid; Y, heat-killed yeast.

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to express a high phagocytic activity towards Y by retinoic acid (RA) as well as by 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) (Goldman, 1984a,b). Acquisition of the high phagocytic phenotype was not associated with induction of mannose receptors in the cells that lack these receptors in their untreated phenotype (Stahl & Gordon, 1982; Goldman, 1984a). In the present study, it is shown that RA and $1,25(OH)_2D_3$ induce the expression of a trypsin-sensitive β-glucan-inhibitable receptor that recognizes Y for binding and phagocytosis. Using the phagocytosis of Y as a marker for β-glucan-receptor expression, receptor specificity for polysaccharides, its rate of removal from the surface and protein synthesis-dependent reappearance were analysed.

MATERIALS AND METHODS

Chemicals

$1,25(OH)_2D_3$ was obtained from Hoffman-La Roche Inc. (Nutley, NJ). β-All-trans-retinoic acid (RA), and dexamethasone were obtained from Sigma Chemical Co. (St Louis, MO) and kept dissolved in ethanol at −24°. Cycloheximide, α-mannan, laminarin, glucan particles from baker's yeast (Sigma-glucan^P), zymosan, agarose, type III, were also from Sigma. Dextran T-40 was from Pharmacia Fine Chemicals (Piscataway, NJ). Soluble glucan secreted from *Sclerotium rolfsii* was a gift of Dr I. Chet. L-[4,5-^{3}H]leucine (36 Ci/mmol) was obtained from Amersham International (Amersham, Bucks).

P388D1 cell growth and differentiation

P388D1 cells (Dawe & Potter, 1957) were serially passaged (bi-weekly) in Dulbecco's modified Eagle's medium (DMEM) (4·5 g glucose/l; Grand Island Biological Co., Grand Island, NY) supplemented with 10% heat-inactivated horse serum (56° for 30 min) (HI-HS), 1% 100 × non-essential amino acids, 1% glutamine (200 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml) (all from Bio-Lab, Jerusalem, Israel). The cells, both adherent and non-adherent, were removed from the 10-cm diameter Falcon tissue culture dishes by mild streams of the culture medium (Goldman, 1984b) (Falcon Plastics, Oxnard, CA) plated onto 35-mm Falcon tissue culture dishes ($1·25 \times 10^5$ cells unless specified otherwise) in the above complete medium and cultured with or without the added compounds (2 ml final culture volume) for 96 hr. $1,25(OH)_2D_3$ and RA were added for the entire period of 96 hr at a final concentration of 12 nM and 1 μM, respectively, conditions found optimal for induction of Y phagocytosis (Goldman, 1984a,b).

Assay of phagocytosis

The standard assay designed to evaluate effects of various agents on phagocytosis consisted of two steps: preincubation of cells with or without the test compounds in PBS (0·8 ml, 20 min, 37°), and a further incubation with added suspensions of heat-killed yeast cells (Y) (*Saccharomyces cerevisiae*) in PBS ($7·5 \times 10^7$ Y/ml, 0·2 ml per dish) for 30 min at 37°. Variations from this procedure are specified in the legends to figures or tables. The cell monolayers were than washed with PBS (22°), fixed with 2·5% glutaraldehyde in PBS, and stained (Giemsa). The number of ingested Y per cell was assessed using light microscopy (× 1000). Two- to three-hundred cells were enumerated in each of triplicate cultures. The SD was in the range of 5–15%.

Preparation of particulate and soluble glucan

Baker's yeast were digested sequentially with boiling NaOH and HCl and extracted with isopropyl alcohol, essentially as described elsewhere (Di Luzio *et al.*, 1979). The glucan particles were hydrolysed further by boiling for 2 hr in 50% formic acid (FA). The residue was washed with water and boiled in water for 3 hr to hydrolyse the formate ester bonds (Sasaki *et al.*, 1976). The particles thus obtained (glucan^P) were of a smaller size than the former step particles, which resemble intact yeast cell walls. Soluble glucan was prepared from glucan particles (without the 50% FA treatment) by the method of Sasaki *et al.* (1976). Two conditions for glucan solubilization were used: (i) 90% FA at 95° for 20 min, and (ii) 80% FA at 85° for 20 min. FA was removed by flash evaporation. The residue was hydrolysed in water (3 hr in a boiling water bath), and the water-soluble glucan (glucan^S) separated from the insoluble glucan by centrifugation and filtration (Millipore 0·45 μm). The product was dried by flash evaporation. Glucan^S was fractionated according to solubility of its mixed polymers in ethanol/water solutions (4°). Glucan^1 precipitated at 70% ethanol, glucan^2 precipitated at 80% ethanol and glucan^3 precipitated at 90% ethanol. The 90%-ethanol supernatant was dried and the residue dissolved in water. The glucan oligomers were fractionated on a Sephadex G-15 column (50 cm × 0·8 cm diameter, 35 ml bed volume, 0·5 ml fractions, equilibrated and eluted with water). The phenol sulphuric acid method (Hodge & Hofreiter, 1962) was used to determine the concentration of glucose equivalents in glucan fractions. The ratio of reducing sugar determined by the Somogy–Nelson method (Hodge & Hofneiter, 1962) to glucose equivalents gave an estimate of the size of the chain length of the glucan polymers (oligomers).

Treatment of glucan^S and mannan with various glycosidases

Glucan^S (150 μg/ml) and mannan (50 mg/ml) were dissolved in 5 mM citrate, 0·15 M NaCl, pH 5·2, incubated for 4 hr at 37° with β-glucosidase (type 1, Sigma) (3 U/ml mannan and 1 U/ml glucan), with the β-glucanase laminarase (penicillium sp.; Sigma) (1 U/ml mannan and 0·4 U/ml glucan) or dialysed α-mannosidase (Jack bean; Sigma) (3 U/ml mannan and 0·9 U/ml glucan). The units are those given by Sigma. The activities of the glycosidases were also assessed using *P*-nitrophenyl-glycosides under the treatment conditions described for the polysaccharides, and as described elsewhere (Czop & Austen, 1985b). The laminarase (β-glucanase) did not hydrolyse *P*-nitrophenyl β-glucopyranoside. For assessment of interference with Y phagocytosis, the treated mannan solutions were diluted 10-fold and the treated glucan^S solutions were diluted 15-fold. Blanks of mannan and glucan in buffer, and of the enzymes in buffer, did not have any effect on Y phagocytosis.

RESULTS

Specific inhibition by β-glucan of the RA- and $1,25(OH)_2D_3$-inducible Y phagocytosis by P388D1 cells

RA and $1,25(OH)_2D_3$, each alone (Goldman, 1984a,b) and in synergism, (Goldman, 1985) induce the capability to phagocytose Y in P388D1 cells which is only marginally expressed (2–10%) in non-treated P388D1 cells. Soluble and particulate β-glucan from *Saccharomyces cerevisiae*, laminarin (*Laminaria digitata*) and a soluble β-glucan from *Sclerotium rolfsii* (not

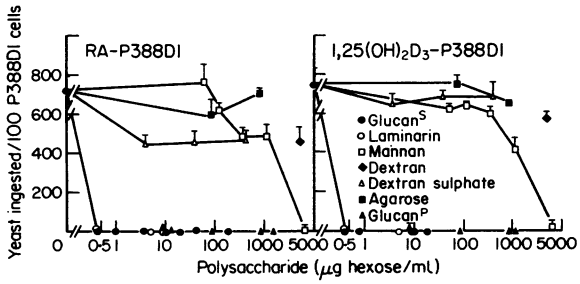

Figure 1. The effect of various polysaccharides on the phagocytosis of Y by RA- and 1,25(OH)$_2$D$_3$-treated P388D1 cells. Means of triplicate cultures ± SD.

shown) inhibited at <0·5 μg/ml the phagocytosis of Y (100% inhibition, Fig.1a,b). Other polysaccharides, such as agarose, dextran and dextran sulphate, had either no effect or a limited inhibitory activity at concentrations up to 1–5 mg polysaccharide/ml. Mannan showed an inhibitory activity at comparatively high concentrations, i.e. 40% inhibition at 1 mg/ml and a 100% inhibition at 5 mg/ml (Fig.1a,b). Treatment of mannan with β-glucanase totally abrogated its inhibitory activity for Y phagocytosis. β-Glucosidase and α-mannosidase did not affect the inhibitory potential of mannan. β-Glucanase was also the only enzyme of the three glycosidases tested that abrogated the inhibitory activity of soluble glucan (Table 1). Glucan^S, obtained by partial hydrolysis of glucan particles using 80% or 90% FA, was separated into four fractions. In the 80% FA hydrolysate, 35%, 44%, and 9% were precipitated in 70%, 80%, and 90% ethanol, respectively, the other 12% remained soluble in 90% ethanol. In the 90% FA hydrolysate, 23%, 34% and 10% precipitated in 70%, 80% and 90% ethanol, respectively, and 33% remained soluble. The average chain length ($\overline{DPn}$) of the glucan polymers solubilized by 90% FA and precipitated in 70%, 80% and 90% ethanol, was 34, 23·5 and 15·5 glucose monomers per chain, respectively, assuming a linear polymer. The fraction that remained soluble in 90% ethanol was further fractionated on a Sephadex G-15 column to yield oligosaccharides ranging from $\overline{DPn}$ 12·6 to 2·8, and glucose.

Figure 2 shows that the higher the chain length of the soluble glucan polymer, the more effective its inhibitory activity on Y phagocytosis. The concentration of glucan at which a 50% inhibition of Y phagocytosis by RA-P388D1 cells was observed (Kip) was <0·02 μg/ml for glucan^1, about 0·05 μg/ml for glucan^2 and about 1·1 μg/ml for glucan^3. On a molar basis (as opposed to the weight basis given), the inhibitory capacity of the various polymers would assume a greater differential. Further decrease in chain length yielded a steep reduction in the inhibitory potential. A glucan fraction with $\overline{DPn}$13·8 had a Kip of 9·3 μg/ml for Y phagocytosis by RA-P388D1 cells, whereas oligomers of $\overline{DPn}$ 12·6 down to $\overline{DPn}$ 3 exerted, in the same system, inhibitory effects in the range of 20% at 1–30 μg/ml ($\overline{DPn}$ 12·6), 20–200 μg/ml ($\overline{DPn}$8) and 100–1000 μg/ml ($\overline{DPn}$ 3). Assuming a molecular weight of about 6000 for glucan^1 and a concentration of 0·01 μg glucose/ml (Fig. 2a,b), as that required for a 50% inhibition of phagocytosis, the Kip for this fraction would be about 2 nM. A similar correlation between chain length and inhibitory capacity was observed with fractions of laminarin (a water soluble β-glucan) obtained by ethanol precipitation as above. Soluble glucan was also shown to inhibit the phagocytosis of zymosan and Sigma-glucan^P by RA-P388D1 cells (Table 2).

Table 1. Effect of glycosidases on the phagocytosis inhibiting potential of mannan and glucan

Polysaccharide*	Glycosidase†	No. Y ingested/100 P388D1 cells ± SD	
		RA-P388D1	1,25(OH)$_2$D$_3$-P388D1
—	—	711 ± 52	646 ± 56
Mannan	—	302 ± 56	345 ± 44
Mannan	β-Glucosidase	262 ± 21	328 ± 54
Mannan	β-Glucanase	706 ± 21	550 ± 36
Mannan	α-Mannosidase	314 ± 70	383 ± 40
Glucan^S	—	19 ± 2	10 ± 1
Glucan^S	β-Glucosidase	10 ± 3	15 ± 4
Glucan^S	β-Glucanase	495 ± 50	654 ± 61
Glucan^S	α-Mannosidase	17 ± 9	12 ± 5

* P388D1 cells were incubated for 20 min at 37° with 0·8 ml of glycosidase-treated and untreated mannan (5 mg/ml) and glucan^S (10 μg/ml). Y ($1·5 \times 10^7$ in 0·2 ml) was then added for 30 min at 37°.

† For conditions see the Materials and Methods.

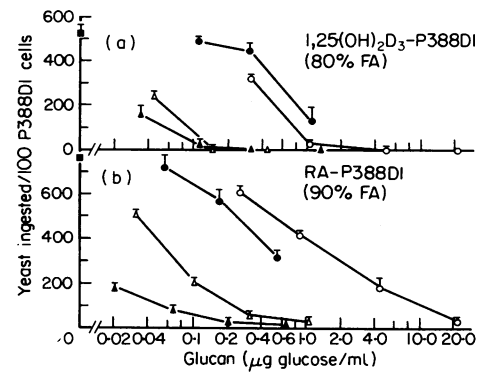

Figure 2. Effect of glucan^S and its ethanol-precipitated fractions on the phagocytosis of Y by RA-P388D1 and 1,25(OH)$_2$D$_3$-P388D1 cells. (a) and (b) an 80% and 90% formic acid (FA) hydrolysate of glucan particles; (●) denotes the total glucan^S preparation; (▲), (△) and (○) denote the fractions precipitated at 70%, 80% and 90% ethanol, respectively. Means of triplicate culture ± SD.

Characterization of the interaction of glucan with RA- and 1,25(OH)$_2$D$_3$-treated cells

Figure 3 shows that the effect of glucan on the phagocytosis of Y did not require its presence in the medium during the phagocytic assay. A short incubation with glucan^1, 30 seconds up to 2–5 min at either 4° or 37°, followed by two washes in PBS, sufficed to render the cells incapable of interaction with Y; neither phagocytosis nor Y attachment were brought about. RA-P388D1 cells recovered slowly from the glucan-mediated state of suppressed phagocytosis (Fig. 3b). Two hours and four hours

Table 2. Effect of glucan[2] on the phagocytosis of heat-killed yeast, zymosan and glucan particles

Particle*	Glucan[2] (μg/ml) —	0·5	2·0	10·0
Heat-killed yeast	488±11†	118±21	67±12	32±6
Sigma-glucan[P]	324±66	138±24	118±20	99±38
Zymosan	185±28	84±3	35±10	38±3

* RA-P388D1 cells were incubated with 0·8 ml of glucan[2] of the specified concentration (in PBS) for 20 min at 37°. The particles (0·2 ml of $7{\cdot}5 \times 10^7$/ml) were then added for 30 min (37°).

† Means of triplicate cultures ±SD.

after the removal of glucan from the medium, phagocytosis of Y amounted to 17% and 40% of control values, respectively. The recovery of the phagocytic capability depended on protein synthesis since cycloheximide at concentrations that did not affect cell viability inhibited the recovery of the phagocytic capability of the cells (Fig. 3c). The degree of inhibition of protein synthesis, as reflected by [^{3}H]leucine incorporation in the presence and absence of cycloheximide, throughout the whole 5 hr of the recovery period is given in Fig. 3d. Incubation of RA-P388D1 cells in DMEM containing 5 μg/ml cycloheximide for 7 hr prior to the assay of phagocytosis resulted in only a small (23%) inhibitory effect on Y phagocytosis (493±25, and 381±37 Y ingested per 100 control and cycloheximide-treated RA-P388D1 cells, respectively.) Thus, the slow rate of recovery of the phagocytic capability after glucan treatment and its inhibition by a protein synthesis inhibitor imply that glucan induces the removal or inactivation of a protein(s) from the cell or the cell surface that is required for the process of Y recognition and/or phagocytosis, and that its turnover or secretion in the absence of glucan is slow. Y ingestion was inhibited 90% in both RA-P388D1 and $1,25(OH)_2D_3$-P388D1 cells pretreated with 200 U of trypsin in DMEM (30 min, 37°) and washed with DMEM-10% HI-HS prior to assay of phagocytosis. A mild fixation of RA-P388D1 cells with paraformaldehyde resulted in a 100% inhibition of Y phagocytosis. The Y cells were recognized, however, and formed stable associations with the fixed cells (Table 3). Attachment of Y was inhibited by glucan[1]; this inhibition of Y recognition could be brought about under conditions that presumably prevent receptor internalization.

DISCUSSION

Three elements could be involved, in principle, in the acquired means of recognition and ingestion of Y and zymosan by RA- and $1,25(OH)_2D_3$-treated cells; recognition via cell wall-associated polysaccharides, mannan and glucan or by means of C3b/C3bi fixed on their surface as a result of local opsonization mediated by P388D1-secreted complement components and their activating proteins.

The present study established that Y ingestion (and attachment) is practically 100% inhibited by particulate and soluble β-glucan from a variety of sources. Other polysaccharides, such as dextran, dextran sulphate, agarose, and β-glucanase-treated mannan, did not suppress Y phagocytosis. The inhibition of Y ingestion by soluble glucan was concentration dependent, with a 50% inhibition by glucan[1] in the nM range. This concentration range is compatible with binding of carbohydrates to specific macrophage receptors (Stahl & Gordon, 1982; Shepherd *et al.*, 1984). The rather steep decline in the potency of soluble glucans of decreasing chain length to inhibit Y phagocytosis may reflect the differential in their ability to react simultaneously with more than one receptor. Glucan polymers of $\overline{DPn}$ 34 and $\overline{DPn}$ 22 are more likely to interact with more than one receptor than lower molecular weight polymers ($\overline{DPn} \leqq 15{\cdot}5$). Receptor cross-linking by di- or polyvalent ligands may lead to pinocytosis and loss of receptor activity from the cell surface. The actual structural requirements of the receptor-binding sites are not known, and it is possible that the low molecular weight oligomers ($\overline{DPn} \leqq 12{\cdot}6$) that do not provide strong inhibitory capacity (about 20% under the experimental conditions used) for Y phagocytosis are also recognized by the receptor-binding site, but since they are not likely to induce receptor cross-linking and internalization they are removed from the receptors by a multivalent ligand such as a Y particle.

The kinetics of inhibition of Y phagocytosis by glucan, the persistence of its effect after a thorough wash of the cells, the slow rate of recovery of the phagocytic capability and its dependence on protein synthesis suggest that glucan binding to cell-surface receptors leads to their removal from the cell surface (either by pinocytosis or shedding) or their practical blockade. Resumption of function requires the synthesis and reappearance of receptors on the cell surface.

C3 and its activating proteins are continuously synthesized and secreted in macrophages and macrophage cell lines (Zimmer *et al.*, 1982; Goodrum, 1987). The possibility that glucan inhibits Y ingestion by interfering with local opsonization by secreted proteins rather than by binding to specific receptors is rather unlikely since: (i) soluble glucan in contrast to particulate glucan does not have the capacity to activate complement (Czop & Austen, 1985c); (ii) glucan can be washed away prior to Y addition; (iii) cycloheximide treatment, which only partially inhibits the synthesis and secretion of C3 (Zimmer *et al.*, 1982; Goodrum, 1987) and has little effect on Y phagocytosis by $1,25(OH)_2D_3$ and RA-treated P388D1 cells (23% after 7-hr pretreatment), totally abrogates the recovery of phagocytosis after glucan[1] treatment; and (iv) paraformaldehyde treatment, which inhibits Y phagocytosis and is likely to inhibit C3 synthesis and secretion (Ross *et al.*, 1985), does not interfere with a β-glucan-inhibitable Y attachment to the fixed cells. The above constitutes indirect evidence of the exclusion of local opsonization as a major mechanism of Y phagocytosis by RA- and $1,25(OH)_2D_3$-treated P388D1 cells.

It is of note that while this paper was being reviewed, Janusz, Austen & Czop (1986) reported on the fractionation of yeast glucan into high and low molecular weight fractions of soluble glucan and showed that the various fractions inhibit human monocyte phagocytosis of unopsonized zymosan. Phagocytosis was inhibited by oligoglucosides as small as $\overline{DPn}$ 11 and $\overline{DPn}$ 5 and required short preincubation periods (2–5 min).

The contribution of a mannose receptor to the interaction of Y with RA- or $1,25(OH)_2D_3$-P388D1 cells was conclusively negated by the finding that glucanase treatment of mannan totally abolished its suppressive effect on Y phagocytosis, corroborating an earlier finding that P388D1 cells do not express mannose receptors (Stahl & Gordon, 1982), and that the

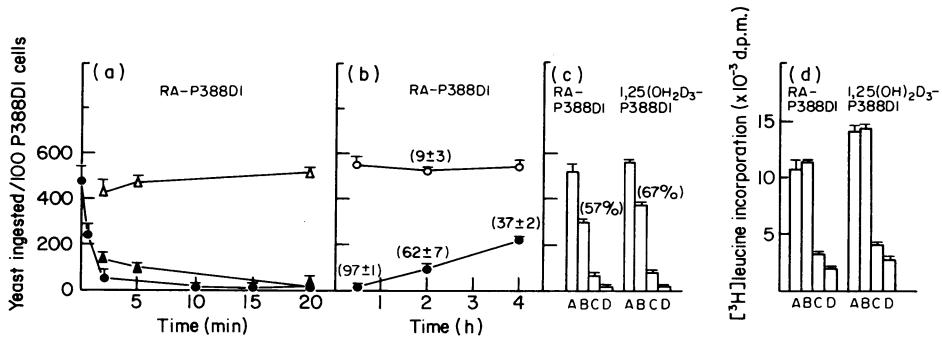

Figure 3. Dynamics of the glucan inhibitable receptor. (a) Kinetics of glucan-mediated inhibition of Y phagocytosis. RA-P388D1 cells were incubated with glucan[1] (2 μg/ml) for 30 seconds up to 20 min, at: (●) 37° and (▲) on ice. (△) Cells incubated on ice without glucan. (b) Rate of recovery from glucan-mediated inhibition. Cells were treated with glucan[1] (2 μg/ml) (●) or PBS (○) for 20 min at 37°. After washing twice in PBS, the cells were incubated in DMEM for the specified time, at which Y were added for 30 min (percentage empty cells). (c) Dependence of recovery of glucan-inhibitable phagocytosis on protein synthesis. Cells were incubated in PBS with (B–D) or without (A) glucan[1] (2 μg/ml). The cells were washed twice in PBS, and incubated in DMEM (37°) for 5 hr. A and B no cycloheximide. C and D, 0·5 μg/ml and 5 μg/ml of cycloheximide, respectively (percentage recovery of Y phagocytosis.) (a) to (c) means of triplicate cultures ±SD. (d) Inhibition of protein synthesis by cycloheximide. Cells were treated in parallel to (c). [^{3}H]leucine (10 μCi/ml) was added at the beginning of the 5-hr recovery period. After 5 hr, the cells were treated (10 min) with 10% TCA (4°), washed with 5% TCA (4°) and 100% ethanol. The residue was dissolved in 0·1 N NaOH and counted. Results are means of [^{3}H]leucine incorporation into TCA precipitable material in triplicate cultures ±SD.

Table 3. Effect of paraformaldehyde on Y recognition by RA-P388D1 cells

Treatment	Glucan	Y ingestion or attachment (Y/100 cells ± SD)‡
Control*	−	493 ± 17
	+	12 ± 5
Paraformaldehyde†	−	352 ± 11
	+	55 ± 20

* The cells were preincubated for 20 min at 37° with or without glucan[1] (2 μg/ml), and Y were added for additional 30 min at 37°.

† Cells were treated for 15 min at 22° with 0·15% paraformaldehyde, washed with PBS and incubated for 5 min with PBS, 1% BSA. The cells were then incubated with or without glucan[1] (2 μg/ml) for 5 min at 22° and Y were added for 30 min at 22°.

‡ All the cell-associated Y were ingested in the non-treated cells and attached in the paraformaldehyde-treated cells.

latter are not induced in the RA- and 1,25(OH)$_2$D$_3$-treated cells (Goldman, 1984a,b). The above is also in line with the work of Czop & Austen (1985a) who showed that the inhibitory effect of high concentrations of yeast mannan on zymosan phagocytosis by human monocytes was abrogated by prior treatment of mannan with β-glucanase.

In a recent study characterizing the β-glucan receptor of murine peritoneal macrophages, I have shown that the inhibitory effect of mannan on Y phagocytosis by thioglycollate-elicited mouse peritoneal macrophages (Goldman, 1987), which in contrast to RA- and 1,25(OH)$_2$D$_3$-treated P388D1 cells (Goldman, 1985) and fresh human monocytes (Shepherd *et al.*, 1982) express mannose receptors (Stahl & Gordon, 1982), was also abrogated by β-glucanase treatment. Furthermore, the mannose receptors do not synergize with the β-glucan receptors in Y phagocytosis (Goldman, 1987).

Comparison of the specificity and characteristics of the trypsin-sensitive β-glucan-specific receptor that mediates the binding and phagocytosis of Y by murine macrophages (Goldman, 1987; this paper) with the trypsin-sensitive β-glucan receptor that was described in human monocytes (Czop & Austen, 1985a) and human polymorphonuclear leucocytes (Williams *et al.*, 1986) suggests that these are probably analogous receptors.

Glucan receptors in phagocytes appear to mediate a very important aspect of their function *in vivo* and *in vitro*. Glucan, both particulate and soluble, has been shown to exert profound beneficial effects on the ability of laboratory animals to cope with malignancy and immunosuppression, and to activate murine and human monocytes and macrophages for both intracellular and extracellular killing of bacteria, parasites and tumour cells (DiLuzio, 1985; Bögwald, Johnson & Seljelid, 1982). Recent studies showed that occupation of β-glucan receptors can trigger the secretion of leukotrienes from human monocytes (Czop & Austen, 1985b) and H_2O_2 from murine granulocytes (Morikawa *et al.*, 1985). A better understanding of the characteristics of the β-glucan receptor, its up and down regulation, and the events triggered by receptor occupancy will no doubt be of relevance in the current attempts to use glucan as a therapoietic agent.

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