# GLUCAN INDUCES CYTOKINE SECRETION FROM MURINE MACROPHAGES AND INHIBITS LPS BINDING

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## Abstract

Glucan administration induces multiple effects in animals which may involve cytokine secretion and induction of a Th1 immune response. We examined the secretion of selected cytokines by murine macrophage cell lines induced by exposure to scleroglucan and laminarin, using lipopolysaccharide (LPS) as a positive control. We examined TNFα as an example of an inflammatory cytokine, IL12 as a cytokine that induces differentiation of CD4 lymphocytes to Th1 cells, and IL10 as an anti-inflammatory cytokine. We also examined binding of these glucans to the macrophage cell lines using inhibition of LPS binding using ligand binding kinetics flow cytometry. We found that scleroglucan induced TNFα secretion from RAW264.7 cells and J774A.1 cells; and IL12 (p40) from J774A.1 cells, but did not cause IL10 secretion. Laminarin did not cause cytokine secretion. Both scleroglucan and laminarin blocked LPS binding to macrophages. We conclude that glucans cause cytokine secretion by murine macrophages and that they bind to these cells using LPS receptor(s).

## Introduction

Glucans are polyglucose compounds in which glucopyranosyl rings are attached in chains which may be linked in α or β positions. They are a constituent of many organic materials which cause inflammatory lung disease, such as hypersensitivity pneumonitis (HP) and sick building syndrome (Rylander et al, 1994; Rylander et al, 1992). The (1→3)-β-D-glucans, found in fungal and some bacterial cell walls, have numerous immunomodulatory effects.

Murine CD4+ cells can be divided into Th1 or Th2 subsets by their patterns of cytokine secretion. Th1 cells preferentially secrete interferon-γ (IFNγ), and tumor necrosis factor-β (TNFβ); activate macrophages; are responsible for cell-mediated immunity reactions and CTL (cytotoxic lymphocytes); and provide help for $IgG_{2a}$ production. Th2 CD4+ cells secrete IL4, IL5, IL9, IL10 and IL13; provide help for immunoglobulin (particularly IgE and $IgG_1$) secretion; enhance eosinophil production, survival, and activity; and promote mast cell proliferation and maturation. Cytokines secreted by one CD4+ subset inhibit the development of the reciprocal subset leading to a predominance of one of the subsets and polarization of the antigen specific immune response (Finkelman, 1995). IL12 and IFNγ are particularly important in inducing differentiation of Th1 cells, and IL4 in inducing differentiation of Th2 cells, from Thp (Th precursor) cells. IL12, a product of macrophages, dendritic cells (Heufler et al, 1996) and polymorphonuclear leukocytes (Cassatella et al, 1995), is a very important inducer of Th1 development (Trinchieri and Scott, 1994).

Since glucan administration increases cell mediated immune responses and macrophage activation can be the result of Th1 immune responses, it is likely that glucan's effects on macrophages are mediated by cytokines secreted by Th1 cells.

Therefore, we postulate that the development of Th1 cells after glucan administration is promoted by the secretion of IL12 by macrophages and that glucan induced IL12 secretion is enhanced by LPS via interaction with CD14.

## Methods

The J774A.1 and RAW264.7 cell lines were obtained from ATCC and maintained in Dulbecco's MEM. The J774A.1 cell line was chosen as it secretes cytokines (Kirikae et al, 1994; Kirikae et al, 1994), expresses CD14 (Matsuura et al, 1994) and binds to glucan (Muller et al, 1996). RAW264.7 cells secreted large amounts of TNFα and IL10 and small amounts of IL12 and were used to examine TNFα and IL10 secretion. Cells were allowed to adhere for 2 to 4 hours in 96 well plates before exposure to LPS or glucan. All results are from supernatants of 3-4 x $10^6$ cells/ml.

Lipopolysaccharide (LPS) from Salmonella minnesota (Sigma Chemical Co) was dissolved in pyrogen free Dulbeco's Minimal Essential Media (DMEM) at a concentration of 5 mg/ml, stored at -70$^0$C and diluted in endotoxin free saline before use.

Cytokines: IL12 (p40), TNFα, and IL10 were measured by ELISA techniques.

Glucans: Scleroglucan and laminarin provided by Dr. David Williams, East Tennessee State University School of Medicine, were diluted in DMEM.

Inhibition of LPS-FITC binding: Human peripheral blood monocytes or murine macrophages are exposed to fluorescein-labeled LPS (LPS-FITC, Sigma) and analyzed by flow cytometry to determine the effect of glucan upon the median fluorescence intensity of bound LPS as a function of time.

## Results

### LPS induced murine macrophage cytokine secretion

**TNFα:** Scleroglucan could induce TNFα secretion. Figure 1 demonstrates dose related TNFα secretion from J774A.1

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cells. Laminarin did not induce TNFα secretion (data not shown).

**IL10:** The RAW264.7 cell line secretes substantial amounts of IL10 in response to LPS with optimal response at 10 μg/ml LPS. Neither scleroglucan or laminarin induced IL10 secretion.

**IL12:** The J774A.1 cell line secretes IL12 with optimal secretion of the p40 component of IL12 at 6 hour at ≥ 1.0 μg/ml LPS. The threshold for LPS induced IL12 secretion is 1 ηg/ml.

Figure 1 indicates that scleroglucan can induce IL12 (p40) secretion from the J774A.1 cell line. These data are the first demonstration that J774A.1 cells can secrete IL12. The threshold for IL12 secretion (25 μg/ml) is less than TNFα secretion (100 μg/ml). Laminarin did not cause secretion of either cytokine (concentration up to 800 μg/ml, data not shown).

This demonstrates that glucans can induce IL12 and TNFα secretion by murine macrophage cell lines in a dose related response and that glucans differ considerably in their ability to induce secretion of this cytokine.

**Glucan blocks LPS-FITC binding to Macrophages**

We utilized flow cytometry to determine LPS-FITC binding to macrophages/ monocytes. Details of the technique are presented in the Methods section. We found that scleroglucan can inhibit LPS-FITC binding to human monocytes.

We then used the same techniques to describe the interaction of the J774A.1 murine macrophage cell line, LPS and 2 different glucans. Figures 2 and 3 demonstrate blocking of LPS-FITC binding to murine macrophages by scleroglucan and laminarin, strongly suggesting that glucan binds to these cells using the same receptor as LPS.

## Discussion

Glucans have multiple immunomodulatory effects. They exhibit marked phagocytic and killing stimulatory effects on macrophages (Browder et al, 1988; Williams et al, 1987), stimulatory effects on T and B lymphocytes (Haba et al, 1976; Williams, et al., 1987), and neutrophils (Morikawa et al, 1985), act as adjuvants (Williams et al, 1978; Williams and Di Luzio, 1980; Williams et al, 1989), decrease survival of bone marrow transplant recipients (i.e. increase resistance to homologous bone marrow) (Wooles and Di Luzio, 1962) and enhance delayed type hypersensitivity reactions (Di Luzio, 1985). Glucans can induce granulomas (Johnson et al, 1984), increase protection against various infectious processes (Browder et al, 1984; Williams et al, 1983; Williams et al, 1982; Williams and Di Luzio, 1979), exhibit anti-tumor effects (Williams et al, 1985), and modulate immune response (Di Luzio, 1985). Glucans also increase macrophage secretion of IL-1 and GM-CSF (Di Luzio, 1985; Sherwood et al, 1987). Although many of the effects of glucan on macrophages are direct, there is evidence that some of glucan's effects may be mediated through lymphocytes (Di Luzio, 1985).

Glucans can induce secretion of many different cytokines. Yadomae and colleagues demonstrated increased secretion of IL1α, IL6 and TNFα from a macrophage cell line exposed to grifolan, a soluble glucan preparation (Adachi et al, 1994). Another soluble glucan preparation from the fungus Sclerotinia sclerotiorum, induced secretion of IL6 and TNFα from murine alveolar macrophages and expression of membrane IL1α *in vitro* (Sakurai et al, 1996) and IL1α production *ex vivo* (Sakurai et al, 1995).

Lipopolysaccharide is a constituent of many gram negative bacteria which is responsible for multiple effects on cells and organ systems in disease syndromes such as sepsis and adult respiratory disease syndrome. LPS binds to serum LPS binding protein (LBP), an acute phase protein, to form the LBP-LPS complex which transports LPS to CD14, the high affinity LPS receptor on macrophages. CD14 is a 53 to 55-kDa glycoprotein which exists either in a glycosyl-phosphotidylinositol anchored membrane bound form or in a soluble form. After binding of the LBP-LPS complex to membrane CD14, various intracellular tyrosine kinases are phosphorylated (Hambleton et al, 1996; Schumann et al, 1996), resulting in disassociation of NFκB from IκB, activation of cytoplasmic NFκB and degradation of IκB (Gupta et al, 1996). NFκB translocates to the nucleus where it binds to regulatory elements which activate gene transcription and induce production of the cytokines (in particular IL6, IFNγ, IL1β and TNFα) (Dentener et al, 1993), and changes of other cell properties (such as adhesion molecules in neutrophils), typical of the response to LPS. Soluble CD14 can substitute for LBP in transport of LPS to membrane CD14 (Hailman et al, 1996). This mechanism seems to be important in activating (i.e. inducing secretion of proinflammatory cytokines) cells that do not express the membrane bound form of CD14 (Frey et al, 1992; Pugin et al, 1995). LPS can also upregulate the expression of CD14 on macrophages (Hopkins et al, 1995), providing a positive feedback loop for LPS induced inflammation. LPS induces production of many different cytokines (including IL12) by monocytes and macrophages (Chensue et al, 1995; D'Andrea et al, 1992; DeKruyff et al, 1997; Heinzel et al, 1994; Skeen et al, 1996; Wysocka et al, 1995).

Microbial products other than glucan and LPS interact with macrophages through CD14. Bacterial peptidoglycans are polymers of cross linked glucopeptides from bacterial cell walls which are related to glucans. Peptidoglycans *in vivo* produce many of the same systemic effects as LPS such as fever and the acute phase response due to the release of cytokines from macrophages and other cells (Weidemann et al, 1994). *In vitro*, peptidoglycans are potent activators of macrophages and B cells (Dziarski, 1980; Gupta et al, 1995).

Peptidoglycans induce activation of NFκB and degradation of IκB in murine macrophage RAW264.7 cells via interaction of the polysaccharide portion of peptidoglycan with CD14 (Gupta, et al., 1996). Peptidoglycans also induce human peripheral blood monocyte secretion of IL1 and IL6 via CD14 (Weidemann, et al., 1994). However there are differences between the interaction of LPS and CD14 and the interaction of peptidoglycan and CD14 in that LBP does not enhance the interaction of peptidoglycan and CD14 (Mathison et al, 1992; Weidemann, et al., 1994).

Glucan also activates macrophages to secrete cytokines in a synergistic manner with LPS (Adachi, et al., 1994). The above suggests that both glucan and LPS might mediate their effects on cells (or at least macrophages) via interaction with CD14.

Normal human alveolar macrophages (AM) express CD14, although to a lesser extent (approximately 50%) than peripheral blood monocytes. Of interest, a greater proportion of alveolar macrophages (AM) from patients with hypersensitivity pneumonitis express CD14 than AM from normal subjects or those with idiopathic pulmonary fibrosis and sarcoidosis, another granulomatous interstitial lung disease (Hoogsteden et al, 1989; Pforte et al, 1993). LPS induced release of TNFα, IL6 and IL8 from alveolar macrophages is dependent on CD14 (Dentener, et al., 1993).

The data presented in this paper indicate that glucans can induce secretion of IL12 and TNFα, but not IL10 from macrophage cell lines. This is compatible with induction of a Th1 type immune response. In addition, glucans can block binding of LPS to macrophages, suggesting that LPS and glucans utilize similar receptors (such as CD14).

## Summary

We found that glucans can induce secretion of IL12, TNFα , but not IL10 from macrophage cell lines. This is compatible with induction of a Th1 type immune response. In addition, glucans can block binding of LPS to macrophages, suggesting that LPS and glucans utilize similar receptors.

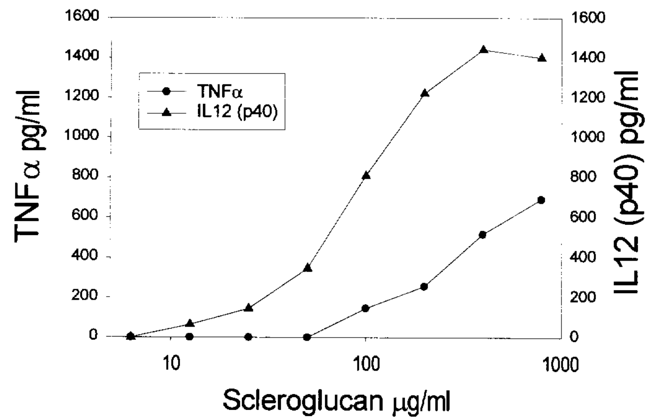


Figure 1. TNFα and IL12 secretion from J774KA.1 cells. Laminarin did not cause secretion of either cytokine (concentration up to 800 μg/ml, data not shown).

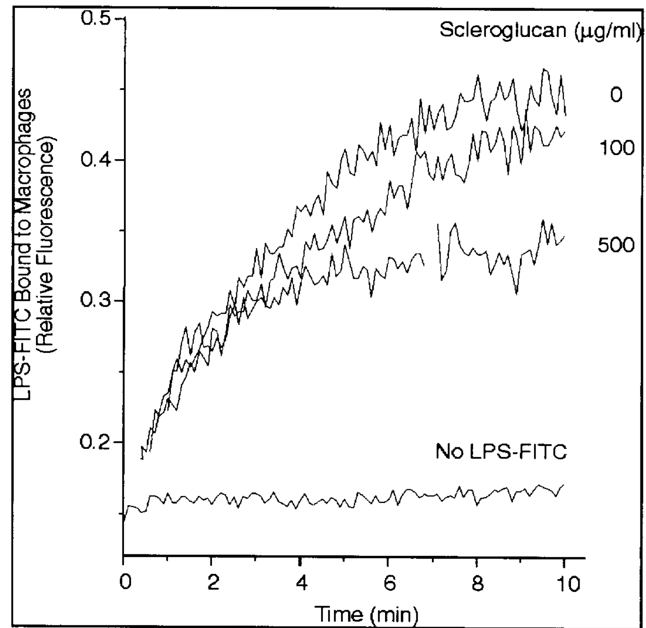


Figure 2. Scleroglucan blocks LPS-FITC binding to J774A. 1 cells in a dose dependent manner.

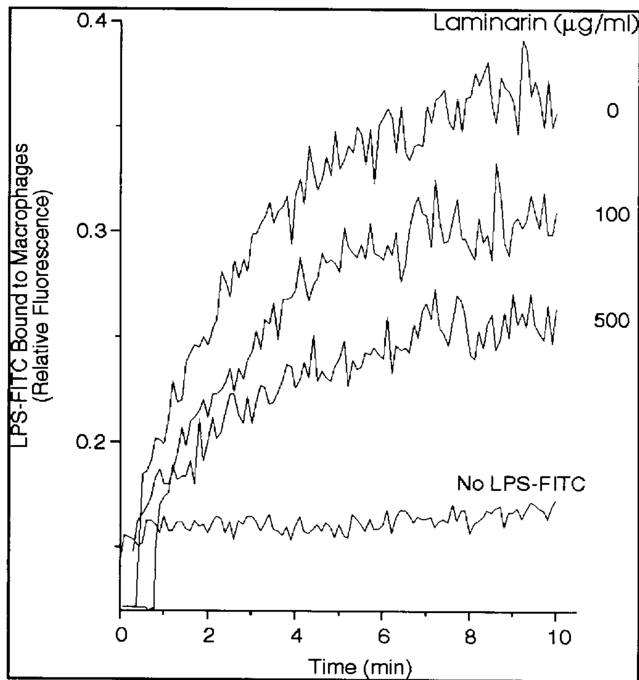


Figure 3. Laminarain blocks LPS-FITC binding to J77D.A.1 cells in a dose dependent manner.

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