

# Cross-Linking of the $\beta$ -Glucan Receptor on Human Monocytes Results in Interleukin-1 Receptor Antagonist But Not Interleukin-1 Production

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The  $\beta$ -glucan receptor, found on monocytes and neutrophils, binds glucose polymers derived from fungi. Ligands for the receptor have various immunomodulatory effects, including increased microbicidal killing activity. We have investigated the effect of  $\beta$ -glucans on the production of interleukin-1 (IL-1) and its naturally occurring inhibitor, the IL-1 receptor antagonist (IL-1Ra). Particulate  $\beta$ -glucan induced IL-1Ra production from human peripheral blood mononuclear cells (PBMC) but did not stimulate IL-1 $\beta$  synthesis or gene expression in these same cells. Monomeric (soluble)  $\beta$ -glucan did not induce IL-1Ra production. However, when preincubated with PBMC, monomeric  $\beta$ -glucan

significantly ( $P < .01$ ) reduced particulate  $\beta$ -glucan induction of IL-1Ra by 40%, suggesting that crosslinking of  $\beta$ -glucan receptors is required for induction of IL-1Ra. In support of this, monomeric  $\beta$ -glucan immobilized on plastic surfaces stimulated IL-1Ra production. Vitamin D<sub>3</sub>, which increases the functional capacity of  $\beta$ -glucan receptors, increased IL-1Ra production induced by particulate  $\beta$ -glucan, whereas dexamethasone suppressed IL-1Ra synthesis. Because of their differential effects on cytokine production,  $\beta$ -glucans may be used to therapeutic advantage in the diseases in which IL-1 is implicated.

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**T**HE  $\beta$ -GLUCAN RECEPTOR is a trypsin-sensitive receptor on monocytes and other cells to which bind glucose polymers derived from fungi.<sup>1</sup> Activities associated with the  $\beta$ -glucan receptor include the opsonin-independent clearance of yeast and fungi, the release of lysosomal enzymes, and the production of leukotrienes from human monocytes.<sup>2,3</sup> Ligands for the  $\beta$ -glucan receptor have immunomodulatory activities. For example, lentinan, a  $\beta$ -glucan derived from the Japanese mushroom *Lentinus edodes*, has antitumor activity in animals,<sup>4</sup> stimulates interleukin-1 (IL-1) activity in human monocytes,<sup>5</sup> and augments the in vivo generation of alloreactive cytotoxic T cells.<sup>6</sup> Ongoing clinical trials suggest that  $\beta$ -glucan receptor ligands enhance nonspecific resistance to infection.

IL-1 is implicated in a number of disease states such as septic shock, rheumatoid arthritis, and leukemia.<sup>7</sup> A naturally occurring IL-1 inhibitor, the IL-1 receptor antagonist (IL-1Ra) can reduce or prevent a variety of diseases in animals and the effects of IL-1 in vitro.<sup>8</sup> A potential mechanism of action of immunomodulators may be to modify the balance between IL-1 and IL-1Ra. Therefore, we studied the effect of particulate and soluble  $\beta$ -glucans derived from the yeast *Saccharomyces cerevisiae* on the production of IL-1Ra and IL-1 $\beta$  by peripheral blood mononuclear cells (PBMC). We demonstrate that  $\beta$ -glucans exert a differential effect on cytokine production and that the ability of a  $\beta$ -glucan to cross-link its receptor is related to cytokine production.

## MATERIALS AND METHODS

**Materials.** The following materials were purchased: Ficoll type 400, formaldehyde, EDTA and lipopolysaccharide (*Escherichia coli*, serotype 055:B5; Sigma Chemical Company, St Louis, MO); Hypaque-M, 90% (Winthrop Pharmaceuticals, New York, NY); sterile 0.9% saline (Abbott Laboratories, Rockford, IL); RPMI 1640 culture medium (Whittaker M.A. Bioproducts, Walkersville, MD); polypropylene tubes (12  $\times$  75 mm) and 24-well polystyrene plates (Becton Dickinson Laboratories, Lincoln Park, IL); penicillin and streptomycin (GIBCO, Grand Island, NY); dexamethasone (LypheMed, Inc, Rosemont, IL); guanidine isothiocyanate (BRL, Life Technologies, Gaithersburg, MD); agarose and cesium chloride (International Biotechnology, Inc, New Haven, CT); nylon hybridization transfer membranes (Hybond-N, Amersham Corp, Arlington Heights, IL); random primed DNA labeling kits (Boehringer Mannheim, Mannheim, Germany); (<sup>32</sup>P)dCTP (New England Nuclear,

Boston, MA). Human IgG, clinical grade suitable for intravenous injection, and human serum albumin (HSA) were gifts of Hyland Laboratories (Duarte, CA).

1,25-Dihydroxyvitamin D<sub>3</sub> (vitamin D<sub>3</sub>) was a gift of Dr Maurice Pechet (Research Institute of Medicine and Chemistry, Cambridge, MA). The cDNA probes were gifts of the following: IL-1Ra from Dr A. Shaw (Glaxo Institute for Molecular Biology, Geneva, Switzerland) and chicken  $\beta$ -actin from Dr B. Huber (Tufts University, Boston, MA).

Zymocel is a particulate poly-(1-6)- $\beta$ -D-glucopyranosyl-(1-3)- $\beta$ -D-glucopyranose polymer derived from a nonrecombinant proprietary strain of the yeast, *S cerevisiae*. Betafectin is a soluble hydrolysis product of Zymocel, which has an average molecular weight of 80 kD. They were gifts of Dr Gary Ostroff (Alpha-Beta Technologies, Inc, Worcester, MA).

**Study population.** The study was approved by the Human Investigation Review Committee of the New England Medical Center Hospitals. Informed consent was obtained from each subject. Healthy male and female volunteers 20 to 50 years of age taking no medications were recruited from laboratory and office personnel.

**PBMC.** PBMC were isolated from the blood of healthy human volunteers as previously described.<sup>9</sup> In brief, after isolation by Ficoll-Hypaque centrifugation and washing in 0.9% saline, PBMC (final density of  $2.5 \times 10^6$ /mL) were resuspended in serum-free RPMI containing penicillin (100 U/mL), streptomycin (100  $\mu$ g/mL), and HEPES (0.01 mol/L). RPMI was subjected to ultrafiltration to remove cytokine-inducing substances prior to use.<sup>10</sup> PBMC were cultured with Betafectin, Zymocel, vitamin D<sub>3</sub> ( $1 \times 10^{-8}$  mol/

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L), LPS (10 ng/mL), or human IgG (10  $\mu$ g/mL) at 37°C in a 5% CO<sub>2</sub> atmosphere in polypropylene tubes or, if stated, in polystyrene plates, for 24 or 48 hours. The concentrations of the latter two stimuli induce maximal IL-1Ra production by PBMC.<sup>9</sup>

**Immobilization of Betaflectin.** Betaflectin (100  $\mu$ g/mL) in coating buffer (0.1 mol/L NaHCO<sub>3</sub>, pH 9.2) or coating buffer alone was added to 16-mm wells of polystyrene plates overnight at 4°C. The contents of the wells were removed and replaced with 1% HSA for 2 hours at room temperature. The wells were rinsed three times with RPMI before culture of PBMC.

**Cytokine determinations.** IL-1Ra and IL-1 $\beta$  concentrations were determined using specific radioimmunoassays.<sup>9,11</sup> The sensitivities were 80 to 160 and 30 to 80 pg/mL, respectively. The IL-1 $\beta$  radioimmunoassay (RIA) detects both the precursor and mature forms of IL-1 $\beta$ .<sup>12</sup> Samples with undetectable levels of cytokine were assigned a cytokine concentration equal to the lower limit of detection of the RIA.

**RNA analysis.** PBMC were centrifuged and the pellets were lysed with 4 mol/L guanidine isothiocyanate in 2% sarkosyl, 0.01 mol/L EDTA, and 1%  $\beta$ -mercaptoethanol. Total cellular RNA was obtained by centrifugation through 5.7 mol/L cesium chloride in 0.1 mol/L EDTA, followed by precipitation with 2.5 volumes of absolute ethanol and 0.1 volume of 3 mol/L sodium acetate per standard methods.<sup>13</sup> For Northern analysis, RNA was electrophoresed in a 1.2% agarose gel containing 6.6% formaldehyde as a denaturing agent and was transferred by capillary blotting onto nylon membranes. The membrane-bound RNA was fixed by short wave UV irradiation and sequentially hybridized to the following (<sup>32</sup>P)dCTP-labeled cDNA probes: an 800-bp fragment of human IL-1Ra cDNA subcloned into pUC8, a 1,075-bp fragment of human IL-1 $\beta$  precursor cDNA subcloned into pGEM2, and the full length of chicken  $\beta$ -actin cDNA subcloned into pGEM3. Membranes were then subjected to autoradiography at -70°C using intensifying screens.

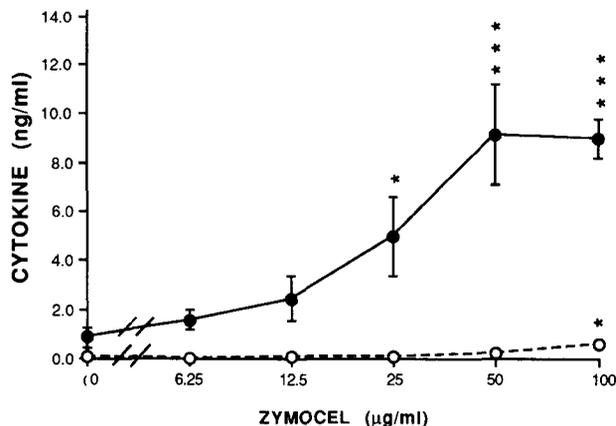
**Statistics.** Two-tailed paired *t*-tests and analysis of variance (ANOVA) using Fisher's least significant difference were performed using Stat-View software (Abacus Concepts, Inc, Calabasas, CA) on a Macintosh Classic computer (Apple Computer, Inc, Cupertino, CA). Data are expressed as mean  $\pm$  SEM.

## RESULTS

**Effect of engagement of the  $\beta$ -glucan receptor by particulate ligand on cytokine production.** To determine the effect of stimulation of the  $\beta$ -glucan receptor on cytokine production, PBMC were cultured in the presence of increasing concentrations of the particulate  $\beta$ -glucan, Zymocel. After 24 hours, total IL-1Ra and IL-1 $\beta$  were measured. As shown in Fig 1, Zymocel stimulated IL-1Ra production by PBMC in a dose-dependent manner ( $P < .001$ ), reaching maximal production (9.1  $\pm$  2.1 ng/mL) at a concentration of 50  $\mu$ g/mL. In comparison, the concentration of IL-1Ra synthesized by parallel cultures of PBMC stimulated with lipopolysaccharide (LPS) was 9.4  $\pm$  0.7 ng/mL. Unstimulated PBMC produced 0.9  $\pm$  0.4 ng/mL.

IL-1 $\beta$  production in the same PBMC cultures stimulated with Zymocel was negligible (0.03  $\pm$  0.03 to 0.6  $\pm$  0.1 ng/mL over the same concentration range). Only at a concentration of Zymocel of 100  $\mu$ g/mL was there significant IL-1 $\beta$  production ( $P < .05$  by ANOVA). In contrast, these cells produced IL-1 $\beta$  (3.9  $\pm$  0.5 ng/mL) in response to LPS (10 ng/mL).

**Priming of IL-1 $\beta$  production by Zymocel.** Certain stim-



**Fig 1.** Effect of Zymocel on cytokine production. PBMC were cultured in serum-free RPMI in the presence of increasing concentrations of Zymocel. After 24 hours, the cultures were harvested and assayed for total (●) IL-1Ra and (○) IL-1 $\beta$ . Each point represents the mean  $\pm$  SEM from three individuals. \* $P < .05$ , \*\*\* $P < .001$  (ANOVA) for control v Zymocel.

uli, which alone do not induce IL-1 $\beta$  synthesis, will increase the amount of IL-1 $\beta$  produced in response to low concentrations of LPS or IL-1 itself.<sup>12,14,15</sup> This phenomenon is termed priming. To determine if Zymocel possessed the ability to prime PBMC for LPS-induced IL-1 $\beta$  synthesis, PBMC were cultured with RPMI, Zymocel (12.5  $\mu$ g/mL), LPS (0.1 ng/mL), or Zymocel plus LPS. After 24 hours, IL-1 $\beta$  was found in low concentrations (0.06 and 0.061 ng/mL) in response to RPMI or Zymocel, respectively. In contrast, LPS-induced IL-1 $\beta$  production was significantly augmented by the presence of Zymocel (from 2.02  $\pm$  0.60 to 3.06  $\pm$  0.66 ng/mL, respectively;  $n = 5$ ;  $P = .02$ ).

**Effect of soluble  $\beta$ -glucan on cytokine production.** To compare the effect of engagement of the  $\beta$ -glucan receptor with soluble and particulate ligand, PBMC were cultured with Betaflectin (20 to 125  $\mu$ g/mL), a soluble derivative of Zymocel. After 24 hours, total IL-1Ra and IL-1 $\beta$  synthesis were measured (Table 1). Although PBMC produced both cytokines in response to LPS, there was no increased synthesis of IL-1Ra or IL-1 $\beta$  by cells cultured with Betaflectin.

**Specific inhibition by Betaflectin of Zymocel-induced IL-1Ra production.** Soluble  $\beta$ -glucan preparations inhibit the effects of particulate  $\beta$ -glucans in several models,<sup>2,3,16,17</sup> presumably by occupying the  $\beta$ -glucan receptor without triggering a response. We studied whether this occurred for Zymocel-induced IL-1Ra production. PBMC were preincubated at 37°C for 1 hour with RPMI or Betaflectin (20 to 125  $\mu$ g/mL). Subsequently, Zymocel was added, with the concentration of Betaflectin being maintained. PBMC cultured with RPMI or Betaflectin only served as controls. After 24 hours, total IL-1Ra was measured (Fig 2A). Betaflectin (20  $\mu$ g/mL) significantly reduced Zymocel-induced IL-1Ra production by 28% ( $P < .05$ ). Maximal inhibition of 40% ( $P < .01$ ) was achieved at a Betaflectin concentration of 50  $\mu$ g/mL. Higher concentrations of Betaflectin did not increase the degree of inhibition further.

Table 1. Inability of Betaflectin to Induce IL-1Ra or IL-1 $\beta$ 

	RPMI	LPS (10 ng/mL)	Betaflectin		
			20 $\mu$ g/mL	50 $\mu$ g/mL	125 $\mu$ g/mL
IL-1Ra (ng/mL)	0.21 $\pm$ 0.01	4.9 $\pm$ 0.36	0.21 $\pm$ 0.03	0.23 $\pm$ 0.01	0.34 $\pm$ 0.10
IL-1 $\beta$ (ng/mL)	0.03 $\pm$ 0	5.4 $\pm$ 0.27	0.03 $\pm$ 0	0.03 $\pm$ 0	0.03 $\pm$ 0

To investigate whether these results were caused by a non-specific or toxic effect of Betaflectin, PBMC were preincubated at 37°C for 1 hour with RPMI or Betaflectin (20 to 125  $\mu$ g/mL). Subsequently, cells were stimulated with LPS, IgG, or Zymocel (50  $\mu$ g/mL) with the concentration of Betaflectin being maintained. After 24 hours, total IL-1Ra was measured (Fig 2B). The presence of Betaflectin did not influence the amount of IL-1Ra produced by PBMC cultured with RPMI, LPS (5.5  $\pm$  0.5 v 5.4  $\pm$  0.6 ng/mL) or IgG (5.9  $\pm$  0.5 v 5.9  $\pm$  0.1 ng/mL). However, Zymocel-stimulated IL-1Ra was reduced by 35% ( $P < .001$ ) in the presence of Betaflectin.

#### Effect of immobilized Betaflectin on IL-1Ra produc-

tion. The results described above suggest that both engagement and cross-linking of the  $\beta$ -glucan receptor are necessary to trigger the synthesis of IL-1Ra. To test this hypothesis, the effect of immobilized Betaflectin on IL-1Ra synthesis was studied. Polystyrene wells were coated with Betaflectin (100  $\mu$ g/mL) or coating buffer and subsequently blocked with 1% HSA. PBMC were then added. After 24 hours, IL-1Ra production was assessed. Cells cultured in soluble Betaflectin produced no more IL-1Ra than did cells cultured in RPMI only (Fig 3). However, PBMC cultured in the presence of immobilized Betaflectin produced significantly more IL-1Ra than those cultured with the soluble form (2.2  $\pm$  0.1 and 0.3  $\pm$  0.1 ng/mL, respectively;  $P < .001$ ). In comparison, PBMC stimulated by immobilized Betaflectin produced less IL-1Ra than those stimulated by Zymocel, 50  $\mu$ g/mL (4.4  $\pm$  0.1 ng/mL,  $P < .01$ ).

*Influence of vitamin D<sub>3</sub> on Zymocel-induced cytokine induction.* Vitamin D<sub>3</sub> increases the functional expression of the  $\beta$ -glucan receptor.<sup>16</sup> Therefore, PBMC were cultured in the presence of vitamin D<sub>3</sub> ( $1 \times 10^{-8}$  mol/L) for 24 hours, after which time Zymocel (50  $\mu$ g/mL) was added, while maintaining the same concentration of vitamin D<sub>3</sub>. After an additional 24 hours, the cultures were harvested and total IL-1Ra and IL-1 $\beta$  were measured. Culture of PBMC with vitamin D<sub>3</sub> resulted in a 60% increase in Zymocel-induced IL-1Ra production ( $P < .01$ ; Fig 4). In unstimulated PBMC, vitamin D<sub>3</sub> alone caused a small but statistically significant increase in IL-1Ra production compared with

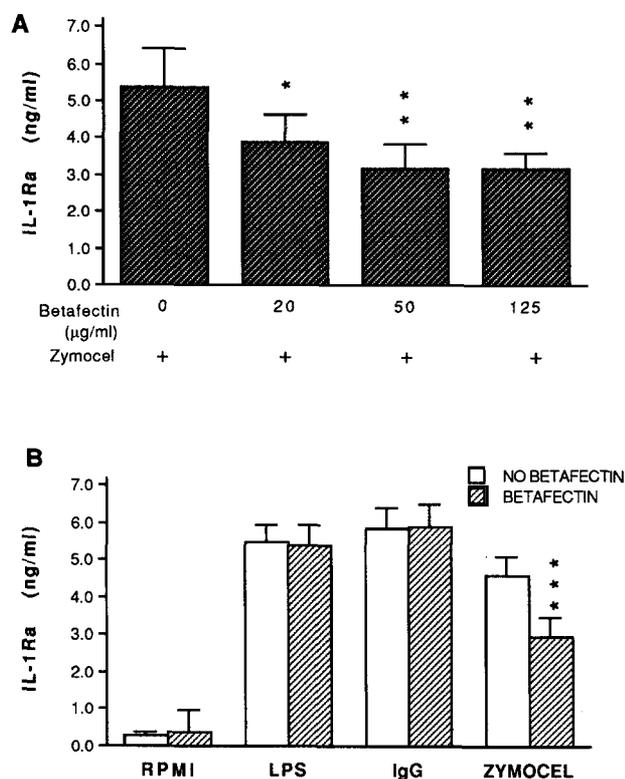


Fig 2. Betaflectin inhibits Zymocel-induced IL-1Ra production. (A) PBMC from three donors were preincubated with Betaflectin (20 to 125  $\mu$ g/mL) for 1 hour before the addition of Zymocel (50  $\mu$ g/mL). After 24 hours, total IL-1Ra was measured. (B) PBMC from 12 donors were stimulated with LPS (10 ng/mL), IgG (10  $\mu$ g/mL) or Zymocel (50  $\mu$ g/mL). Parallel cultures contained Betaflectin (50  $\mu$ g/mL), added 1 hour before the other stimuli. After 24 hours, total IL-1Ra was measured. Each bar represents the mean  $\pm$  SEM. (A) and (B) were analyzed by ANOVA and paired *t*-test, respectively. \* $P < .05$ ; \*\* $P < .01$  for Zymocel v Zymocel plus Betaflectin; \*\*\* $P < .001$  for without v with Betaflectin.

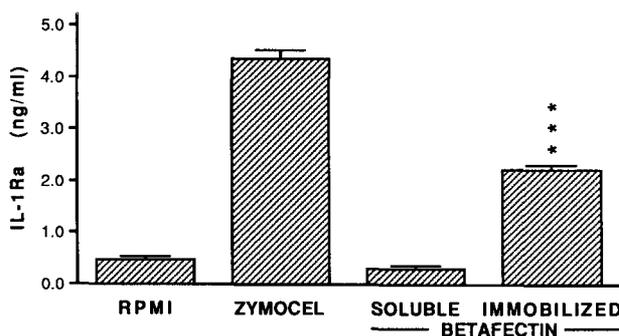
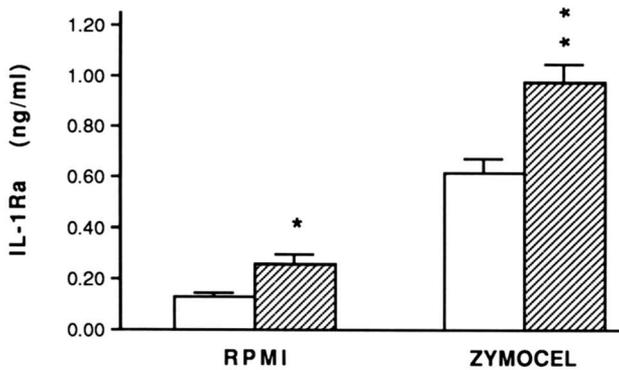


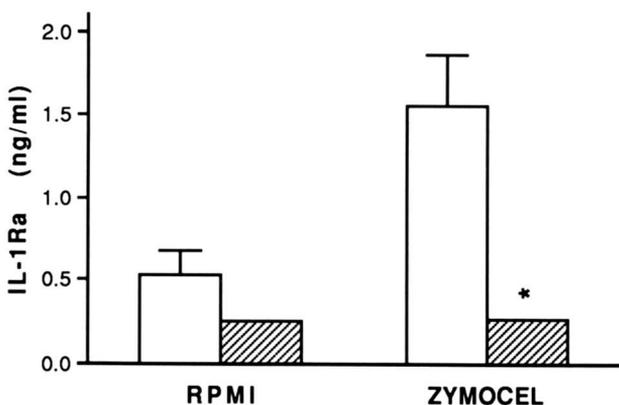
Fig 3. Induction of IL-1Ra by immobilized Betaflectin. Polystyrene wells were coated with Betaflectin (100  $\mu$ g/mL) and blocked with 1% HSA. PBMC were cultured in wells coated with immobilized Betaflectin or in control wells treated with coating buffer only and blocked with 1% HSA. To each well, Betaflectin (100  $\mu$ g/mL) was added. For comparison, PBMC were cultured in RPMI (negative control) or Zymocel (50  $\mu$ g/mL). After 24 hours, the cultures were harvested and total IL-1Ra was measured. Each bar represents the mean  $\pm$  SEM from 4 individuals. \*\*\* $P < .001$  for soluble v immobilized Betaflectin.



**Fig 4.** Effect of vitamin D<sub>3</sub> on the production of Zymocel-induced cytokines. PBMC were cultured with vitamin D<sub>3</sub> ( $1 \times 10^{-8}$  mol/L). After 24 hours, cells were stimulated with Zymocel (final concentration of 50  $\mu$ g/mL), maintaining the same concentration of vitamin D<sub>3</sub>. After an additional 24 hours of culture, the cells were harvested and total IL-1Ra was measured. Each bar represents the mean  $\pm$  SEM from three individuals. \* $P < .05$ , \*\* $P < .01$  for vitamin D<sub>3</sub> v control. (□) No vitamin D<sub>3</sub>; (▨) vitamin D<sub>3</sub>.

controls ( $0.26 \pm 0.03$  and  $0.13 \pm 0.01$  ng/mL, respectively;  $P < .05$ ). However, vitamin D<sub>3</sub> had no effect on IL-1 $\beta$  production by unstimulated PBMC or those cultured with Zymocel.

**Effect of dexamethasone on Zymocel-induced IL-1Ra production.** Dexamethasone inhibits the functional expression of the  $\beta$ -glucan receptor.<sup>17</sup> To determine its effect on Zymocel-induced IL-1Ra production, PBMC were preincubated with dexamethasone ( $2 \times 10^{-5}$  mol/L) for 24 hours. Dexamethasone alone had no effect on IL-1Ra production from unstimulated cells (data not shown). After the preincubation period, PBMC were cultured for an additional 24 hours with Zymocel in the presence or absence of dexamethasone ( $1 \times 10^{-5}$  mol/L). Total IL-1Ra was measured (Fig 5).



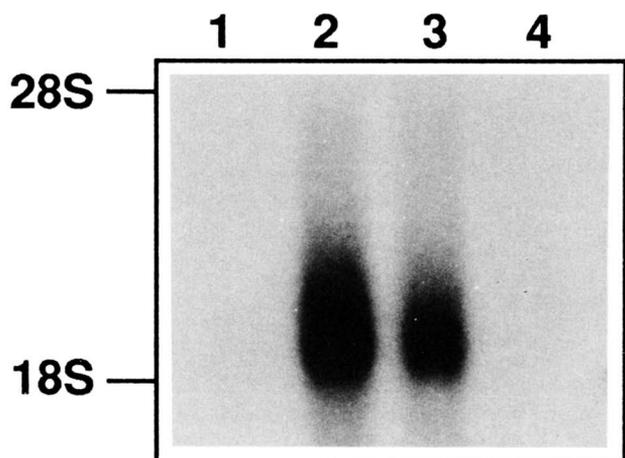
**Fig 5.** Effect of dexamethasone on Zymocel-induced IL-1Ra production. PBMC were cultured with dexamethasone ( $2 \times 10^{-5}$  mol/L). After 24 hours, Zymocel (final concentration of 50  $\mu$ g/mL) was added, with adjustment of the concentration of dexamethasone to  $1 \times 10^{-5}$  mol/L. After an additional 24 hours of culture, the cells were harvested and total IL-1Ra was measured. Each bar represents the mean  $\pm$  SEM from three individuals. \* $P < .05$  for without v with dexamethasone. (□) No dexamethasone; (▨) dexamethasone.

Dexamethasone reduced Zymocel-induced IL-1Ra production by 83% ( $P = .05$ ).

**IL-1Ra and IL-1 $\beta$  gene expression after culture with  $\beta$ -glucans.** The production of IL-1 $\beta$  is under both transcriptional and translational control<sup>12,18,19</sup> such that large amounts of mRNA may be present in PBMC without synthesis of significant amounts of the cytokine protein. Therefore, we examined if  $\beta$ -glucans triggered cytokine mRNA transcription in the absence of cytokine synthesis. PBMC were cultured for 4 hours with RPMI, LPS, Zymocel, or Betafectin. Total RNA was isolated and subjected to Northern hybridization. Steady-state IL-1Ra mRNA levels were undetectable in PBMC cultured with RPMI or Betafectin (Fig 6). However, PBMC cultured with LPS or Zymocel contained elevated steady-state levels of mRNA for IL-1Ra. Steady-state IL-1 $\beta$  levels were detectable in PBMC cultured with LPS but not with Zymocel or Betafectin (data not shown).

## DISCUSSION

Activation of the  $\beta$ -glucan receptor has modulatory effects on host defense systems.<sup>4,6</sup> The present studies further define the influence of two defined  $\beta$ -glucans, Zymocel and Betafectin. Zymocel, a particulate form of  $\beta$ -glucan, induced IL-1Ra but not IL-1 $\beta$  production. Betafectin, a soluble monomeric  $\beta$ -glucan that is derived from Zymocel, did not induce IL-1Ra, IL-1 $\beta$ , or gene expression of these cytokines. Cross-linking of the  $\beta$ -glucan receptor seems necessary for the induction of IL-1Ra because (1) Betafectin, the monomeric  $\beta$ -glucan, inhibited Zymocel-induced IL-1Ra production in a dose-dependent and specific fashion, and (2) PBMC exposed to immobilized but not soluble Betafectin produced IL-1Ra. Vitamin D<sub>3</sub> exerted differential effects on cytokine synthesis by increasing  $\beta$ -glucan-induced IL-1Ra synthesis but not  $\beta$ -glucan-induced IL-1 $\beta$  production.



**Fig 6.** Steady-state mRNA levels for IL-1Ra. PBMC were cultured for 4 hours with RPMI (lane 1), LPS (lane 2), Zymocel (50  $\mu$ g/mL; lane 3) or Betafectin (50  $\mu$ g/mL; lane 4). Twelve micrograms of RNA was applied to each lane. Comparable amounts of total RNA were detected in all lanes by ethidium bromide staining (data not shown). Similar data were obtained in a second study using a different donor.

The present studies add new information to the differential regulation of IL-1 $\beta$  and IL-1Ra production. Zymocel induced amounts of IL-1Ra comparable to that induced by LPS, whereas it did not induce significant amounts of IL-1 $\beta$ . This stimulation is not a simple matter of phagocytosis because uptake of particles triggers the synthesis of both cytokines. In addition, vitamin D<sub>3</sub> augmented Zymocel-induced IL-1Ra but not IL-1 $\beta$  production, as mentioned above. These findings add to those of other studies<sup>9,19</sup> that demonstrate differential production of IL-1Ra and IL-1 $\beta$  under various conditions.

The priming effect of Zymocel for IL-1 $\beta$  production by LPS-stimulated cells is similar to that observed for other stimuli or conditions. These include recombinant C5a<sup>14,15</sup> and the culture of monocytes on glass.<sup>12</sup> In those studies, C5a or adherence to glass served as a strong transcriptional signal for IL-1 $\beta$  gene expression without translation into IL-1 $\beta$  protein. A second signal, such as LPS, was necessary for translation. However, it is unlikely the mechanism accounts for Zymocel-primed LPS-induced IL-1 $\beta$  synthesis because Zymocel alone did not act as a transcriptional signal.

The cross-linking of surface receptors by specific ligands that trigger signal transduction has been well documented in a variety of cells. For example, the culture of human monocytes on immobilized adhesion molecules or immobilized MoAbs to the receptors for the adhesion molecules induce the production of IL-1 $\beta$  and tumor necrosis factor- $\alpha$ .<sup>20</sup> In another example, goat anti-mouse IgG cross-links murine anti-CD-3 on T cells and induces transient adhesion to intracellular adhesion molecule substrates.<sup>21</sup> Early work by Arend et al showed that immobilized but not soluble immune complexes induced an IL-1 inhibitor, later renamed IL-1Ra.<sup>22</sup> These experiments suggest that, like the  $\beta$ -glucan receptor, cross-linking of the Fc $\gamma$  receptor facilitates IL-1Ra production.

The present work showed that not only occupancy but cross-linking of the  $\beta$ -glucan receptor was necessary for induction of IL-1Ra synthesis. Betafectin, a monomeric, 80-kD hydrolysis product of Zymocel, shares with its particulate parent the same glucopyranosyl structure, yet does not induce IL-1Ra. However, its binding to the  $\beta$ -glucan receptor has been shown in several studies and in the present experiments, Betafectin specifically prevented Zymocel-induced IL-1Ra production. Soluble, monomeric  $\beta$ -glucans inhibit the ingestion of yeast particles and the subsequent release of lysosomal enzymes and leukotrienes.<sup>3,16</sup> This inhibition is related to the size of the soluble  $\beta$ -glucan.<sup>2,17</sup> The smallest  $\beta$ -glucan that inhibits the  $\beta$ -glucan receptor is a heptaglucoside.<sup>23</sup> Presumably, Betafectin is acting as a competitive inhibitor by binding to the  $\beta$ -glucan receptor and hindering the binding and receptor cross-linking of Zymocel. We postulate that Betafectin lacks the size or degree of branching necessary for cross-linking of its receptor and is therefore incapable of stimulating IL-1Ra synthesis. If this were the case, we reasoned that immobilized Betafectin fixed on a surface (the culture plate) would span a distance sufficient to cross-link  $\beta$ -glucan receptors. If Betafectin molecules were in the proper spacing configuration, cross-link-

ing of the receptors would transduce the signal to initiate IL-1Ra gene and protein expression. This, in fact, occurred because immobilized Betafectin induced significant amounts of IL-1Ra.

Vitamin D<sub>3</sub> has several effects on the immune system. It enhances the interferon gamma-induced expression of Ia antigen on a murine macrophage cell line<sup>24</sup> and decreases the IL-2 activity produced by phytohemagglutinin-activated lymphocytes.<sup>25</sup> In general, treatment of monocytes or the monocyte/macrophage cell line, U937, with vitamin D<sub>3</sub> increases IL-1 production.<sup>25-27</sup> Additionally, vitamin D<sub>3</sub> causes maturational changes in U937 cells, and augmented the production of IL-1-like activity stimulated by conditioned medium.<sup>28</sup> In THP-1 cells, a human macrophage line, vitamin D<sub>3</sub> enhanced the production of IL-1 $\beta$  when stimulated by activated fixed T cells.<sup>29</sup> In contrast, Tsoukas et al found that vitamin D<sub>3</sub> decreased IL-1 $\beta$  and IL-1 $\alpha$  synthesis by human monocytes cocultured with phytohemagglutinin-stimulated lymphocytes.<sup>30</sup> In the present studies, we have found that vitamin D<sub>3</sub> augmented Zymocel-induced IL-1Ra production. A component of this increase may be caused by increased expression or function of the  $\beta$ -glucan receptor. Another possible mechanism for the increased production of IL-1Ra in the presence of vitamin D<sub>3</sub> is enhanced or accelerated maturation of the monocytes. Cells of the monocyte-macrophage lineage have an increased capacity for IL-1Ra production when exposed to differentiating agents such as granulocyte-macrophage colony-stimulating factor.<sup>9,31,32</sup>

In summary, we have described a new property associated with stimulation of the  $\beta$ -glucan receptor, namely, the induction of IL-1Ra without IL-1 $\beta$  production by PBMC. Because administration of IL-1Ra reduces the severity of infectious, inflammatory, or hematologic diseases,<sup>8</sup> further study of  $\beta$ -glucan-mediated induction of IL-1Ra is warranted.

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