[Frontiers in Bioscience 11, 2264-2274, September 1, 2006]

G_i proteins regulate lipopolysaccharide and *Staphylococcus Aureus* induced cytokine production but not (1→3)-Beta-D-glucan induced cytokine suppression

Hongkuan Fan [1], David L. Williams [2], Kevin F. Breuel [3], Basilia Zingarelli [4], Giuseppe Teti [5], George E Tempel [1], Perry V Halushka [6], and James A Cook [1]

[1] Department of Neuroscience, [6]Pharmacology, and [6]Medicine, Medical University of South Carolina, Charleston, South Carolina, 29425, [2] Department of Surgery, [3]Obstetrics and Gynecology, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, Tennessee, 37614, [4] Division of Critical Care Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, [5] Department of Experimental Pathology and Microbiology, Medical University of Messina, Messina, Italy

TABLE OF CONTENTS

1. ABSTRACT

Previous studies have demonstrated that bacterial lipopolysaccharide (LPS) and heat killed *Staphylococcus aureus* (SA) activation of inflammatory cells depended in part upon activation of heterotrimeric Gi proteins. It has also been shown that (1→3) beta-D-glucan can suppress inflammatory cell activation by microbial products although the cellular mechanism of the glucan effect remains to be clearly defined. We hypothesized that Gi proteins function as a common convergent signaling pathway for both LPS and SA leading to monocyte mediator production. Additionally, we hypothesized that soluble glucan suppresses LPS and SA induced cytokine production via Gi protein coupled signaling. Human THP-1 promonocytic cells were pretreated with pertussis toxin (PTx, 100ng/ml or 1 microgram/ml) 6 hours prior to stimulation with LPS (10 microgram/ml) and SA (10 microgram/ml) and/or soluble glucan (10 microgram/ml). Both LPS and SA significantly ($p<0.05$) induced cytokine production IL-6 >TNF alpha >IL-1 beta >GM-CSF >IL-10 >IFN gamma. The induction of these cytokines was significantly ($p<0.05$) suppressed by PTx. Glucan treatment alone had no effect on cytokine production but suppressed ($P<0.05$) LPS and SA induced cytokines. PTx further augmented ($p<0.05$) the inhibitory effect of glucan on the LPS and SA induced cytokine expression. The data support the hypothesis that Gi proteins function as a common signaling protein for both LPS and SA induction of pro-and anti-inflammatory cytokines and that soluble glucan effectively suppresses cytokine production to the microbial stimuli. In contrast, the effect of soluble glucan on inhibiting cellular activation by LPS and SA is Gi protein independent.

2. INTRODUCTION

Septic shock is most commonly induced by gram-negative and gram-positive bacteria (1,2). Activation of inflammatory cells by bacteria or their cell wall components induces pro-inflammatory mediators, which may play a central role in septic shock. The membrane glycolipid LPS appears to be the main mediator of immune responses to gram-negative bacteria while several gram-positive bacterial components including peptidoglycans, lipoteichoic acid and lipoproteins on the cell surface mediate activation (3-5).

Macrophage pro-inflammatory cytokine gene induction by LPS, and *Staphylococcus aureus* (SA) occurs through activation of different cell receptors. The first identified LPS receptor CD14, expressed on the surface of macrophage and monocytes, binds LPS with high affinity (6-8). Toll-like receptor 4 (TLR4) also plays a critical role in the LPS mediated immune response (9,10). The gram-positive bacteria SA induced signaling pathways are mainly mediated primarily through Toll-like receptor 2 (TLR2) (3,11).

The extent to which gram-negative and gram-positive bacteria show common post-receptor signaling is an area of active investigation. LPS signals through the TLR4/CD14/MD2 cell receptor complex while SA signals through the TLR2/CD14 pathway (12). Although, they interact with different receptors, LPS and SA affect common downstream signaling proteins in the TLR signaling cascade, such as MyD88, IRAK1, and TRAF6 leading to downstream pro-inflammatory gene expression

(12). Despite the fact that both agonists activate similar signaling proteins and cytokines (13,14), there are differences in the signaling pathways. For instance, blockade of the p38 pathway with antibodies prevents LPS-induced TNF-alpha production, but has no effect on SA-induced TNF-alpha production (14). LPS signaling involves MD2 while SA does not (15). TLR4 ligands unlike TLR2 ligands can also signal through a MyD88 independent pathway leading to inflammatory gene expression (16,17).

Hereotrimeric Gi proteins are a novel class of post-receptor binding proteins involved in LPS signaling. Gi proteins co-immunoprecipitate with the CD14 receptor (18). Studies utilizing PTx that specifically inhibits receptor-Gi coupling by catalyzing ADP-ribosylation have demonstrated inhibition of LPS-induced transcription of TNF-alpha, IL-1-beta mRNA, activation of mitogen-activated protein (MAP) kinase, p38, JNK, ERK1/2 and production of prostaglandin E_2 (PGE_2), thromboxane B_2 (TxB_2), and NO in different cell types (19-22). Gi proteins appear to modulate signaling of both gram-negative and gram-positive microbial stimuli. It has also been observed that SA induced cytokine expression was inhibited by mastoparan, which functions as a G_i protein antagonist (23). Also our previous studies have demonstrated that PTx inhibited LPS and SA induced TNF-alpha production in murine J774.1A cells and human promonocytic THP-1 cells (24). However, the effect of inhibition of Gi proteins on synthesis of other cytokines has not been investigated.

Glucans are natural product biological response modifiers composed of (1→3)-beta-D-linked polymers of glucose units (25). In animal studies glucans have been shown to be effective in attenuating experimental peritonitis (26-29). Clinical studies suggest that administration of glucans to trauma and surgical patients will stimulate conversion from anergy, decrease the incidence of septic complications, and improve survival (30-33). Soluble glucan mitigates the severity of *in vivo* endotoxemia (34) and SA infection (35) in rodents. *In vivo* treatment with soluble glucans has been shown to suppress *ex vivo* LPS, staphylococcal enterotoxin B and toxic shock syndrome toxin induction of TNF-alpha, IFN-gamma, IL-2 and IL-6 in murine macrophages or lymphocytes (36,37). However, the molecular mechanisms whereby glucan mediates its cellular effects remains to be clearly defined. Recently, soluble glucan has been shown to activate cells through binding to dectin-1, scavenger and TLR2 receptor (38-41). Scavenger receptors function through Gi protein coupled signaling pathways (42) and inhibition of Gi proteins has been shown to inhibit SA (a TLR2 ligand) activation (24). This suggests that the cellular effects of soluble glucan may be mediated, in part, by Gi protein coupled signaling.

In the present study, we examined the effect of inhibition of Gi protein function on LPS and SA activation of multiple pro-inflammatory cytokines (IL-6, TNF-alpha, IL-1-beta, and IFN-gamma), the anti-inflammatory cytokine IL-10 and GM-CSF in human promonocytic THP-1 cells. Additionally, we examined the effect of soluble glucan on LPS and SA induced mediator production and the possible involvement of Gi proteins.

3. MATERIAL AND METHODS

3.1. Cell culture

THP-1 cells (American Type Culture Collection, Manassas, VA) were cultured in RPMI 1640 medium (Cellgro Mediatech Inc., Herndon, VA), supplemented with 10% heat inactivated fetal calf serum (FCS) (Sigma, St. Luis, MO), 50U/ml penicillin, 50microgram/ml streptomycin (Cellgro Mediatech Inc., VA) in 150cm^2 tissue culture flasks and maintained at 37°C in 5% CO_2, 95% incubator air. The confluent THP-1 cells within 10 passages were used for experiments.

3.2. Cell stimulation

THP-1 cells at a density of 1×10^6 cells/ml were pretreated with or without PTx (List Biological Laboratories Inc., Campbell, CA) or vehicle at a concentration of 100ng/ml or 1 microgram/ml for 6 h. The cells were washed and treated with or without soluble (1→3) beta-D-glucan together with LPS (10 microgram/ml) from *Salmonella enterititidis* (Sigma, St. Louis, MO), or heat killed SA (10 microgram/ml, heat killed SA were prepared as described previously 43) for 24 h.

Viability was quantitated by Trypan Blue exclusion. LPS, SA, PTx, and soluble (1→3) beta-D-glucan at the concentrations of drugs do not affect cell viability (>95% viability).

3.3. Luminex Analysis

TNF-alpha, IFN-gamma, IL-1–beta, IL-6, IL-10, and GM-CSF were measured using an enzyme-linked immunosorbant assay (ELISA) with luminex ELISA kits (Biosource, Camarillo, CA)

3.4. Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM). Statistical significance was determined by analysis of variance (ANOVA) with Fisher's probable least-squares difference test using Statview software (SAS Institute Inc., Cary, NC). $p< 0.05$ was considered significant.

4. RESULTS

4.1. Effects of PTx on LPS or SA induced cytokine production

To test the hypothesis that G_i proteins are involved in both gram-negative and gram-positive bacteria induced pro-inflammatory cytokine release, human promonocytic THP-1 cells were pretreated with PTx (100ng/ml or 1 microgram/ml) followed by stimulation with LPS, or SA. PTx has no affect on cytokine production. LPS and SA significantly induced IL-6, TNF-alpha, IL-1-beta, GM-CSF, IL-10, and IFN-gamma production. PTx inhibited LPS induced IL-6 production by 80±4% and 87±1% (n=3, $p<0.05$, Figure 1A), TNF-alpha production by 93±1% and 95±2% (n=3, $p<0.05$, Figure 2A), IL-1-beta

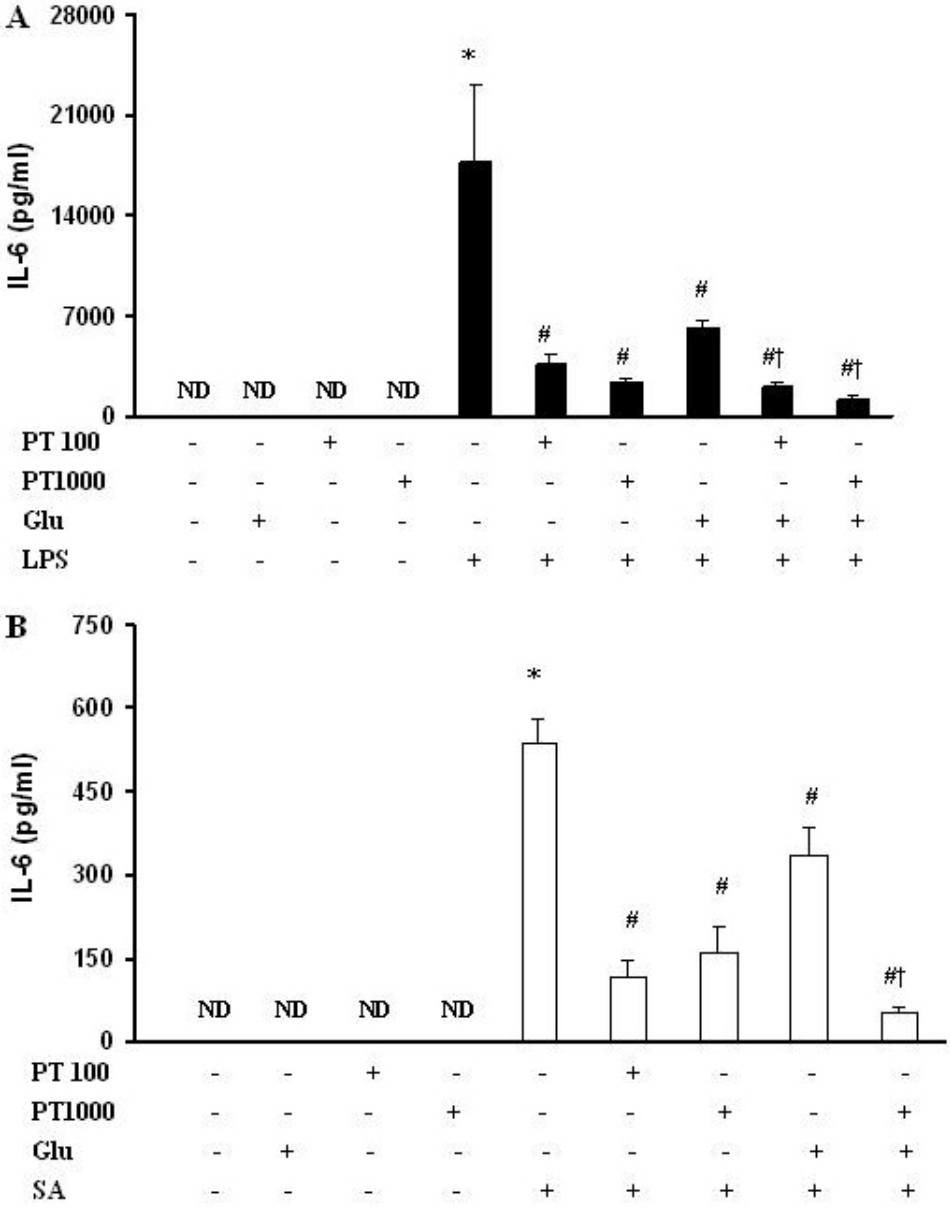

Figure 1. Effects of PTx and glucan pretreatment on LPS and SA induced IL-6 production in THP-1 cells. THP-1 cells were pretreated with PTx (100ng/ml or 1 microgram/ml) for 6 h followed by stimulation with LPS (10 microgram/ml) (A), or heat killed SA (10 microgram/ml) (B) with or without (1→3) beta-D-glucan (10μg/ml) for 24 h. LPS and SA induced IL-6 production was analyzed by luminex analysis. Data represent the mean ± SE from three independent experiments. *, p< 0.05 compared to basal group. #, p < 0.05 compared with LPS or SA stimulated group.†, p<0.05 compared with glucans pretreated group.

production by 65±5% and 73±2% (n=3, p<0.05, Figure 3A), GM-CSF production by 43±4% and 51±2% (n=3, p<0.05, Figure 4A), IL-10 production by 90±3% and 91±2% (n=3, p<0.05, Figure 5A), and IFN-gamma production by 9±1% and 10±2% (n=3, p<0.05, Figure 6A). PTx inhibited SA induced IL-6 production by 79±6% and 70±9% (n=3, p<0.05, Figure 1B), TNF-alpha production by 80±6% and 59±14% (n=3, p<0.05, Figure 2B), IL-1-beta production by 40±3% and 22±15% (n=3, p<0.05, Figure 3B), GM-CSF production by 29±1% and 23±6% (n=3, p<0.05, Figure 4B), IL-10 production by 78±3% and 69±7% (n=3, p<0.05, Figure 5B), and IFN-gamma production by 30±5% and 23±5% (n=3, p<0.05, Figure 6B).

4.2. Effects of (1→3) beta-D-glucan on LPS or SA induced cytokine production

To investigate the effect of glucan on LPS and SA induced cytokine production, human promonocytic THP-1 cells were stimulation with LPS or SA together with

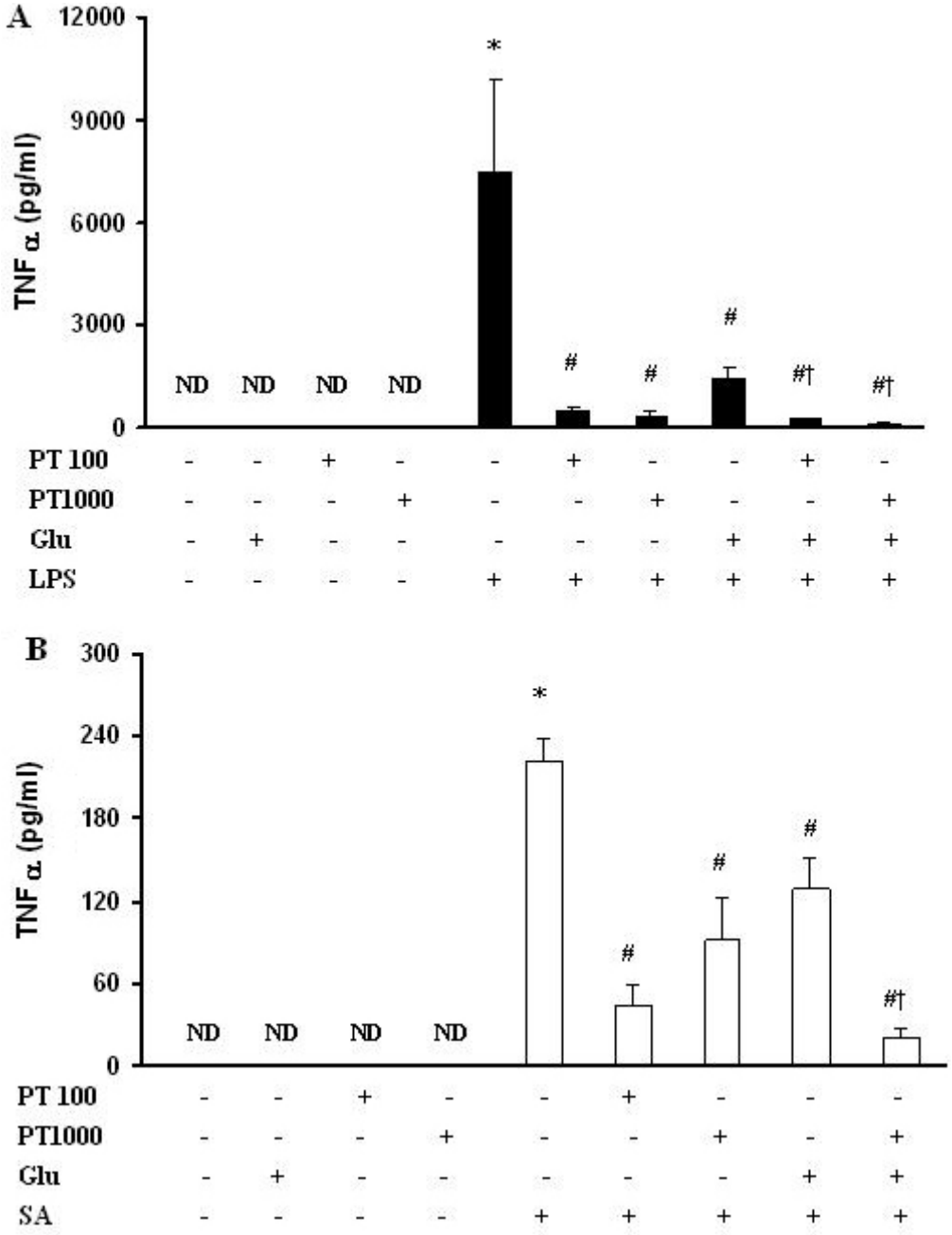

Figure 2. Effects of PTx and glucan pretreatment on LPS and SA induced TNF-alpha production in THP-1 cells. THP-1 cells were pretreated with PTx (100ng/ml or 1 microgram/ml) for 6 h followed by stimulation with LPS (10 microgram/ml) (A), or heat killed SA (10 microgram/ml) (B) with or without (1→3) beta-D-glucan (10 microgram/ml) for 24 h. LPS and SA induced TNF-alpha production was analyzed by luminex analysis. Data represent the mean ± SE from three independent experiments. *, $p< 0.05$ compared to basal group. #, $p < 0.05$ compared with LPS or SA stimulated group.†, $p<0.05$ compared with glucans pretreated group.

or without glucan (10 microgram/ml) or vehicle. Glucan had no effect on cytokine production. However, glucan inhibited LPS- induced IL-6 production by 65±3% (n=3, $p<0.05$, Figure 1A), TNF-alpha production by 81±4% (n=3, $p<0.05$, Figure 2A), IL-1-beta production by 67±3% (n=3, $p<0.05$, Figure 3A), GM-CSF production by 32±2% (n=3, $p<0.05$, Figure 4A), IL-10 production by 76±2% (n=3, $p<0.05$, Figure 5A), and IFN-gamma production by 10±2% (n=3, $p<0.05$, Figure 6A). Glucan inhibited SA-induced IL-6 production by 37±9% (n=3, $p<0.05$, Figure 1B), TNF-alpha production by 42±10% (n=3, $p<0.05$, Figure 2B), IL-1-beta production by 32±4% (n=3, $p<0.05$, Figure 3B), GM-CSF production by 19±2% (n=3, $p<0.05$, Figure 4B), IL-10 production by 48±3% (n=3, $p<0.05$,

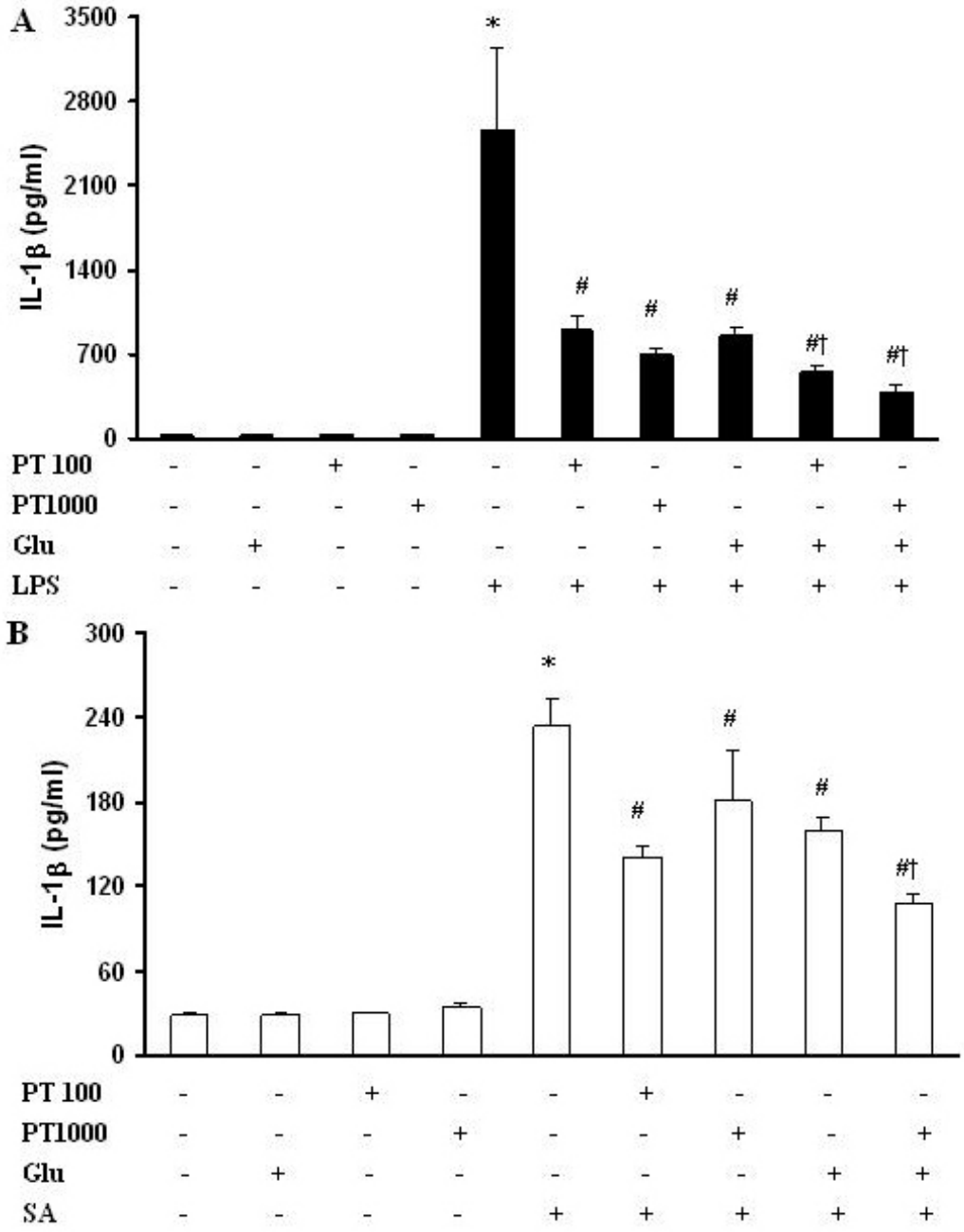

Figure 3. Effects of PTx and glucan pretreatment on LPS and SA induced IL-1-beta production in THP-1 cells. THP-1 cells were pretreated with PTx (100ng/ml or 1 microgram/ml) for 6 h followed by stimulation with LPS (10 microgram/ml) (A), or heat killed SA (10 microgram/ml) (B) with or without (1→3) beta-D-glucan (10 microgram/ml) for 24 h. LPS and SA induced IL-1-beta production was analyzed by luminex analysis. Data represent the mean ± SE from three independent experiments. *, $p< 0.05$ compared to basal group. #, $p < 0.05$ compared with LPS or SA stimulated group.†, $p<0.05$ compared with glucans pretreated group.

Figure 5B), and IFN-gamma production by 16±0% (n=3, $p<0.05$, Figure 6B).

4.3. Effects of PTx on glucan induced suppression of cytokine production

To further investigate the involvement of Gi proteins in glucan induced suppression of cytokine production, human promonocytic THP-1 cells were pretreated with PTx (100ng/ml or 1 microgram/ml) followed by stimulation with LPS or SA with glucan (10 microgram/ml). PTx further decreased glucan induced suppression of LPS induced IL-6 production by 68±4% and 80±4% (Figure 1A), TNF-alpha production by 82±1% and

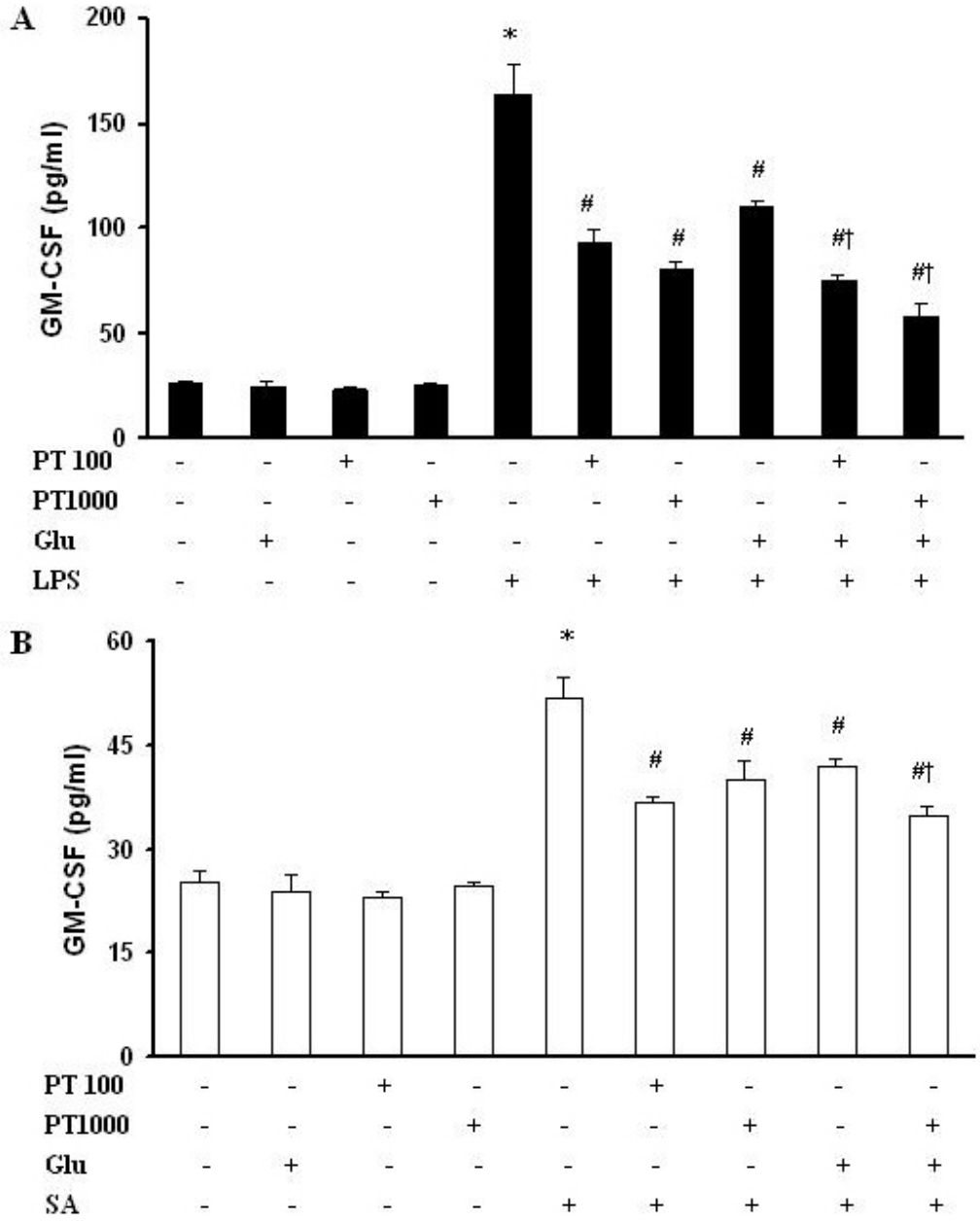

Figure 4. Effects of PTx and glucan pretreatment on LPS and SA induced GM-CSF production in THP-1 cells. THP-1 cells were pretreated with PTx (100ng/ml or 1 microgram/ml) for 6 h followed by stimulation with LPS (10 microgram/ml) (A), or heat killed SA (10 microgram/ml) (B) with or without (1→3) beta-D-glucan (10 microgram/ml) for 24 h. LPS and SA induced GM-CSF production was analyzed by luminex analysis. Data represent the mean ± SE from three independent experiments. *, $p< 0.05$ compared to basal group. #, $p < 0.05$ compared with LPS or SA stimulated group.†, $p<0.05$ compared with glucans pretreated group.

91±2% (Figure 2A), IL-1 beta production by 44±5% and 55±7% (Figure 3A), GM-CSF production by 32±2% and 48±6% (n=3, $p<0.05$, Figure 4A), IL-10 production by 77±3% and 84±3% (Figure 5A), and IFN-gamma production by 8±4% and 12±2% (n=3, $p<0.05$, Figure 6A). PTx further decreased glucan induced suppression of SA induced IL-6 production by 84±3% (n=3, $p<0.05$, Figure 1B), TNF-alpha production by 84±5% (n=3, $p<0.05$, Figure 2B), IL-1-beta production by 32±4% (n=3, $p<0.05$, Figure 3B), GM-CSF production by 17±3% (Figure 4B), IL-10 production by 72±5% (n=3, $p<0.05$, Figure 5B), and IFN-gamma production by 28±4% (n=3, $p<0.05$, Figure 6B).

5. DISCUSSION

Lipopolysaccharide and SA induce synthesis of a

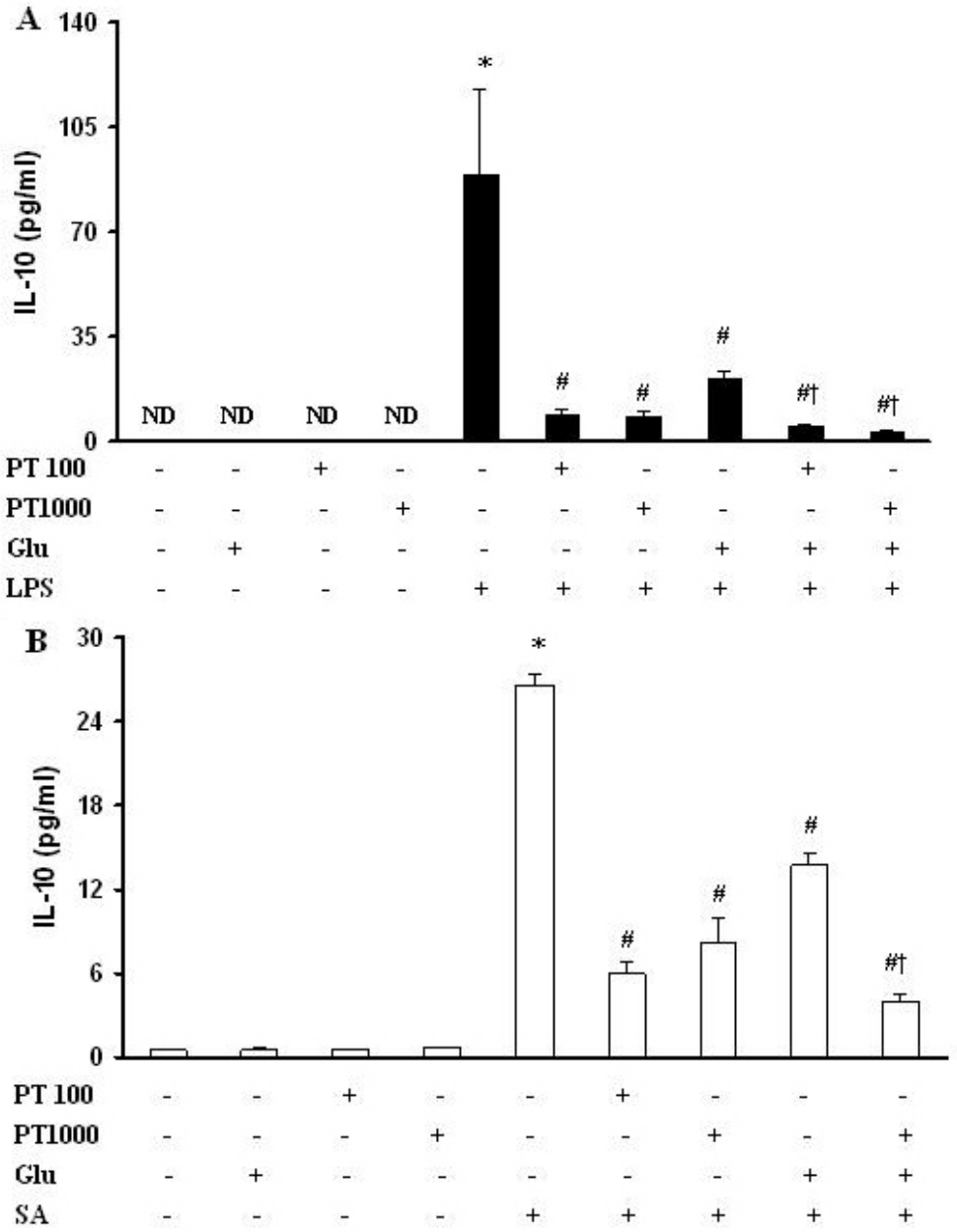

Figure 5. Effects of PTx and glucan pretreatment on LPS and SA induced IL-10 production in THP-1 cells. THP-1 cells were pretreated with PTx (100ng/ml or 1 microgram/ml) for 6 h followed by stimulation with LPS (10 microgram/ml) (A), or heat killed SA (10 microgram/ml) (B) with or without (1→3) beta-D-glucan (10 microgram/ml) for 24 h. LPS and SA induced IL-10 production was analyzed by luminex analysis. Data represent the mean ± SE from three independent experiments. *, $p< 0.05$ compared to basal group. #, $p < 0.05$ compared with LPS or SA stimulated group.†, $p<0.05$ compared with glucans pretreated group.

variety of cytokines by human THP-1 cells. These microbial stimuli induce the expression of cytokines in rank order potency IL-6>TNF-alpha>IL-1-beta>GM-CSF>IL-10>IFN-gamma. Interestingly, in all cases LPS was a more potent stimulus compared to SA suggesting a predominant TLR4 driven response of the THP-1 monocytes. PTx significantly inhibited both LPS and SA induced cytokine production. These data confirm our previous findings that Gi proteins regulate TLR4 and TLR2 ligand induction of TNF-alpha in monocytes and macrophages (24), and importantly extend these studies demonstrating that Gi proteins also regulate other pro-inflammatory cytokines, the anti-inflammatory cytokine IL-10, and the growth factor GM-CSF. Soluble glucan also suppressed mediator production induced by both LPS and SA. Inhibition of Gi protein function with PTx further

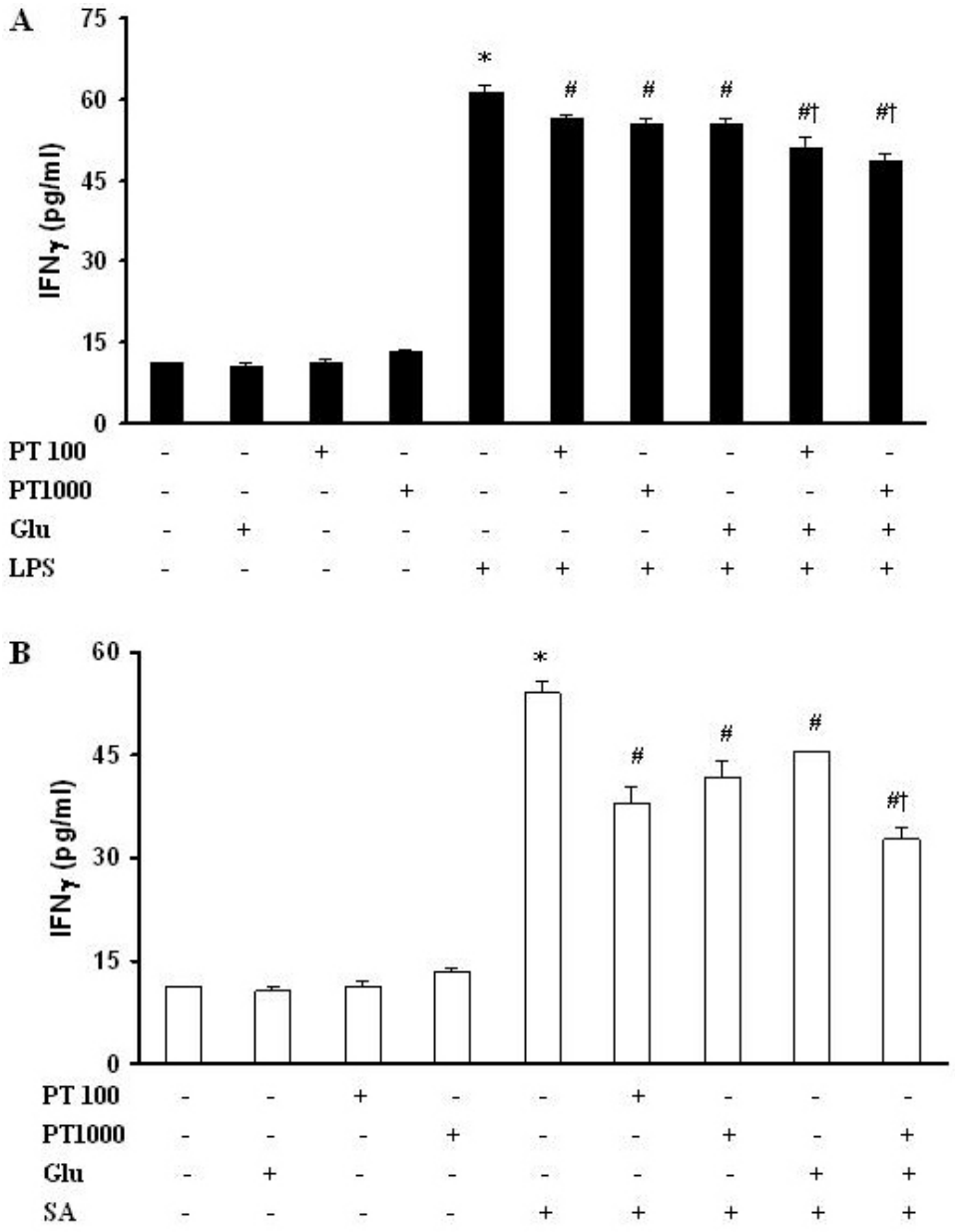

Figure 6. Effects of PTx and glucan pretreatment on LPS and SA induced IFN-gamma production in THP-1 cells. THP-1 cells were pretreated with PTx (100ng/ml or 1 microgram/ml) for 6 h followed by stimulation with LPS (10 microgram/ml) (A), or heat killed SA (10 microgram/ml) (B) with or without (1→3) beta-D-glucan (10 microgram/ml) for 24 h. LPS and SA induced IFN-gamma production was analyzed by luminex analysis. Data represent the mean ± SE from three independent experiments. *, $p< 0.05$ compared to basal group. #, $p < 0.05$ compared with LPS or SA stimulated group.†, $p<0.05$ compared with glucans pretreated group.

augmented the glucan-induced suppression of LPS and SA induced cytokine production.

The findings that PTx blocked both LPS and SA induced multiple pro-inflammatory cytokines, a growth factor, and the anti-inflammatory cytokine IL-10 suggest that Gi proteins function as proximal signal transducers for both TLR4 and TLR2 ligands. In a recent study, Lentschat *et al*. (44) also concluded that Gi proteins function as proximally in the signaling cascade since Gi protein inhibition with mastoparan inhibited both MyD88 dependent and independent signaling in murine macrophages. In the latter study Gi protein inhibition did not affect activation with the soluble TLR2 ligand Pam3Cys. These findings are not in agreement with findings that PTx or mastoparan blocked the TLR2 ligand SA induced activation (23, 24). The results may suggest differences in the mechanism whereby PTx and mastoparan

inhibit Gi protein function or to species differences ie. human monocytic cells vs murine macrophage. Another possibility is that particulate phagocytizable TLR2 ligands, ie. heat-killed SA, may activate Gi protein coupled signaling pathway not activated by soluble TLR2 ligands, ie. Pam3Cys.

Our studies demonstrate that soluble glucan alone had no effect on the induction of the cytokines. These observations are consistent with other studies that showed that soluble glucan had no effect on *in vitro* cytokine production by human monocytes (45) or in cultured murine BMC2.3 cells (46). Soluble glucan effectively ameliorated all cytokines induced by LPS and SA. The glucan inhibition of cellular activation by TLR2 and TLR4 ligands may be a mechanism whereby glucan improves survival in polymicrobial sepsis (47). Glucan has only a modest, but significant, effect on inhibiting IFN-gamma. Previous studies have demonstrated that soluble glucan induced IFN-gamma in mice (48). It has been suggested that the ability to induce IFN-gamma or, as in the present study, to induce a relatively modest inhibition relative to other cytokines may be beneficial in immuno-suppressed syndromes exhibiting a predominant Th2 response. Soluble glucans have been shown to bind to dectin-1 recognition domains, which in turn interact with TLR2 to transduce signaling via the TLR2/MyD88/NF–kappa-B pathway (49). In addition to dectin, class A scavenger receptors also bind to glucan (50). Scavenger receptors transduce signaling by Gi proteins (42). Thus, it is reasonable to assume that glucan transduces signaling, in part, through a Gi protein dependent mechanism. However, our results demonstrated that PTx consistently augmented glucan induced suppression of cytokine production. These data suggest that the pathways of inhibition by glucan and PTx appear to be independent. Furthermore, this is the first report that glucan mediated effects on LPS or SA cytokine induction are Gi protein independent. It was also noted that the inhibitory effect of soluble glucan was more pronounced on LPS induced cytokine production than it was for SA induced cytokine production. However, there was one exception, IFN-gamma. Glucan was more effective at inhibiting SA induced IFN-gamma than LPS induced IFN-gamma production.

Down regulation of IKK kinase and degradation of I-kappa-B-alpha are possible cellular mechanisms whereby soluble glucan inhibit LPS activation (47). Our previous studies have shown that PTx inhibition of HEK 293 cells transfected with constitutively active TLR4 inhibited ERK 1/2 activation, but not NF-kappa-B activation (51). Similarly PTx failed to inhibit LPS induced NF-kappa-B translocation in TLR4 transfected CHO cells (22). Thus the inhibitory effect of glucan on NF-kappa-B signaling pathway may be independent of Gi protein coupled signaling activated by LPS and SA.

The phosphatidylinositol-3 kinase (PI3 Kinase) pathway has been implicated in glucan-induced suppression of the NF-kappa-B signaling pathway (52). Williams, Li and colleagues (52,53) have demonstrated that glucan induced stimulation of PI3 kinase suppresses pro-inflammatory responses associated with inflammatory sepsis and/or improved survival. The catalytic subunit of type 1A PI3 kinase engages Gi proteins, which regulates inflammation and chemotaxis (54). However, the role of Gi protein in regulating PI3 kinase activity remains controversal (55, 56). Additionally, it was recently shown that inhibition of Gi protein function with mastoparan failed to inhibit TLR4 or TLR2 activation of Akt, a downstream protein activated by PI3 kinase (44). Thus it is feasible that glucan induced suppression of cytokine production occurs through Gi protein independent pathways leading to PI3 kinase activation.

These data support the concept that convergent Gi signaling occurs between TLR4 and TLR2 in human monocytic lineage cells treated with LPS or SA. However, since the suppression of microbial stimuli with soluble glucan occurs independent of Gi proteins, these observations raise the interesting possibility that conjoint therapeutic approaches targeted at inhibiting Gi protein function coupled with soluble glucan may provide superior beneficial effects in treating polymicrobial sepsis.

6. ACKNOWLEDGEMENTS

This work was supported in part by NIH GM27673, NIH GM53522, and NIH GM67202

7. REFERENCES

1. Bone R.C.: The pathogenesis of sepsis. *Ann Intern Med* 115, 457-469 (1991)
2. Karima R., S. Matsumoto, H. Higashi, & K. Matsushima: The molecular pathogenesis of endotoxic shock and organ failure. *Mol Med Today* 5, 123-132 (1999)
3. Yoshimura A., E. Lien, R.R. Ingalls, E. Tuomanen, R. Dziarski, & D. Golenbock: Cutting edge: recognition of Gram-positive cell wall components by the innate immune system occurs via Toll-like receptor 2. *J Immunol* 163, 1-5 (1999)
4. Schwandner R., R. Dziarski, H. Wesche, M. Rothe, C. & J. Kirschning: Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. *J Biol Chem* 274, 17406-17409 (1999)
5. Brightbill H.D., D.H. Libraty, S.R. Krutzik, R.B. Yang, J.T. Belisle, J.R. Bleharski, M. Maitland, M.V. Norgard, S.E. Plevy, S.T. Smale, P.J. Brennan, B.R. Bloom, P.J. Godowski, & R.L. Modlin: Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. *Science* 285, 732-736 (1999)
6. Wright S.D., R.A. Ramos, P.S. Tobias, R.J. Ulevitch, & J.C. Mathison: CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* 249, 1431-1433 (1990)
7. McGinley M.D., L.O. Narhi, M.J. Kelley, E. Davy, J. Robinson, M.F. Rohde, S.D. Wright, & H.S. Lichenstein: CD14: physical properties and identification of an exposed site that is protected by lipopolysaccharide. *J Biol Chem* 270, 5213-5218 (1995)
8. Juan T.S., E. Hailman, M. J. Kelley, L.A. Busse, E. Davy, C. J. Empig, L.O. Narhi, & S.D. Wright: Lichenstein HS. Identification of a lipopolysaccharide binding domain

in CD14 between amino acids 57 and 64. *J Biol Chem* 270, 5219-5224 (1995)
9. Poltorak A., X. He, I. Smirnova, M.Y. Liu, C.V. Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, M. Freudenberg, P. Ricciardi-Castagnoli, B. Layton, & B. Beutler: Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 282, 2085-2088 (1998)
10. Qureshi S.T., L. Lariviere, G. Leveque, S. Clermont, K. J. Moore, P. Gros, & D. Malo: Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4) *J Exp Med* 189, 615-625 (1999)
11. Takeuchi O., K. Hoshino, & S. Akira: Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to Staphylococcus aureus infection. *J Immunol* 165, 5392-5396 (2000)
12. Imler J.L., & J.A. Hoffmann: Toll receptors in innate immunity. *Trends Cell Biol* 11, 304-311 (2001)
13. Ellingsen E.A., S. Morath, T. Flo, A.B. Schromm, T. Hartung, C. Thiemermann, T. Espevik, D.T. Golenbock, S.J. Foster, R. Solberg, A.O. Aasen, & J.E. Wang: Induction of cytokine production in human T cells and monocytes by highly purified lipoteichoic acid: involvement of Toll-like receptors and CD14. *Med Sci Monit* 8, BR149-156 (2002)
14. Rabehi L., T. Irinopoulou, B. Cholley, N. Haeffner-Cavaillon, & M.P. Carreno: Gram-positive and gram-negative bacteria do not trigger monocytic cytokine production through similar intracellular pathways. *Infect Immun* 69, 4590-4599 (2001)
15. Schroder N.W., S. Morath, C. Alexander, L. Hamann, T. Hartung, U. Zahringer, U.B. Gobel, J.R. Weber, & R.R. Schumann: Lipoteichoic acid (LTA) of Streptococcus pneumoniae and Staphylococcus aureus activates immune cells via Toll-like receptor (TLR)-2, lipopolysaccharide-binding protein (LBP), and CD14, whereas TLR-4 and MD-2 are not involved. *J Biol Chem* 278, 15587-15594 (2003)
16. Kawai T., O. Takeuchi, T. Fujita, J. Inoue, P.F. Muhlradt, S. Sato, K. Hoshino, & S. Akira: Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes. *J Immunol* 167, 5887-5894 (2001)
17. Akira S, & K. Hoshino: Myeloid differentiation factor 88-dependent and -independent pathways in toll-like receptor signaling. *J Infect Dis* 187, S356-363 (2003)
18. Solomon K.R., E.A. Kurt-Jones, R.A. Saladino, A.M. Stack, I.F. Dunn, M. Ferretti, D. Golenbock, G.R. Fleisher, & R.W. Finberg: Heterotrimeric G proteins physically associated with the lipopolysaccharide receptor CD14 modulate both in vivo and in vitro responses to lipopolysaccharide. *J Clin Invest* 102, 2019-2027 (1998)
19. Daniel-Issakani S., A.M. Spiegel, & B. Strulovici: Lipopolysaccharide response is linked to the GTP binding protein, Gi2, in the promonocytic cell line U937. *J Biol Chem* 264, 20240-20247. (1989)
20. Wang J., M. Kester, M. & J. Dunn: Involvement of a pertussis toxin-sensitive G-protein-coupled phospholipase A2 in lipopolysaccharide-stimulated prostaglandin E2 synthesis in cultured rat mesangial cells. *Biochim Biophys Acta* 963, 429-435 (1988)
21. Zhang X., & D.C. Morrison: Lipopolysaccharide-induced selective priming effects on tumor necrosis factor alpha and nitric oxide production in mouse peritoneal macrophages. *J Exp Med* 177, 511-516 (1993)
22. Ferlito M., F. Squadrito, P.V. Halushka, & J.A. Cook: Signal transduction events in Chinese hamster ovary cells expressing human CD14; effect of endotoxin desensitization. *Shock* 15, 291-296 (2001)
23. Bocker U., T. Manigold, J.M. Watson, M.V. Singer, & S. Rossol: Regulation of Staphylococcus aureus-mediated activation of interleukin-18 in peripheral blood mononuclear cells. *Eur Cytokine Netw* 12, 631-638 (2001)
24. Fan H., G. Teti, S. Ashton, K. Guyton, G.E. Tempel, P.V. Halushka, & J.A. Cook: Involvement of G(i) proteins and Src tyrosine kinase in TNFalpha production induced by lipopolysaccharide, group B Streptococci and Staphylococcus aureus. *Cytokine* 22, 126-133 (2003)
25. Ensley H.E., B. Tobias, H.A. Pretus, R.B. McNamee, E.L. Jones, I.W. Browder, & D.L. Williams: NMR spectral analysis of a water-insoluble (1-->3)-beta-D-glucan isolated from Saccharomyces cerevisiae. *Carbohydr Res* 258, 307-311 (1994)
26. Williams D.L., A. Mueller, & W. Browder: Glucan-based macrophage stimulators: a review of their anti-infective potential. *Clin Immunother* 5, 392-399 (1996)
27. Williams D.L., E.R. Sherwood, I.W. Browder, R.B. McNamee, E.L. Jones, J. Rakinic, & N.R. Di Luzio: Effect of glucan on neutrophil dynamics and immune function in Escherichia coli peritonitis. *J Surg Res* 44, 54-61 (1988)
28. Williams D.L., I.W. Browder, & N.R. Di Luzio: Immunotherapeutic modification of Escherichia coli--induced experimental peritonitis and bacteremia by glucan. *Surgery* 93, 448-454 (1983)
29. Browder W., D. Williams, E. Sherwood, R. McNamee, E. Jones, & N. DiLuzio: Synergistic effect of nonspecific immunostimulation and antibiotics in experimental peritonitis. *Surgery* 102, 206-214 (1987)
30. Browder W., D. Williams, H. Pretus, G. Olivero, F. Enrichens, P. Mao, & A. Franchello: Beneficial effect of enhanced macrophage function in the trauma patient. *Ann Surg* 11, 605-612 (1990)
31. Babineau T.J., A. Hackford, A. Kenler, B. Bistrian, R.A. Forse, P.G. Fairchild, S. Heard, M. Keroack, P. Caushaj, & P. Benotti: A phase II multicenter, double-blind, randomized, placebo-controlled study of three dosages of an immunomodulator (PGG-glucan) in high-risk surgical patients. *Arch Surg* 129, 1204-1210 (1994)
32. Babineau T.J., P. Marcello, W. Swails, A. Kenler, B. Bistrian, R.A. Forse: Randomized phase I/II trial of a macrophage-specific immunomodulator (PGG-glucan) in high-risk surgical patients. *Ann Surg* 220, 601-609 (1994)
33. de Felippe Junior J., M. da Rocha e Silva Junior, F.M. Maciel, M. Soares Ade, & N.F. Mendes: Infection prevention in patients with severe multiple trauma with the immunomodulator beta 1-3 polyglucose (glucan). *Surg Gynecol Obstet* 177, 383-388 (1993)
34. Vereschagin E.I., A.A. van Lambalgen, M.I. Dushkin, Y.S. Schwartz, L. Polyakov, A. Heemskerk, E. Huisman, L.G. Thijs, & G.C. van den Bos: Soluble glucan protects against endotoxin shock in the rat: the role of the scavenger receptor. *Shock* 9, 193-198 (1998)

35. Liang J., D. Melican, L. Cafro, G. Palace, L. Fisette, R. Armstrong, M.L. Patchen: Enhanced clearance of a multiple antibiotic resistant Staphylococcus aureus in rats treated with PGG-glucan is associated with increased leukocyte counts and increased neutrophil oxidative burst activity. *Int J Immunopharmacol* 20, 595-614 (1998)
36. Kernodle D.S., H. Gates, & A.B. Kaiser: Prophylactic anti-infective activity of poly-[1-6]-beta-D-glucopyranosyl-[1-3]-beta-D-glucopryanose glucan in a guinea pig model of staphylococcal wound infection. *Antimicrob Agents Chemother* 42, 545-549 (1998)
37. Soltys J., & M.T. Quinn: Modulation of endotoxin- and enterotoxin-induced cytokine release by in vivo treatment with beta-(1,6)-branched beta-(1,3)-glucan. *Infect Immun*. 67, 244-252 (1999)
38.Brown G.D., & S. Gordon: Immune recognition. A new receptor for beta-glucans. *Nature* 413, 36-37 (2001)
39. Brown G.D., J. Herre, D.L. Williams, J.A. Willment, A.S. Marshall, & S. Gordon: Dectin-1 mediates the biological effects of beta-glucans. *J Exp Med* 197, 1119-1124 (2003)
40. Rice P.J., J.L. Kelley, G. Kogan, H.E. Ensley, J.H. Kalbfleisch, I.W. Browder, & D.L. Williams: Human monocyte scavenger receptors are pattern recognition receptors for (1-->3)-beta-D-glucans. *J Leukoc Biol* 72, 140-146 (2002)
41. Williams D.L., T. Ha, C. Li, J.H. Kalbfleisch, J. Schweitzer, W. Vogt, & I.W. Browder: Modulation of tissue Toll-like receptor 2 and 4 during the early phases of polymicrobial sepsis correlates with mortality. *Crit Care Med* 31, 1808-1818 (2003)
42. Connelly M.A., & D.L. Williams: Scavenger receptor BI: a scavenger receptor with a mission to transport high density lipoprotein lipids. *Curr Opin Lipidol* 15, 287-295 (2004)
43. Cuzzola M., G. Mancuso, C. Beninati, C. Biondo, F. Genovese, F. Tomasello, T.H. Flo, T. Espevik, & G. Teti: Beta 2 integrins are involved in cytokine responses to whole Gram-positive bacteria. *J Immunol* 164, 5871-5876 (2000)
44. Lentschat A., H. Karahashi, K.S. Michelsen, L S. Thomas, W. Zhang, S.N. Vogel, & M. Arditi: Mastoparan, a G protein agonist peptide, differentially modulates TLR4- and TLR2-mediated signaling in human endothelial cells and murine macrophages. *J Immunol* 174, 4252-4261 (2005)
45. Poutsiaka D.D., M. Mengozzi, E. Vannier, B. Sinha, & C.A. Dinarello: Cross-linking of the beta-glucan receptor on human monocytes results in interleukin-1 receptor antagonist but not interleukin-1 production. *Blood* 82, 3695-3700 (1993)
46. Adams D.S., S.C. Pero, J.B. Petro, R. Nathans, W.M. Mackin, & E. Wakshull: PGG-Glucan activates NF-kappaB-like and NF-IL-6-like transcription factor complexes in a murine monocytic cell line. *J Leukoc Biol* 62, 865-873 (1997)
47. Williams D.L., T. Ha, C. Li, J. Laffan, J. Kalbfleisch, & W. Browder: Inhibition of LPS-induced NFkappaB activation by a glucan ligand involves down-regulation of IKKbeta kinase activity and altered phosphorylation and degradation of IkappaBalpha. *Shock* 13, 446-452 (2000)
48. Sherwood E.R., T.K. Varma, R.Y. Fram, C.Y. Lin, A.P. Koutrouvelis, & T.E. Toliver-Kinsky: Glucan phosphate potentiates endotoxin-induced interferon-gamma expression in immunocompetent mice, but attenuates induction of endotoxin tolerance. *Clin Sci* (Lond) 101, 541-550 (2001)
49. Yang R.B., M.R Mark, A. Gray, A. Huang, M.H. Xie, M. Zhang, A. Goddard, W.I. Wood, A.L. Gurney, & P.J. Godowski: Toll-like receptor-2 mediates lipoolysaccharide -induced cellular signalling *Nature* 395, 284-288 (1998)
50. Linehan S.A., L. Martinez-Pomares, & S. Gordon: Mannose receptor and scavenger receptor: two macrophage pattern recognition receptors with diverse functions in tissue homeostatis and host defense. *Adv Exp Med Biol* 479, 1-14 (2000)
51. Fan H., O.M. Peck, G.E. Tempel, P.V. Halushka, & J.A. Cook: Toll-like receptor 4 coupled GI protein signaling pathways regulate extracellular signal-regulated kinase phosphorylation and AP-1 activation independent of NFkappaB activation. *Shock* 22, 57-62 (2004)
52. Li C., T. Ha, J. Kelley, X. Gao, Y. Qiu, R.L. Kao, W. Browder, & D.L. Williams: Modulating Toll-like receptor mediated signaling by (1-->3)-beta-D-glucan rapidly induces cardioprotection. *Cardiovasc Res* 61, 538-547 (2004)
53. Williams D.L., C. Li, T. Ha, T. Ozment-Skelton, J.H. Kalbfleisch, J. Preiszner, L. Brooks, K. Breuel, & J.B. Schweitzer: Modulation of the phosphoinositide 3-kinase pathway alters innate resistance to polymicrobial sepsis. *J Immunol* 172, 449-456 (2004)
54. Dekker L.V., & A.W. Segal: Perspectives: signal transduction. Signals to move cells. *Science* 287, 982-983 (2000)
55. Hirsch E., V.L. Katanaev, C. Garlanda, O. Azzolino, L. Pirola, L. Silengo, S. Sozzani, A. Mantovani, F. Altruda, & M.P. Wymann: Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation. *Science* 287, 1049-1053 (2000)
56. Bommakanti R.K., S. Vinayak, & W.F. Simonds: Dual regulation of Akt/protein kinase B by heterotrimeric G protein subunits. *J Biol Chem* 275, 38870-38876 (2000)

Key Words: G_i protein, LPS, *Staphylococcus aureus*, Glucan, Cytokine

Send correspondence to: James A Cook, Professor of Physiology, Department of Physiology and Neuroscience, 173 Ashley Ave., BSB Room 403, Charleston, SC 29425, Tel: 843-792-2978, Fax number: 843-792-1066, E-mail: cookja@musc.edu

http://www.bioscience.org/current/vol11.htm