OVERVIEW OF (1→3)-β-GLUCAN CHEMISTRY, IMMUNOLOGY AND TOXICOLOGY

David L. Williams
Department of Surgery
James H. Quillen College of Medicine
East Tennessee State University
Johnson City, TN

Abstract

Glucans are (1→3)-β-D-glucose polymers that are found in the cell wall of fungi, bacteria and plants. Glucans, isolated from natural sources, are known to stimulate humoral and cell-mediated immunity in humans and animals. In addition to the potent immune stimulatory effects of (1→3)-β-D-glucans, there are a number of toxicological effects associated with exposure to the water insoluble, micro-particulate form of the polymer. Recent investigations have also suggested a potential role for (1→3)-β-D-glucans in inhalational toxicity. Specifically, (1→3)-β-D-glucans have been implicated in the symptomatology associated with "sick building" syndrome. The mechanisms by which (1→3)-β-D-glucans mediate immune stimulation and, perhaps, toxicity are currently under investigation. It is now established that (1→3)-β-D-glucans are recognized by macrophages and, perhaps, neutrophils and NK cells via a (1→3)-β-D-glucan specific receptor. Following receptor binding, glucan modulates macrophage cytokine expression. Herein, we review the chemistry, immunobiology and toxicity of (1→3)-β-D-glucans and how it may relate to sick building syndrome.

Introduction

Glucans are polymers of glucose that are found widely distributed throughout the biosphere (1). Specifically, glucans are found in the cell wall of plants, bacteria and fungi, as minor constituents of fungal cytosol and as polymers which are secreted into the environment by glucan producing microorganisms (1). Glucans can be broadly classified according to the type of intrachain linkage of the polymer, *i.e.* α- or β-linked (1). The β-linked glucans are the predominant form found in fungi (1). It is the fungal derived (1→3)-β-D-glucans which have been reported to modulate various aspects of immunity (2-5). Recently, it has been proposed that glucans may play a role in the symptoms associated with sick building syndrome (6-15). This is due, in part, to their immunomodulatory activity and presence in the cell wall of fungi, which are frequently associated with "sick buildings". In the fungal cell wall (1→3)-β-D-glucans are linked to proteins, lipids and other carbohydrates such as mannan (1). The specific role for glucans in the physiology of fungi is not clearly understood. However, it is generally considered that the primary role for these polymers is a structural one, *i.e.* they play a role in maintaining the rigidity and integrity of the fungal cell wall (1). The glucan polymers in the fungal cell wall may form a meshwork, due to the presence of (1→6)-β-D-glucopyranose side-chain branches, which may connect adjacent (1→3)-β-D-glucan polymers (1). The basic structure of a non-branched (1→3)-β-D-glucan polymer and a (1→3)-β-D-glucan polymer with single (1→6)-β side-chain branches are shown in Figure 1. Glucan polymers can exist as a single polymer strand with a helical conformation (single helix) or as a stable complex of three polymer strands forming a triple helix (16). The triple helix, which appears to be the preferred form, is stabilized by extensive hydrogen bonding at the O-2 hydroxyl (17-20).

This report will focus on the: i) growing evidence which implicates (1→3)-β-D-glucans in the symptoms associated with sick building syndrome (6-15); ii) immunologic effects of (1→3)-β-D-glucans and iii) mechanisms by which (1→3)-β-D-glucans may modulate macrophage activity and subsequently immunity.

Potential Role of (1→3)-β-D-Glucans in Symptoms Related to Sick Building Syndrome.

Rylander, Fogelmark, Goto and colleagues (6-15) have recently speculated on the potential role of (1→3)-β-D-glucans in the pulmonary symptoms associated with "sick building" syndrome. In 1992, Rylander *et al.* reported that airborne (1→3)-β-D-glucan may be related to symptoms in sick buildings (6). These workers measured airborne levels of glucan and endotoxin in four buildings where complaints about indoor air quality had been registered. A control building, where no similar complaints were recorded, was assessed in an identical manner. Elevated levels of both glucan and endotoxin were recorded in the suspected "sick buildings", when compared to the control building. There was a statistically significant correlation between elevated levels of glucan and dry cough and itchy skin in the sick buildings. In addition, there was a statistically significant correlation between airborne endotoxin and skin rashes. The authors correctly pointed out that the data acquired was based on a limited set of samples. Further, these workers noted that the data acquired did not establish a cause-and-effect relationship between the presence of glucan and/or endotoxin and the symptoms reported in the buildings. Nevertheless, these data suggested a potential role for glucan and/or endotoxin involvement in the symptoms associated with sick building syndrome.

To test the hypothesis that airborne glucan and/or endotoxin play a role in sick building symptomatology, Rylander, Fogelmark and co-workers (7,9,11,14,15) have studied the effects of these agents under controlled laboratory conditions. Fogelmark *et al.* (14) have examined the acute pulmonary toxicity of inhaled (1→3)-β-D-glucan and endotoxin on guinea pig lung inflammatory cells. In this study, a crude glucomannan preparation, zymosan, a

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:285-288 (1996)
National Cotton Council, Memphis TN

purified (1→3)-β-D-glucan, curdulan, and a branched (1→3)-β-D-glucan, schizophyllan, were evaluated. While inhalational exposure to endotoxin increased lung lavage and wall inflammatory cells, inhaled curdulan either had no effect or reduced inflammatory cell infiltrate. When curdulan and endotoxin were administered conjointly, the inflammatory response to endotoxin was reduced (14). Subsequent studies by Fogelmark *et al.* (15) have extended these observations. In this study, guinea pigs were chronically exposed to repeated inhalations of curdulan and/or endotoxin for a period of up to 5 weeks. Chronic exposure to curdulan did not significantly alter lung lavage cell number. As expected, chronic endotoxin exposure increased the number of lung lavage cells for all cell types examined (15). When curdulan and endotoxin were combined a significant increase in the number of macrophages, lymphocytes and neutrophils was observed, when compared to either endotoxin or curdulan alone (15). Pulmonary histopathology was also evaluated in control, endotoxin, curdulan and endotoxin + curdulan exposed guinea pigs. A significant increase in alveolar infiltrate, interstitial cell infiltrate and alveolar wall thickening was noted (15). The authors concluded that the chronic curdulan and endotoxin inhalation resulted in a synergistic effect on lung lavage cells and pulmonary histopathology. These workers also speculated that exposure to both agents was necessary for induction of a pulmonary response in sick buildings. Taken together these studies suggest a potential role for (1→3)-β-D-glucan and endotoxin as mediators of sick building symptoms. An important caveat in these studies concerns the use of a purified (1→3)-β-D-glucan, such as curdulan, as the stimulus. In order to test the hypothesis that glucans mediate, in part, the symptoms associated with sick buildings, it is essential to examine the effect of a "chemically defined" glucan preparation under controlled laboratory conditions, such as those described above. However, in the real world scenario of "sick buildings" the glucans that would be encountered, would in all likelihood be part of a fungal cell wall where they would be complexed to proteins, lipids and other carbohydrates. The pathophysiological effect of such a complex mixture will be difficult to elucidate. Additional research is required to address this important issue.

Immunologic effects of (1→3)-β-D-glucans

The ability of naturally occurring complex polysaccharide polymers to modulate immunity has been well documented (2-5). In 1959, Benacerraf and Sebestyn demonstrated that zymosan, a glucomannan isolated from *Saccharomyces cerevisiae*, would produce marked hyperplasia and hyperfunctionality of fixed tissue macrophages (21). Di Luzio (22), Cutler (23), Kelly and colleagues (24) confirmed and extended the work of Benacerraf and Sebestyn (21). In 1961, Di Luzio and Riggi (25) demonstrated that glucan, a (1→3)-β-D-linked glucopyranose polymer, was the macrophage stimulating agent in zymosan. Since that time, numerous studies have shown that (1→3)-β-D-glucan polymers will enhance the functional status of macrophages (26-29), neutrophils (30,31) and other immunocytes. These observations have stimulated investigation into the potential biomedical applications of polymeric (1→3)-β-D-glucans (4). Unfortunately, there were also toxicological effects associated with the systemic administration of these agents. Upon initial isolation from yeast, (1→3)-β-D-glucans are usually water insoluble, micro-particulates (16). Systemic (IV) administration of glucan micro-particulates is associated with hypertrophy and hyperplasia of macrophage rich organs such as the liver, lung and spleen. Specifically, granuloma formation is observed (32). Interestingly, many workers have reported that if (1→3)-β-D-glucans are converted to a water soluble form the immunologic activity is preserved, but the undesirable side-effects are eliminated (33).

The studies by Rylander, Fogelmark and colleagues (7,9,11,14,15) suggest that a potential role for (1→3)-β-D-glucans in pulmonary symptomatology may include synergy with endotoxin. Therefore, an important aspect of glucan's immunobiological activity, which may be of particular relevance to sick building syndrome, is its adjuvant effect. Numerous studies have documented the ability of (1→3)-β-D-glucans to exert adjuvanticity when combined with a variety of bacterial, viral or parasite vaccines (34-41). Glucan has been shown to enhance humoral and cell-mediated immunity to antigens (34-41). In addition, glucans have been demonstrated to exert additive or synergistic effects on immunity when combined with a variety of agents (27,29).

Receptor Mediated Binding of a (1→3)-β-D-Glucan to the Human Macrophage Cell Line, U937.

The cellular and molecular mechanisms by which (1→3)-β-D-glucans modulate immunity are just beginning to emerge. We (27,29) have shown that glucans mediate immunological activity, in part, via macrophage participation. Fogelmark *et al.* (15) have speculated that glucans mediate pulmonary effects due, in part, to their effect on macrophages. The first step in the interaction of glucan with mammalian macrophages is thought to involve the binding of (1→3)-β-D-glucan to a macrophage receptor (42-45). In support of this concept, Czop and co-workers (42-45) have reported the existence of a (1→3)-β-D-glucan specific receptor on human monocytes. Goldman (46) has reported the presence of a (1→3)-β-D-glucan receptor on P388D1 cells, a murine macrophage cell line. Williams *et al.* (31) have extended these observations by reporting a glucan specific receptor on human polymorphonuclear leukocytes. These reports of specific (1→3)-β-D-glucan receptors on mammalian immunocytes offers a tantalizing explanation for the cellular activation induced by glucans. However, the *in vivo* significance of these receptor studies is uncertain, because virtually all of these experiments were limited to *in vitro* phagocytosis and/or phagocytosis

inhibition assays (31,42,43). More importantly, virtually all of these studies employed water insoluble glucans which were not chemically characterized (31,42,43). To conclusively demonstrate the presence of a (1→3)-β-D-glucan specific receptor required a water soluble, chemically characterized, radiolabeled glucan ligand. We have developed a process for incorporating a non-exchangable ^{3}H-label into a chemically pure, well characterized, water soluble glucan, termed "glucan phosphate" (16). Preliminary data with the human promonocytic cell line, U937, indicates that glucan phosphate binding obeys the criterion for specific binding in that competition for the bind sites can be demonstrated in the presence of a 10- fold excess of unlabeled ligand (data in press). Association binding studies were also undertaken. In these experiments, U937 (1 x 10^6 cells) were co-incubated with varying concentrations of ^{3}H-glucan phosphate for 90 minutes (Fig. 2). We observed a rate constant (K_{ob}) of 0.95 min^{-1}, a K_D of 37 µM and a B_{MAX} of 6.5 x 10^7 sites/cell. This indicated a medium affinity receptor and a rapid binding of the ligand. The B_{MAX} value more than accounts for the entire U937 cell surface area. This suggested that the glucan was being internalized following receptor binding. Konopski *et al.* (47) have reported that murine macrophages will bind and internalize a fluorescent labeled glucan. Our observations are consistent with those of Konopski *et al.* (47). We have examined U937 cells by electron microscopy following glucan exposure and observed an increase in phagolysosome formation which is consistent with uptake of glucan (data in press). We concluded that the binding and internalization of glucans by human macrophages is a two-phase process; the first phase is a rapid binding of the glucan ligand to the receptor followed by a slower uptake/internalization phase. In subsequent studies, we examined the specificity of the (1→3)-β-D-glucan receptor on U937 cells. In this series of experiments, U937 (1 x 10^6 cells) was incubated with ^{3}H-glucan phosphate and varying concentrations of unlabeled glucan phosphate, pullulan, a (1→4)-α-linked glucose polymer and schizophyllan (SPG), a branched (1→3)-β-D-glucan polymer (Fig. 3) for 90 min. In this competitive binding study, pullulan did not compete for binding to the (1→3)-β-D-glucan receptor. Schizophyllan showed a 5-fold increase in affinity for the human macrophage (1→3)-β-D-glucan receptor, when compared to non-branched glucan phosphate. These data conclusively demonstrate the presence of a (1→3)-β-D-glucan specific receptor on a human macrophage-like cell line. Further, these data demonstrate that the glucan receptor is specific for (1→3)-β-linked polymers and that the glucan receptor has a higher affinity for one form of glucan over another, *i.e.* branched versus non-branched.

Conclusions

Indirect evidence continues to mount concerning the potential involvement of (1→3)-β-D-glucans in the symptoms associated with sick building syndrome. The data thus far suggests that glucans must act in concert with other etiologic agents, such as endotoxin, in order to mediate symptomatology. Therefore, the primary role of (1→3)-β-D-glucans appears to be as an adjuvant which exerts an additive or synergistic effect when combined with other agents. It is important to note that absolute proof of a cause-and-effect relationship between the presence of airborne (1→3)-β-D-glucans and sick building syndrome has not been established. Additional research is required in order to confirm a role for glucans in this condition.

References

1. Ruiz-Herrera, J. 1991. Biosynthesis of beta-glucans in fungi. *Antonie van Leeuwenhoek*, 60:72-81.

2. Williams, D., Mueller, A., and Browder, W. 1995. Prevention and clinical evaluation of carbohydrate immunopharmaceuticals in the prevention of sepsis and septic sequelae. *The Journal of Endotoxin Research*, 2:203-208.

3. Williams, D.L., Pretus, H.A., McNamee, R.B., et al. 1991. Development, physicochemical characterization and preclinical efficacy evaluation of a water soluble glucan sulfate derived from *Saccharomyces cerevisiae*. *Immunopharmacol.* 22:139-156.

4. Browder, W., Williams, D., Pretus, H., et al. 1990. Beneficial effect of enhanced macrophage function in the trauma patient. *Ann.Surg.* 211:605-613.

5. Williams, D. and Browder, W. 1994. Development of natural product (1→3)-β-D-glucan polymers as immune stimulating pharmaceuticals. *Polym. Adv. Tech.* 5:529-534.

6. Rylander, R., Persson, K., Goto, H., et al. 1992. Airborne Beta-1,3-glucan may be related to symptoms in sick buildings. *Indoor Environ.* 1:263-267.

7. Goto, H., Yuasa, K., and Rylander, R. 1994. (1-3)-β-D-glucan in indoor air, its measurement and in vitro activity. *Amer.J.of Ind.Med.* 25:81-83.

8. Rylander, R., Hsieh, V., and Courteheuse, C. 1994. The first case of sick building syndrome in Switzerland. *Indoor Environ.* 3:159-162.

9. Fogelmark, B., Sjostrand, M., and Rylander, R. 1994. Pulmonary inflammation induced by repeated inhalations of beta(1, 3)-D-glucan and endotoxin. *Int.J.Exp.Pathol.* 75:85-90.

10. Goto, H., Yuasa, K., and Rylander, R. 1994. (1-->3)-beta-D-glucan in indoor air, its measurement and in vitro activity. *Am.J.Ind.Med.* 25:81-83.

11. Rylander, R. and Fogelmark, B. 1994. Inflammatory responses by inhalation of endotoxin and (1-->3)-beta-D-glucan. *Am.J.Ind.Med.* 25:101-102.

12. Rylander, R., Goto, H., Yuasa, K., et al. 1994. Bird droppings contain endotoxin and (1-->3)-beta-D-glucan. *Int.Arch.Allergy Immunol.* 103:102-104.

13. Rylander, R. and Peterson, Y. 1992. Recent Experiences on effects of Endotoxin and β,1-3 Glucan in indoor Air. *Beltwide Cotton Conferences*, 288-289.

14. Fogelmark, B., Goto, H., Yuasa, K., et al. 1992. Acute pulmonary toxicity of inhaled β-1,3-glucan and endotoxin. *Agents Actions*, 35:50-56.

15. Fogelmark, B., Sjostrand, M., and Rylander, R. 1994. Pulmonary inflammation induced by repeated inhalations if β(1,3)-D-glucan and endotoxin. *Int.J.Exp.Path.* 75:85-90.

16. Williams, D.L., McNamee, R.B., Jones, E.L., et al. 1991. A method for the solubilization of a (1-3)-β-D-glucan isolated from *Saccharomyces cerevisiae*. *Carbohyd.Res.* 219:203-213.

17. Ensley, H.E., Tobias, B., Pretus, H.A., et al. 1994. NMR spectral analysis of a water-insoluble (1-3)-β-d-glucan isolated from *Saccharomyces cerevisiae*. *Carbohyd.Res.* 258:307-311.

18. Marchessault, R.H. and Deslandes, Y. 1979. Fine structure of (1-->3)-D-glucans: Curdlan and paramylon. *Carbohyd.Res.* 75:231-242.

19. Deslandes, Y., Marchessault, R.H., and Sarko, A. 1980. Triple-helical structure of (1-->3)-D-glucan. *Macromolecules*, 13:1466-1471.

20. Bluhm, T.L., Deslandes, Y., Marchessault, R.H., et al. 1982. Solid-state and solution conformation of scleroglucan. *Carbohyd.Res.* 100:117-130.

21. Benacerraf, B., Thorbecke, G.J., and Jacoby, D. 1959. Effect of zymosan on endotoxin toxicity in mice. *Proc.Soc.Exp.Biol.Med.* 100:796-799.

22. Di Luzio, N.R. and Morrow, S.H. 1965. The fate of foreign red cells in mice with altered reticuloendothelial function. *Proc.Soc.Exp.Biol.Med.* 119:647-652.

23. Cutler, J.L. 1960. The enhancement of hemolysin production in the rat by zymosan. *J.Immunol.* 84:416-419.

24. Kelly, L.S., Dobson, E.L., Finney, C.R., et al. 1960. Proliferation of the reticuloendothelial system in the liver. *Am.J.Physiol.* 198:1134-1138.

25. Riggi, S.J. and Di Luzio, N.R. 1961. Identification of a reticuloendothelial stimulating agent in zymosan. *Am.J.Physiol.* 200:297-300.

26. Sherwood, E.R., Williams, D.L., McNamee, R.B., et al. 1987. In vitro tumoricidal activity of resting and glucan-activated Kupffer cells. *J.Leukocyte Biol.* 42:69-75.

27. Browder, W., Williams, D., Sherwood, E., et al. 1987. Synergistic effect of nonspecific immunostimulation and antibiotics in experimental peritonitis. *Surgery*, 102:206-214.

28. Williams, D.L. and DiLuzio, N.R. 1980. Glucan-induced modification of murine viral hepatitis. *Science*, 208:67-69.

29. Williams, D.L., Sherwood, E.R., McNamee, R.B., et al. 1987. Chemoimmunotherapy of Experimental Hepatic Metastases. *Hepatology*, 7:1296-1304.

30. Williams, D.L., Sherwood, E.R., Browder, I.W., et al. 1988. Effect of glucan on neutrophil dynamics and immune function in Escherichia coli peritonitis. *J.Surg.Res.* 44:54-61.

31. Williams, J.D., Topley, N., Alobaidi, H.M., et al. 1986. Activation of human polymorphonuclear leucocytes by particulate zymosan is related to both its major carbohydrate components: glucan and mannan. *Immunology*, 58:117-124.

32. Williams, D.L., Sherwood, E.R., McNamee, R.B., et al. 1985. Therapeutic efficacy of glucan in a murine model of hepatic metastatic disease. *Hepatology*, 5:198-206.

33. Williams, D.L., Sherwood, E.R., Browder, I.W., et al. 1988. Pre-clinical safety evaluation of soluble glucan. *Int.J.Immunopharmac.* 10(4):405-411.

34. Holbrook, T.W., Cook, J.A., and Parker, B.W. 1981. Glucan-enhanced immunogenicity of killed erythrocytic stages of *Plasmodium berghei*. *Infec.Immun.* 32(2):542-546.

35. Holbrook, T.W., Cook, J.A., and Parker, B.W. 1981. Immunization against *Leishmania Donovani*: Glucan as an adjuvant with killed promastigotes. *Am.J.Trop.Med.Hyg.* 30(4):762-768.

36. Cook, J.A. and Holbrook, T.W. 1983. Immunogenicity of soluble and particulate antigens from *Leishmania donovani*: Effect of glucan as an adjuvant. *Infec.Immun.* 40(3):1038-1043.

37. Holbrook, T.W. and Cook, J.A. 1983. Non-specific and Specific Stimulation of Resistance Against Leishmania

donovani in C57Bl/6J Mice. *Annals of Clinical and Laboratory Science,* 13(5):411-417.

38. Holbrook, T.W. and Cook, J.A. 1983. Immunization of mice against Leishmania donovani by subcutaneous injections of dead promastigotes. *Am.J.Trop.Med.Hyg.* 32(1):51-53.

39. Benach, J.L., Habicht, G.S., Holbrook, T.W., et al. 1982. Glucan as an adjuvant for a murine Babesia microti immunization trial. *Infec.Immun.* 35(3):947-951.

40. Lasarow, R.M., Williams, D.L., and Theis, J.H. 1992. Humoral response following immunization with *Leishmania infantum* (ex. Oklahoma): A comparison of adjuvant efficacy in the antibody responses of Balb-C mice. *Int.J.Immunopharmacol.* 14:767-772.

41. Williams, D.L., Yaeger, R.G., Pretus, H.A., et al. 1989. Immunization against Trypanosoma cruzi: Adjuvant effect of glucan. *Int.J.Immunopharmac.* 11:403-410.

42. Abel, G. and Czop, J.K. 1992. Stimulation of human monocyte β-glucan receptors by glucan particles induces production of TNFα and IL-β. *Immunopharmac.* 14:1363-1373.

43. Janusz, M.J., Austen, K.F., and Czop, J.K. 1988. Isolation of a unit ligand for beta-glucan receptors of human monocytes. *Intl.J.Immunopharmacol.* 10(S1):89

44. Janusz, M.J., Austen, K.F., and Czop, J.K. 1989. Isolation of a yeast heptaglucoside for monocyte phagocytic β-glucan receptors. *FASEB J.* 3:6421

45. Czop, J.K. and Kay, J. 1991. Isolation and characterization of β-glucan receptors on human mononuclear phagocytes. *J.Exp.Med.* 173:1511-1520.

46. Goldman, R. 1988. Induction of a beta-1,3-D-glucan receptor in P388D1 cells treated with retinoic acid or 1,25-dihydroxyvitamin D_3. *Immunology*, 63:319-324.

47. Konopski, Z., Fandrem, J., Selejid, R., et al. 1995. Interferonγ inhibits endocytosis of soluble snimated β-1,3-D-glucan and neutral red in mouse peritoneal macrophages. *Journal of Interferon and Cytokine Research,* 15:597-603.

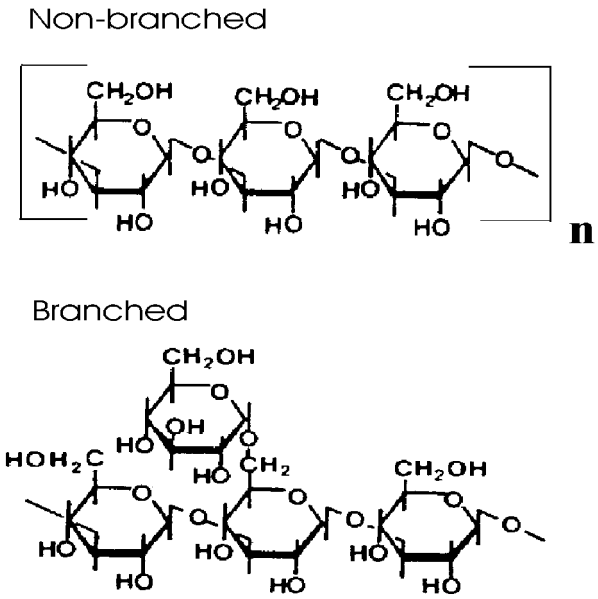

Figure 1. Primary structure of a single, non-branched and a single, branched (1→3)-β-D-glucan polymer. The backbone of the glucan polymer is composed of glucose subunits connected by intrachain glycosidic (1→3)-β-linkages. In a branched (1→3)-β-D-glucan polymer the branches are connected by (1→6)-β-linkages. In this model the side-chain branch is a single glucose subunit. However, side-chains may contain multiple glucose subunits. Glucan polymers can exist as a single polymer strand with a helical conformation (single helix) or as a stable complex of three polymer strands forming a triple helix. The triple helical form is generally considered to be the preferred form in nature.

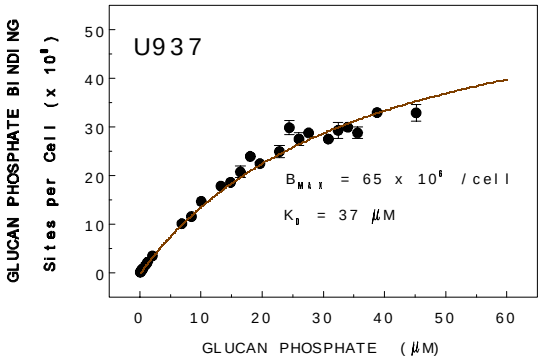

Figure 2. Specific binding and internalization of glucan phosphate to U937 cells. Combined saturation and competitive displacement data yielded specific binding (sites per cell) to U937 as a function of (1→3)-β-D-glucan phosphate molarity at 37°C. We mathematically derived the K_D and B_{MAX} value. The results are given as means and 95% CI of means ± s.e. of at least four replicates and represent data from five separate experiments.

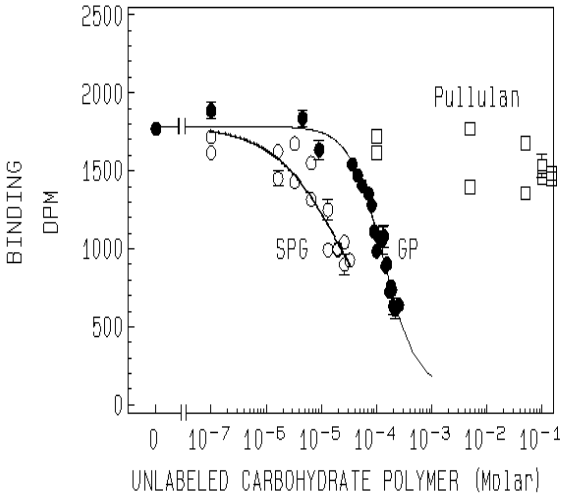

Figure 3. Competitive displacement of tritiated glucan phosphate by unlabeled glucan phosphate and schizophyllan at 37ºC. Pullulan (□) does not compete for the same binding site as unlabeled glucan phosphate (●). Schizophyllan (SPG, ❍) does compete for the same binding site as unlabeled glucan phosphate and has an approximate 5-fold increase in affinity for the binding site. The results are shown as means ± s.e. of at least four replicates from two separate experiments.