Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 2751–2755, May 1985
Cell Biology

Generation of leukotrienes by human monocytes upon stimulation of their β-glucan receptor during phagocytosis

(alternative complement pathway/5-lipoxygenase pathway/zymosan/nonimmune host defense)

JOYCE K. CZOP AND K. FRANK AUSTEN

Department of Medicine, Harvard Medical School, and Department of Rheumatology and Immunology, Brigham and Women's Hospital, Boston, MA 02115

Contributed by K. Frank Austen, December 10, 1984

ABSTRACT **Human monocytes possess a receptor for ingestion of particulate activators of the human alternative complement pathway that functions in the absence of plasma proteins and is distinct from the receptors for Fc-IgG and the major cleavage fragment of the third component of complement (C3b). Incubation of monolayers of monocytes with 1.1×10^6 to 2.2×10^7 glucan particles per ml initiated a phagocytic response comparable to that obtained with zymosan particles, of which β-glucan is a constituent along with mannan. Maximal quantities of 4.93 ± 3.43 ng of leukotriene B_4 (LTB_4) and 0.43 ± 0.23 ng of leukotriene C_4 (LTC_4) (mean ± SD, $n = 3$) were released by 10^6 monocytes stimulated with 1.1×10^7 glucan particles per ml. Preincubation of monocytes with 50 μg of soluble β-glucan per ml reduced subsequent monocyte ingestion of 5×10^6 zymosan particles per ml and 2.2×10^6 glucan particles per ml by 52% and 55%, respectively, and diminished release of LTB_4 by monocytes stimulated with 2×10^8 zymosan particles per ml and 8.6×10^6 glucan particles per ml by 73% and 61%, respectively. Preincubation with 1 mg of soluble mannan per ml had little effect on monocyte phagocytosis or LTB_4 generation in response to either zymosan or glucan particles, and neither soluble β-glucan nor mannan stimulated generation of LTB_4 or LTC_4. The effect of pretreatment of monocytes with soluble β-glucan was time dependent, with the maximal effect being evident within 20 min of pretreatment, and was specific for zymosan or glucan particles in that the LTB_4 and LTC_4 release induced by 2.5 μM calcium ionophore A23187 was unaffected. That both phagocytosis and leukotriene generation are inhibited by soluble β-glucan but not by mannan at a rate compatible with the phagocytic process of monocyte monolayers indicates ligand specificity for a β-glucan receptor. As the β-glucan receptor recognizes particulate activators of the alternative complement pathway, the nonimmune response to a single stimulus induces complement activation, phagocytosis, and leukotriene generation.**

In the absence of plasma proteins, human peripheral blood monocytes phagocytose zymosan particles and other particulate activators of the human alternative complement pathway through a specific trypsin-sensitive receptor that is distinct from that for Fc-IgG or the major cleavage fragment of the third component of complement (C3b) (1–3). Perturbation of this phagocytic receptor with zymosan particles stimulates monocytes to metabolize endogenous arachidonic acid to substantial quantities of leukotriene B_4 (LTB_4) and leukotriene C_4 (LTC_4), whereas comparable perturbation of monocyte phagocytic receptors for Fc-IgG does not (4). The capacity of monocyte monolayers to generate and release leukotrienes in response to zymosan particles is decreased in a dose-dependent fashion by the same low concentrations of trypsin (4) that reduce monocyte ingestion of the particulate activators, zymosan (1), rabbit erythrocytes (1), and desialated sheep erythrocytes (2), whereas the ionophore A23187-induced production of leukotrienes is unaffected. Zymosan particles are yeast cell wall products (5, 6) that are comprised almost exclusively of equal amounts of α-mannan and β-glucan (7, 8). Yeast glucan is an alkali insoluble component of yeast cell walls that consists solely of D-glucose residues, most of which are consecutively joined by β-1,3 linkages (8). Yeast and fungal β-1,3-D-glucans activate human complement proteins (9, 10) and stimulate murine macrophages, as assessed by morphology and incorporation of [^{14}C]glucosamine (10).

Mouse peritoneal macrophages also ingest zymosan particles through an opsonin-independent receptor (11) and release LTC_4 after zymosan stimulation (12). The murine phagocytic response has been reported to occur through mannose receptors since pretreatment of mouse macrophages with various types of mannans inhibits subsequent ingestion of zymosan particles (13). Human monocytes do not possess mannose/fucose receptors (14) and the present study demonstrates that yeast glucan particles initiate a monocyte phagocytic response and generation of leukotrienes. That soluble β-glucan, but not mannan, inhibits both of these receptor-mediated functions when zymosan particles are used as the stimulating agent defines the ligand for these responses and links both phagocytosis and activation of the 5-lipoxygenase pathway to the transmembrane signal of the β-glucan receptor.

MATERIALS AND METHODS

Assessment of Phagocytosis and Leukotriene Generation by Monolayers of Monocytes. Monolayers of human monocytes were prepared on siliconized glass coverslips (1) and assessed for their phagocytic capacities in Tyrode's solution containing 1 mM Ca^{2+}, 5 mM Mg^{2+}, and 0.1% gelatin (TG solution). The preformed monolayers were incubated for 40 min in a humidified CO_2 chamber at 37°C with 0.25 ml of various concentrations of zymosan or glucan particles, washed, fixed (1), and stained with Giemsa. Monolayers were also incubated for the designated times at 37°C with 0.25 ml of various concentrations of soluble glycans or TG solution alone, rinsed twice in a 50-ml volume of TG solution, and layered with 0.25 ml of the specified numbers of glucan or zymosan particles. After 30–40 min of incubation at 37°C, the monocytes were washed, fixed, and stained. The number of particles ingested by at least 300 monocytes per coverslip was determined by direct visual enumeration at ×1000 with a light microscope, and the data are expressed as the percentage of monocytes ingesting ≥1 or ≥3 particles.

For the assessment of leukotriene generation, monolayers

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Abbreviations: C3b, major cleavage fragment of the third component of complement; LTB_4, leukotriene B_4; LTC_4, leukotriene C_4; RP-HPLC, reversed-phase HPLC.

were prepared on 60-mm plastic tissue culture plates (4) and incubated with 1 ml of specified concentrations of zymosan or glucan particles. Monolayers were also preincubated with 1 ml of the soluble glycans or TG solution alone at 37°C, washed three times with 2.5 ml of TG solution, and then exposed to zymosan or glucan particles in 1 ml of TG solution. Calcium ionophore A23187 (Calbiochem–Behring) was dissolved at 10 mM in dimethyl sulfoxide, diluted to 2.5 μM with TG solution, and incubated in volumes of 1 ml for 40 min at 37°C with monolayers pretreated with soluble β-glucan or TG solution alone. The medium from each monocyte monolayer was decanted and centrifuged at 1100 × *g* for 5 min at 25°C and the resulting supernatant was stored at −70°C. The monolayers were washed three times with 2.5 ml of TG solution and incubated with 1 ml of 100% methanol at 4°C for 12 hr. The methanolic extracts were decanted, cleared by centrifugation at 1500 × *g* for 20 min at 4°C, evaporated to dryness under negative pressure in a Speed Vac concentrator (Savant), dissolved in 500 μl of Tris Isogel buffer (0.1 M Tris·HCl/0.14 M NaCl/0.1% gelatin, pH 7.4), and stored at −70°C.

The RIA for LTB_4 was performed in a 250-μl volume containing Tris Isogel buffer, monocyte-derived samples or synthetic LTB_4 (15), [14,15-^{3}H]LTB_4 (32 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear), and immune rabbit plasma to LTB_4 (16). The reaction mixtures were incubated at 37°C for 60 min before the addition of 100 μl of goat anti-rabbit IgG plasma at slight antibody excess. After incubation for 18 hr at 4°C, the immune precipitates were sedimented by centrifugation at 1500 × *g* for 60 min at 4°C, dissolved in 200 μl of 0.1 M NaOH, mixed with 2.5 ml of Hydrofluor (National Diagnostics, Somerville, NJ), and measured for radioactivity in a liquid scintillation counter (Mark III, Tracor Analytic, Elk Grove, IL). Synthetic LTB_4 was detectable on the linear portion of the radioligand binding–inhibition curves at concentrations of 0.05–1.0 ng. The RIA for LTC_4 was carried out in a similar fashion in a 300-μl volume containing monocyte-derived sample or synthetic LTC_4 (17), [14,15-^{3}H]LTC_4 (39 Ci/mmol; New England Nuclear), and immune rabbit plasma to LTC_4 (18). Synthetic LTC_4 was detectable in the linear portion of the radioligand inhibition–binding curves over a dose range of 0.03–1.0 ng.

To assess the metabolism of LTB_4 and LTC_4, monocyte monolayers on Petri dishes were incubated for 40 min at 37°C with 1 ml of TG solution containing 2.2 × 10^7 glucan particles per ml and 62,864 cpm of [^{3}H]LTB_4 or 85,830 cpm of [^{3}H]LTC_4. The monocyte media were decanted and the major portions were centrifuged at 1100 × *g* for 5 min at 25°C. One-hundred-microliter portions of media and supernatants were mixed with 10 ml of Hydrofluor and measured for radioactivity, and 0.7-ml portions of supernatants were mixed with 1 ml of 100% methanol for 30 min at 4°C and cleared by centrifugation at 1500 × *g* for 20 min at 4°C; 1 ml was injected onto a 10-μm C_{18} Ultrasil-ODS column (4.6 × 250 mm, Altex, Berkeley, CA). Reversed-phase HPLC (RP-HPLC) was carried out at a flow rate of 1 ml/min in an isocratic solvent of 65% methanol/34.9% water/0.1% acetic acid (vol/vol), pH 5.6. One-milliliter fractions were collected for 45 min and each fraction and the methanolic starting material was assessed for radioactivity. The column was calibrated with external synthetic standards for the retention times (mean ± SD, n = 4) of LTC_4 (13.0 ± 1.1 min), (5*S*,12*R*)-6-*trans*-LTB_4 (17.6 ± 0.1 min), (5*S*,12*S*)-6-*trans*-LTB_4 (18.5 ± 0.3 min), and LTB_4 (21.1 ± 1.3 min).

Preparation of Zymosan and Glycans and Analysis of Glycans by GLC. Zymosan (Sigma) was boiled for 30 min in 0.15 M NaCl, washed, resuspended in 0.15 M NaCl, and quantitated on a Coulter Counter; it contained 6.75 × 10^7 particles per mg. Glucan, β-glucan, and mannan (Sigma) were dispersed into TG solution, mixed in a Vortex, and passaged through 26-gauge needles. One milligram of glucan contained 4.3 × 10^7 particles by measurements on the Coulter Counter; the particles had diameters of 3–4 μm, as assessed with a cytofluorograph (Ortho Instruments, Westwood, MA) with latex beads as standards. To expedite their solubilization, β-glucan and mannan were incubated for 2 hr at 37°C before use.

To determine the neutral sugar compositions of glucan, β-glucan, and mannan, a 500-μg sample of each was acid hydrolyzed, mixed with 50 μg of *m*-inositol, desalted, reduced, and acetylated as described (19). Two microliters of the resulting solution was injected into an F and M model 402 GC unit (Hewlett–Packard) equipped with a dual-flame hydrogen ionization detector and attached to a glass column (2 m × 2 mm in diameter) packed with Tabsorb (19) and to a chart recorder. The oven temperature was programed from 160°C to 205°C at increments of 2°C/min. Each sugar moiety in the samples was identified by its retention time relative to those of the external standards, fucose, mannose, galactose, glucose, and *m*-inositol, and was quantitated by integration of the area under the peaks with the internal standard *m*-inositol for calibration. Glucan contained 96% glucose and 4% mannose, β-glucan contained 98% glucose and 2% mannose, and mannan consisted of 94% mannose, 2% galactose, 3% glucose, and 1% of an unknown sugar that chromatographed between glucose and *m*-inositol.

RESULTS

Capacity of Particulate Glucan and Zymosan to Stimulate Phagocytosis and Leukotriene Generation by Human Monocytes. Monolayers of monocytes were assessed for their dose-related phagocytosis of 1.1 × 10^6 to 2.2 × 10^7 glucan particles per ml or 1.5 × 10^6 to 3 × 10^7 zymosan particles per ml and for their dose-dependent generation of LTB_4 and LTC_4 after 40 min at 37°C. The percentage of monocytes ingesting ≥1 glucan particle was 42% with 1.1 × 10^6 glucan particles per ml, reached a plateau of 72% with 4.3 × 10^6 per ml, which persisted with 1.1 × 10^7 per ml, and decreased to 45% with 2.2 × 10^7 per ml (Fig. 1*A*). At 1.1 × 10^6 per ml, a target-to-monocyte ratio of 0.5:1, glucan stimulated release of 0.91 ng of immunoreactive LTB_4 per 10^6 monocytes and 0.39 ng of LTC_4 per 10^6 monocytes and these values increased to maximal levels of 4.04 ng and 0.70 ng of LTB_4 and LTC_4, respectively, per 10^6 monocytes with 1.1 × 10^7 glucan particles per ml (Fig. 1*B*). The maximal values of released LTB_4 and LTC_4 were obtained with 1.1 × 10^7 glucan particles per ml and were 4.93 ± 3.43 ng per 10^6 monocytes (mean ± SD, n = 3) and 0.43 ± 0.23 ng per 10^6 monocytes, respectively. The percentage of monocytes ingesting ≥1 zymosan particle continued to increase in a dose-dependent manner over the entire range of zymosan inputs and reached a level of 76% with 3 × 10^7 zymosan particles per ml (Fig. 1*C*). At 3 × 10^6 per ml, zymosan particles stimulated release of 0.19 ng of LTB_4 and 0.40 ng of LTC_4 per 10^6 monocytes and these values increased to 0.91 ng of LTB_4 and 0.67 ng of LTC_4 per 10^6 monocytes with 3 × 10^7 zymosan particles per ml (Fig. 1*D*). Methanolic extracts of unstimulated and stimulated monocyte monolayers were not different in their content of immunoreactive LTB_4 or immunoreactive LTC_4 and the values obtained were at the limits of detection.

To determine if the decreased release of LTB_4 by monocytes interacted with 2.2 × 10^7 glucan particles per ml was due to the catabolism of LTB_4 or LTC_4, duplicate monolayers of monocytes were incubated for 40 min at 37°C with 2.2 × 10^7 glucan particles per ml and 62,864 cpm of [^{3}H]LTB_4 or 85,830 cpm of [^{3}H]LTC_4. Methanolic extracts of supernatants from these monolayers contained 71% of the cpm of [^{3}H]LTB_4 added and 72% of the cpm of [^{3}H]LTC_4 added and RP-HPLC resolved single peaks containing 88% of the

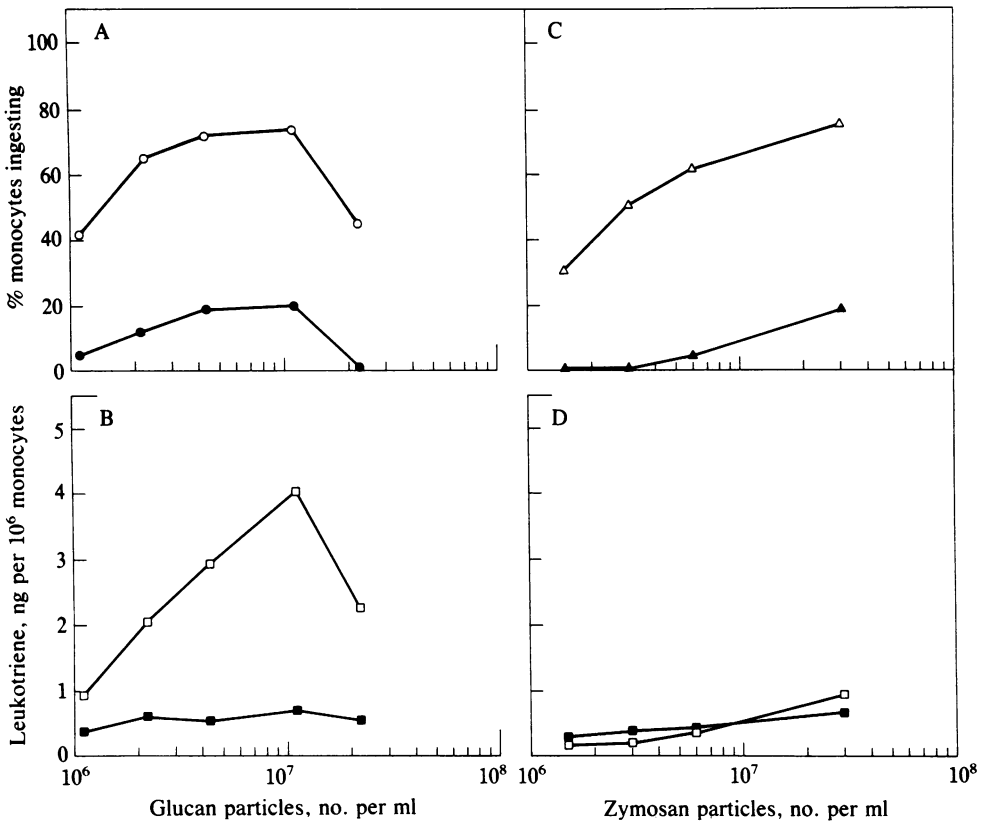

FIG. 1. Dose-dependent effects of glucan particles and zymosan particles on the capacity of adherent monocytes from the same donor to phagocytose ≥1 (○) and ≥3 (●) glucan particles (*A*) or ≥1 (△) and ≥3 (▲) zymosan particles (*C*) and dose effects on the release of immunoreactive LTB_4 (□) and LTC_4 (■) in response to glucan (*B*) or zymosan (*D*) particles. The phagocytic data are expressed as the percentage of ingesting monocytes in single determinations on coverslips and the leukotriene measurements are plotted as mean ng per 10^6 monocytes from duplicates on Petri dishes.

[^{3}H]LTB_4 and 79% of the [^{3}H]LTC_4 applied. To determine if LTB_4 or LTC_4 was adsorbed by glucan particles, 1 ml of 2.2 × 10^7 glucan particles per ml and 65,487 cpm of [^{3}H]LTB_4 or 81,946 cpm of [^{3}H]LTC_4 were incubated with gentle agitation for 40 min at 37°C in the CO_2 chamber and then centrifuged for 5 min at 1100 × *g* at 25°C. The supernatants contained 89% of the [^{3}H]LTB_4 and 92% of the [^{3}H]LTC_4. Thus, glucan particles at a concentration in excess of the concentration that stimulates optimal leukotriene generation and phagocytosis did not induce monocyte catabolism of either LTB_4 or LTC_4 and did not adsorb released leukotrienes.

Effects of Pretreatment of Monocytes with Soluble β-Glucan and α-Mannan on Subsequent Particulate Glucan- and Zymosan-Mediated Phagocytosis and Leukotriene Generation. Monolayers of monocytes were incubated at 37°C for 40 min with β-glucan or α-mannan at 50 μg/ml to 1 mg/ml, washed, and assessed for their phagocytosis of 2.2 × 10^6 and 8.6 × 10^6 glucan particles per ml or of 5 × 10^6 and 2 × 10^8 zymosan particles per ml after 40 min at 37°C. For leukotriene measurements, corresponding monolayers of monocytes were prepared on Petri dishes, treated with each concentration of the soluble glycans for 40 min at 37°C, washed, and incubated for 40 min at 37°C with 8.6 × 10^6 glucan particles per ml or 2 × 10^8 zymosan particles per ml, an input shown previously to stimulate maximal levels of LTB_4 and LTC_4 (4). With 2.2 × 10^6 glucan particles per ml, pretreatment with 100 μg of β-glucan per ml decreased the percentage of ingesting monocytes from 62% to plateau levels of 25%, and with 8.6 × 10^6 glucan particles per ml the decrease was from 74% to 51%; 1 mg of mannan per ml had no effect (Fig. 2*A*). Monocytes stimulated with 8.6 × 10^6 glucan particles per ml released 4.03 ng and 0.60 ng of LTB_4 and LTC_4, respectively, per 10^6 monocytes and these levels decreased to 1.58 ng of LTB_4 and 0.20 ng of LTC_4 for monocytes pretreated with 100 μg of β-glucan per ml, but generation was unaffected for monocytes pretreated with as much as 1 mg of mannan per ml (Fig. 2*B*). Soluble β-glucan and mannan did not stimulate monocyte release of either LTB_4 or LTC_4. In three additional experiments, pretreatment of monocytes with 100 μg of β-glucan per ml for 40 min decreased the subsequent release of LTB_4 by monocytes incubated with 8.6 × 10^6 glucan particles per ml from 2.62 ± 1.31 ng per 10^6 (mean ± SD, *n* = 3) to 1.20 ± 0.74 ng per 10^6 monocytes. In one of

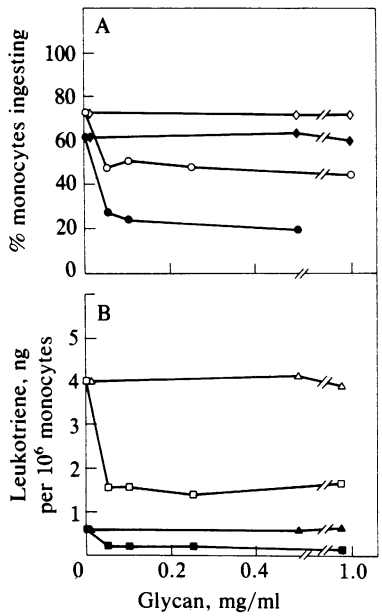

FIG. 2. Dose-dependent effects of pretreatment with soluble β-glucan (○, ●) and mannan (◇, ◆) on the percentages of adherent monocytes subsequently ingesting ≥1 glucan particle at inputs of 2.2 × 10^6 per ml (●, ◆) and 8.6 × 10^6 per ml (○, ◇) (*A*) and dose-dependent effects of pretreatment with β-glucan (□, ■) and mannan (△, ▲) on the subsequent release of immunoreactive LTB_4 (□, △) and LTC_4 (■, ▲) by 8.6 × 10^6 glucan particles per ml (*B*). The data represent a single experiment performed simultaneously with monocytes from the same donor used for the study depicted in Fig. 3.

these experiments, glucan particles stimulated the release of 2.68 and 2.60 ng of LTB_4 per 10^6 monocytes treated without and with 1 mg of mannan per ml, respectively. Preincubation with 100 μg of β-glucan per ml reduced the percentages of monocytes ingesting ≥1 zymosan particle from 60% to 20% and from 78% to 70% with inputs of 5×10^6 and 2×10^8 zymosan particles per ml, respectively (Fig. 3*A*). Mannan at 1 mg/ml did not alter phagocytosis. Zymosan particles at 2×10^8 per ml, a target-to-monocyte ratio of about 100:1, stimulated monocyte release of 4.45 ng of LTB_4 and 2.27 ng of LTC_4 per 10^6 cells and this was decreased to 1.34 ng of LTB_4 and 1.69 ng of LTC_4 per 10^6 monocytes by the pretreatment of monocytes with 100 μg of β-glucan per ml (Fig. 3*B*). Mannan had no effect at lesser concentrations but at 1 mg/ml decreased the subsequent zymosan-induced release of LTB_4 and LTC_4 to 3.5 ng and 2.07 ng, respectively, per 10^6 monocytes.

To determine the rate of inhibition by soluble β-glucan, monolayers were preincubated with 100 μg of β-glucan per ml for 0–60 min at 37°C, washed, and assessed for their release of LTB_4 and LTC_4 after a 40-min incubation with 8.6×10^6 glucan particles per ml. Pretreatment of monocytes with β-glucan decreased their subsequent release of LTB_4 by glucan particles in a time-dependent fashion from 3.80 ng per 10^6 cells to plateau levels of 1.88 ng per 10^6 cells within 20 min (Fig. 4). To determine if β-glucan mediated inhibition by inactivating the 5-lipoxygenase pathway enzymes necessary for generation of leukotrienes, monolayers were incubated with β-glucan at 100 μg/ml to 1 mg/ml for 40 min at 37°C, washed, and then assessed for their capacity to release LTB_4 and LTC_4 after a 40-min incubation with 2.5 μM ionophore. Preincubation of monocytes from two different donors with as much as 1 mg of β-glucan per ml did not decrease their subsequent ionophore-induced release of either LTB_4 or LTC_4 (Fig. 5). Thus, β-glucan inhibited generation of LTB_4 by monocytes stimulated with glucan particles at a rate consistent with that of a receptor-mediated mechanism, which did not involve inactivation of the 5-lipoxygenase pathway enzymes for either LTB_4 or LTC_4.

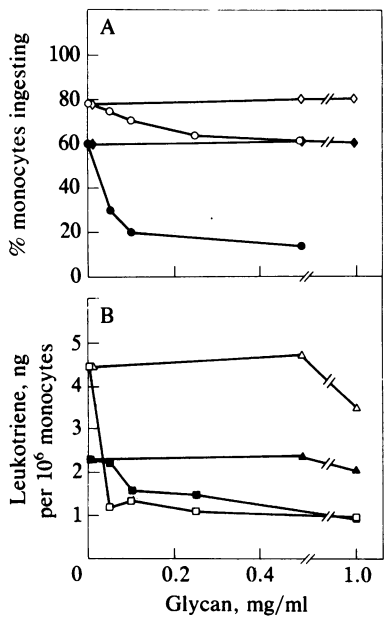

FIG. 3. Dose-dependent effects of pretreatment with soluble β-glucan (○, ●) and mannan (◇, ◆) on the percentages of adherent monocytes subsequently ingesting ≥1 zymosan particle at inputs of 5×10^6 per ml (●, ◆) and 2×10^8 per ml (○, ◇) (*A*) and dose-dependent effects of pretreatment with β-glucan (□, ■) and mannan (△, ▲) on the subsequent release of immunoreactive LTB_4 (□, △) and LTC_4 (■, ▲) by 2×10^8 zymosan particles per ml (*B*).

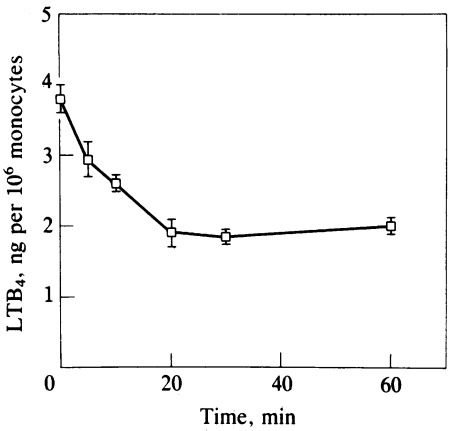

FIG. 4. Time-dependent effects of pretreatment of adherent monocytes with 100 μg of soluble β-glucan per ml on their subsequent release of LTB_4 (□) after a 40-min incubation with 8.6×10^6 glucan particles per ml. The data are the mean of duplicate determinations in a single experiment.

DISCUSSION

The capacity of glucan particles to stimulate human monocyte phagocytosis and the oxidative metabolism of monocyte arachidonic acid to LTB_4 (Fig. 1 *A* and *B*) identifies glycan-mediated responses not previously described. At low target-to-monocyte ratios, glucan particles initiated a phagocytic response similar to that occurring with zymosan particles and an oxidative response that preferentially generated LTB_4. Of the two glycan constituents of zymosan, β-glucan at 50 μg/ml functionally inhibited phagocytosis of 51 μg of glucan per ml (2.2×10^6 particles per ml) and 74 μg of zymosan per ml (5×10^6 per ml) by 55% and 52%, respectively, whereas mannan at 20-fold higher concentrations did not affect ingestion of either particle (Figs. 2*A* and 3*A*). On a weight basis, 200 μg of glucan per ml (8.6×10^6 per ml) and 3 mg of zymosan per ml (2×10^8 per ml) were required to induce comparable levels of LTB_4 release by monocytes and these levels were reduced by 61% and 73%, respectively, by 50 μg of β-glucan per ml but were not affected by 1 mg of mannan per ml (Figs. 2*B* and 3*B*). Therefore, the active constituent of both glucan and zymosan particles that promotes phagocytosis and oxidative generation of LTB_4 is β-glucan. The findings that functional inhibition by soluble β-glucan proceeded at a rate (Fig. 4) comparable to those observed previously for phagocytic responses (1) and did not involve

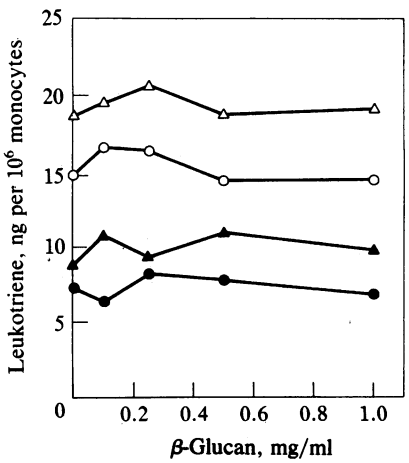

FIG. 5. Dose-dependent effects of β-glucan pretreatment of adherent monocytes from two separate donors on the subsequent release of LTB_4 (△, ○) and LTC_4 (▲, ●) with 2.5 μM calcium ionophore A23187.

inactivation of the 5-lipoxygenase enzymes (Fig. 5) provide further evidence that the inhibitory effects by soluble β-glucan on particulate β-glucan and zymosan are mediated by a monocyte receptor with ligand specificity for β-glucan. The previously described monocyte receptor that directly recognizes and phagocytoses particulate activators of the human alternative complement pathway and that upon stimulation by high inputs of zymosan particles promotes release of leukotrienes (1, 4) is now designated as the β-glucan receptor.

Because the β-glucan receptor mediates phagocytosis in the absence of opsonins (1), it is a clearance mechanism available to the nonimmune host. That this receptor is part of a general nonimmune host defense system is suggested by the capacity of its particulate activators to also initiate rapid cleavage of the third component of complement, C3, through the alternative complement pathway (1, 20, 21). The presence of C3 cleavage fragments, such as C3b (2, 3) or inactivated C3b (iC3b) (22), on particulate activators promotes their adherence to specific monocyte complement receptors and augments their clearance through the β-glucan receptor. The phagocytic response mediated by the β-glucan receptor is also augmented by a second cofactor, a cleavage fragment of human fibronectin (23). Unlike the C3 cleavage fragments that augment phagocytic responses through Fc-IgG (3, 24) or β-glucan (2, 3) receptors, the fibronectin fragment selectively opsonizes particulate activators and binds them to monocyte fibronectin receptors so as to augment clearance mediated solely by the β-glucan receptor (25). That phagocytic stimulation of the monocyte Fc-IgG receptor is more effective than that of the β-glucan receptor in the release of cyclooxygenase products (26), whereas perturbation of the β-glucan receptor preferentially activates the lipoxygenase pathway (4), indicates that these two phagocytic receptors regulate different cellular responses. The major lipoxygenase product released after phagocytic stimulation of the β-glucan receptor is LTB_4 (ref. 4; Fig. 1*B*), which is a potent chemotactic factor (27–29) and which promotes adherence of neutrophils to endothelial cell surfaces (30). The release of LTB_4 by monocytes during nonimmune phagocytosis not only may potentiate recruitment and margination of leukocytes onto the interior surface of blood vessels but also may create a gradient for the entry of leukocytes into the tissue space. Thus, the presence of a particulate activator may rapidly initiate assembly and amplification of a host defense system involving β-glucan receptors whose cellular and mediator components are fully activatable in the absence of adaptive immunity.

This work was supported by Grants AI-22531, AI-10356, AI-17132, AM-20580, and RR-05669 from the National Institutes of Health.

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