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Characteristics of Binding of Interleukin-1 to YT.NCI Cells

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CHARACTERISTICS OF BINDING OF INTERLEUKIN-1 TO YT.NCI CELLS

by

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CHARACTERISTICS OF BINDING OF INTERLEUKIN-1 TO YT.NCI CELLS

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Western Michigan University, 1988

Interleukin-1 (IL-1) demonstrated a concentration dependent, specific binding to the human, large granular lymphocyte cell line, YT.NCI with a dissociation constant of 0.2 nM. There were approximately 500 IL-1 receptors/cell.

IL-1 binding to YT.NCI cells could be inhibited by unlabeled-IL-1 and the plant lectins, WGA and Con A. Lectin inhibition of IL-1 binding could be completely abrogated by exposing lectins to specific simple sugars prior to the addition of ligand.

Tunicamycin, an inhibitor of glycosylation, decreased IL-1 binding to YT cells in a reversible, time- and concentration-dependent manner.

These observations suggest that glycosylation of cell surface proteins may be important for IL-1 receptor orientation, expression, and/or functional ligand binding. Furthermore, the demonstration that specific plant lectins effectively compete with iodinated IL-1 for IL-1 receptor occupancy could facilitate the establishment of conditions for successful isolation and purification of IL-1 membrane receptors via affinity chromatography on immobilized lectins.

ACKNOWLEDGMENTS

I would like to acknowledge my parents, Frank and Mildred Speziale, whose unfaltering faith in me certainly made this endeavor possible. I would also like to take this opportunity to say thanks to my friends and colleagues at work who have continually shown interest in my thesis project and have cheered me on. I would especially like to acknowledge my supervisors at The Upjohn Company, Robert J. Smith, Ph.D., for teaching me scientific thoroughness and excellence, and Jefferson W. Paslay, Ph.D., for encouraging me to expand my horizons and tackle new challenges. My advisor, Leonard C. Ginsberg, Ph.D., and committee members have kept me on target and I'm grateful to them for that.

A special family member, Micheal Kiser, has been at my side for the past few years. His support has been a godsend.

Finally, I would like to dedicate this thesis to my loving daughter, Allyson Haag, whose ever-questioning mind always reminds me why I love science.

Susan C. Speziale

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INTRODUCTION

Interleukin-1 (IL-1) is a cytokine which is produced by a large variety of mammalian cell types, including monocytes, macrophages, keratinocytes, astrocytes and endothelial cells. The first activity ascribed to IL-1 was the ability of culture supernatants from human peripheral blood adherent leukocytes to stimulate proliferation of murine thymocytes, but not immunocompetent peripheral lymphocytes (Gery, Gershon & Waksman, 1972). Gery et al. (1972) also demonstrated that IL-1 synergized with the mitogens, concanavalin A and phytohemagglutinin to augment murine thymocyte and splenic T cell proliferation. This initial activity labeled IL-1 as a lymphocyte activating factor (LAF) and remains a standard for ascribing IL-1-like activities to various molecules. Since then, IL-1 has been attributed with other biological functions and in vitro activities, such as: induction of acute phase protein production by hepatocytes; stimulation of prostaglandins, collagenase and plasminogen activator from chondrocytes, osteoclasts and fibroblasts during inflammation, tissue repair and wound healing; stimulation of immune responsiveness by inducing lymphokine secretion; chemotactic properties; and fever production. Several factors are now known to be biologically, biochemically and structurally similar to IL-1, including epidermal thymocyte activating factor (ETAF), B cell activating factor (BAF), endogenous pyrogen (EP) and catabolin (Oppenheim, Kovacs, Matsushima & Durum, 1986). Mizel, Kilian, Lewis, Paganelli and Chizzonite (1987) demonstrated that IL-1 could stimulate the production

of collagenase and prostaglandins by synovial cells from rheumatoid arthritic patients. Russell, McGuire, Meats, Ebsworth and Beresford (1981) showed that rheumatoid synovium could produce factors which stimulate chondrocytes to degrade their own matrix, resulting in bone loss. In addition, IL-1 has been shown to induce fibroblast proliferation (Schmidt, Mizel, Cohen & Green, 1982), and its production by infiltrating monocytes may contribute to the fibrosis and tissue destruction seen in chronic inflammatory disease. Therefore, IL-1 is thought to be a principle mediator in many normal, as well as pathological, immune and immunoinflammatory processes. A large research effort is on-going to find IL-1 antagonists which may be used as therapeutic agents, particularly in inflammatory diseases.

Two polypeptides have been identified which share IL-1 biological activities, IL-1 alpha and IL-1 beta. These polypeptides have molecular weights of 15-20,000 daltons and have distinct amino acid sequences and isoelectric points (Oppenheim et al., 1986). In spite of the growing body of knowledge on IL-1 structure and function, little is known about intracellular signal transduction mechanisms or regulation of IL-1.

Apparently both IL-1α and IL-1β exert their myriad biological effects by interaction with the same receptor (Dower et al., 1986; Kilian et al., 1986; Matsushima, Akahoshi, Yamada, Furutani & Oppenheim, 1986a). Why nature selected two distinct molecules to carry out similar functions is a mystery that remains to be resolved.

Characteristics of the IL-1 receptor and binding of its ligand are beginning to be elucidated. Molecular weight of the integral membrane protein IL-1 receptor is estimated at 60-100,000 daltons (Dower and Urdal, 1987; Matsushima et al., 1986a), but purification and primary structure have not yet been described. IL-1 receptors have been enumerated on several cell types (Dower and Urdal, 1987) and, compared to other hormone receptors, IL-1 receptor densities are quite small. Mouse thymocytes, spleen cells and lymph node cells reportedly have fewer than 10 IL-1 receptors per cell. Human peripheral blood mononuclear cells have less than 30 IL-1 receptors/cell, human T cells have about 100/cell, while human gingival and dermal fibroblasts have 2000-5000 IL-1 cell surface receptors (Dower and Urdal, 1987). A variety of in vitro cell lines have been analyzed for IL-1 receptors. A subclone of the murine T cell lymphoma EL4 cell line was found to have approximately 10,000 IL-1 receptors/cell, the highest density of IL-1 cell surface receptors reported to date. Investigators screening various human cell lines have found few with relatively high IL-1 receptor densities (Dower and Urdal, 1987). Matsushima, Yodoi, Tagaya and Oppenheim (1986b) recently described an IL-1 receptor density of 7000 receptors/cell on YT cells, a cell line derived from a 15 year old boy with acute lymphoblastic lymphoma and thymoma (Yodoi et al., 1985). YT cells grow continuously in typical suspension culture. This human cell line expressed surface antigens similar to those seen on large granular lymphocytes, known as natural killer cells, such as interleukin-2, Fc receptors for IgG and an antigen found on a subset of

natural killer cells, HNK-1. YT cells also demonstrated killing against several human target cell lines, typical of natural killer-like activity (Yodoi et al., 1985). YT cells were selected for the studies planned in this research project.

The purpose of this master's thesis is to explore IL-1 binding, utilizing tools, such as lectins and an antibiotic inhibitor of glycosylation, which may shed light on the primary structure of the IL-1 receptor. I will attempt to study aspects of IL-1 receptor binding that relate to receptor recognition structures through the use of lectins. Lectins are proteins that have become useful tools for exploring the surface characteristics of numerous mammalian cells and tissues. These proteins were first known as erythrocyte agglutinins and, later, as lectins, because of their ability to distinguish between human blood group antigens. They were more recently redefined (Goldstein, Hughes, Monsigny, Osawa & Sharon, 1980) as proteins that recognize specific sequences of sugar residues that can agglutinate cells or precipitate glytoconjugates, but are not antibodies. Implicit in this definition is that a lectin must have at least 2 sugar-binding sites to provide the capability to cross-link glycoproteins or other glycoconjugates that would result in agglutination or precipitation. Several investigators (i.e., Cuatrecasas, 1973; Monsigny, Roche, Sene, Maget-Dana & Delmotte, 1980) have demonstrated biphasic or more complex binding of lectins to intact cells. Experiments conducted by Monsigny et al. (1980) suggested that sugar-lectin interactions depend on

the structure and density of sugar residues available for binding, as well as on charge effects.

Lectin specificity is defined primarily by the free sugar or sugars that will inhibit the agglutination or precipitation that the lectin induces. However, as Monsigny et al. (1980) and others (Hedo, 1984) have indicated, there are instances where a free, simple sugar will not inhibit lectin activities, but the same sugar moiety found within a glycoconjugate may bind lectin. Table 1 contains a list of the lectins used in experiments reported here and their various sugar specificities.

Since sugar-lectin binding is reversible and can be inhibited by simple sugars, lectins are frequently derivatized, immobilized on solid matrices and thereby used to isolate and purify membrane receptors via a type of affinity chromatography. This technique has been used successfully to isolate a number of receptors, including receptors for acetylcholine, epidermal growth factor, immunoglobulin E and insulin (Hedo, 1984), and thereby helped to establish that these membrane receptors were glycoproteins. The process of selecting a lectin for this purpose involves screening a variety of lectins with different sugar specificities in a binding assay, looking for the ability of free lectin to antagonize binding of the specific ligand to the receptor in question. Part of the purpose of this research is to test a panel of 9 lectins in an intact-cell, IL-1 receptor binding protocol.

In order to further explore the importance of glycoprotein structures in IL-1 binding, the antibiotic tunicamycin was incorporated into cell

Table 1

Carbohydrate Binding Properties of Lectins

Lectin	Plant Source	Sugar Specificity
Concanavalin A (Con A)	Canavalia ensiformis	α-D-mannose α-D-glucose
Lentile Culinaris hemagglutinin (LCH)	Lens culinaris	α-D-mannose α-D-glucose
Mycoplasma gallisepticum agglutinin (MGA)	Mycoplasma gallisepticum	glycophorin (sialyl glycoprotein)
Phytohemagglutinin (PHA)	Phaseolus vulgaris (red kideny bean)	N-acetyl-D-galactosamine
Pokeweed mitogen (PWM)	Phytolacca americana	N-acetyl-β-D-galactosamine
Soybean agglutinin (SBA)	Glycine max	N-acetyl-D-galactosamine
Ulex europaeus agglutinin (UEA)	Ulex europaeus (gorse, UEA I)	α-L-fucose
Wheat germ agglutinin (WGA)	Triticum vulgaris	N-acetyl-β-D-glucosamine
Wisteria floribunda agglutinin (WFA)	Wisteria floribunda	N-acetyl-D-galactosamine

growth media to block protein glycosylation. Tunicamycin inhibits lipid carrier-dependent protein glycosylation by blocking a specific transferase which catalyzes the transfer of N-acetylglucosamine to dolichyl phosphate, the first step in glycosylation of nascent polypeptides (Duksin & Mahoney, 1982). The transfer of oligosaccharide to a newly synthesized protein occurs in the lumen of the endoplasmic reticulum (Keller, 1987). The new glycoprotein can then be transported to other cellular organelles, such as the golgi. Carbohydrate structures on glycoproteins are modified in the golgi, resulting in proteins containing either complex oligosaccharides or simpler ones that are high in mannose. Inhibition of glycosylation by tunicamycin is accompanied by minimal effect on protein synthesis, less than 10% of control, as reported by Chatterjee, Kwiterovich and Sekerke (1979) and Ronnett and Lane (1981). My studies will look at tunicamycin's effect on IL-1 binding in a typical binding assay using iodinated ligand, and on lectin binding via flow cytometry using fluorescently-labeled lectin.

MATERIALS AND METHODS

Materials

YT.NCI Cells

Human YT.NCI cells (YT, obtained from K. Matsushima, NCI, Frederick, MD) were maintained in suspension culture at 37° C, 5% CO2, in RPMI-1640 (GIBCO, Grand Island, NY) containing 10% fetal calf serum (heat-inactivated, Hyclone, Logan, UT), 2 mM L-glutamine (MA Bioproducts, Walkersville, MD), and 50 μg/ml gentamicin (GIBCO). Tunicamycin (Sigma, St. Louis, MO) was dissolved in culture medium. Cell viability was determined by trypan blue dye exclusion.

Recombinant Protein

Recombinant human IL-1β (rhuIL-1β; Batch 12/86) was cloned, expressed in E. coli, isolated, purified, and iodinated at The Upjohn Company, Kalamazoo, MI. Grade I rhuIL-1β (100% pure) was used for iodination, while Grade III (95-99% pure) was used as cold IL-1 for competition studies and in demonstration of non-specific binding (NSB).

Preparation of Iodinated Ligand

Mono-iodo-^{125}I-Bolton Hunter reagent (Amersham, Arlington Heights, IL) was coupled to rhuIL-1β (2 x 10^7 U/mg) according to the published method (Bolton & Hunter, 1973). Iodination of rhuIL-1β was

achieved without loss in bio-activity, as measured by a modification of the interleukin-1/interleukin-2 T-cell assay (Gillis & Mizel, 1981). Specific activity of ^{125}I-rhuIL-1β ranged from 1313-2611 Ci/mmole. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of ^{125}I-rhuIL-1β consistently revealed a single peak of iodinated protein at 20,000 daltons.

Lectins and Sugars

Concanavalin A (Con A) was purchased from Miles Scientific (Naperville, IL). Phytohemagglutinin (PHA, red kidney bean) was obtained from Burroughs Wellcome (HA 17, Dartford, England). The lectins, lentil hemagglutinin (LCH), Mycoplasma gallisepticum agglutinin (MGA), pokeweed mitogen (PWM), soybean agglutinin (SBA) Ulex europaeus agglutinin (UEA), wheat germ agglutinin (WGA) and Wisteria floribunda agglutinin (WFA) were purchased from the Sigma Chemical Co. (St. Louis, MO). N-acetyl-D-glucosamine (NAGlu), N-acetyl-D-galactosamine (NAGal), α-D-mannose (MAN), α-methyl-D-glucoside (MG), α-D-fucose (FUC), and N-acetylneuraminic acid (NAN) were purchased from Sigma. Lectins and sugars were dissolved in receptor binding media at the concentrations indicated in RESULTS.

Methods

Binding Assay

YT cells were harvested by centrifugation at 10°C, washed once in cold receptor binding media, consisting of RPMI-1640, 1 mg/ml BSA (Cohn Fraction V, Miles, Elkhart, IN), 20 mM Hepes (Sigma, St. Louis, MO), and 0.1% sodium azide at pH 7.2, re-suspended at 2 x 10^7/ml in binding media and delivered at 100 µl/tube for assay. Replicate samples containing YT cells and iodinated-rhuIL-1β (50 µl/tube; diluted in binding media) were vortexed and incubated in Skatron macrowell tubes (Skatron, Sterling, VA) in the presence or absence of unlabeled-rhuIL-1β at varying concentrations for 60 minutes at room temperature. Tubes were shaken on an orbital shaker during the incubation. Total reaction mixture volume was 0.2 ml/tube. Cell bound ligand was measured by harvesting the cells (Skatron cell harvester) onto receptor binding paper (Skatron, glass fiber filter) which had been soaked in cold milk (5% Carnation instant non-fat dry milk in PBS with 0.1% sodium azide) for 60 minutes. Unbound ligand was removed by washing the cells on the filter pad with 4 ml cold Tween 20 (0.05% Tween 20 in PBS). Filters were counted in a Packard A800C Gamma Counter. Aliquots of iodinated IL-1 (50 µl) were used to measure "total added" ^{125}I-rhuIL-1β. Controls were included in each experiment to: (1) determine maximum binding in the absence of unlabeled ligand, (2) determine non-specific binding in the presence of

excess unlabeled ligand, and (3) generate a standard competitive inhibition curve using unlabeled free ligand.

Fluorescence

YT cells were washed, re-suspended (1 x 10^7/ml) in fluorescence (FL) media which consisted of Hanks' Balanced Salt Solution, containing 2% fetal calf serum, 25 mM Hepes and .02% sodium azide, and delivered to a Costar U-bottom 96-well microtiter plate (1 x 10^6/100 µl/well). The cells were then incubated with WGA labeled with fluorescein isothiocyanate (WGA-FITC; Polysciences, Inc., Warrington, PA) at 0.1 mg/ml for 60 minutes at 4°C, washed in FL media twice, washed in PBS once and fixed in 1% paraformaldehyde. Fluorescence was measured on an Epics 753 flow cytometer, using an argon ion laser emitting 488 nM wavelength light. For each sample 20,000 cells were examined. Dead cells were excluded via appropriate scatter window gating. Fluorescent signals were displayed via logarithmic amplification.

Calculations

Percent of control binding was calculated, as follows:

$$\frac{\text{cpmCONTROL} - \text{cpmUNKNOWN} \times 100}{\text{cpmCONTROL}} = \% \text{ of Control Binding}$$

Scatchard, an interactive computer program for calculating receptor binding parameter estimates in which both linear and non-linear regression analyses are performed, was used to estimate dissociation constants and receptors/cell (Berzins & Ruppel, 1987). RBPAS, software included in the Scatchard package, was used to calculate IC_{50}'s. Student's t-test was used for comparisons of mean values. Estimates of error were expressed as standard error of the mean (SEM).

RESULTS

Binding of ^{125}I-rhuIL-1β to YT Cells

YT cells (2 x 10^7/ml) were incubated with various amounts of ^{125}I-rhuIL-1β (specific activity 2210 Ci/mmole) in 0.2 ml binding media for 60 minutes at room temperature. As shown in Figure 1, the large granular lymphocyte-like cell line, YT, specifically bound ^{125}I-rhuIL-1β. Binding increased with increased amounts of radiolabel up to the saturation point, approximately 20 fmole/tube. Non-specific binding was determined by the addition of 44 pmole unlabeled rhuIL-1β. Data represent the mean of duplicate samples from a single representative experiment.

Scatchard Analysis

YT cells (2 x 10^7/ml) were incubated with varying amounts of ^{125}I-rhuIL-1β (0.24 pmole/tube at the highest dilution; specific activity 2611 Ci/mmole) in 0.2 ml binding media for 60 minutes at room temperature. Non-specific binding was determined by the addition of 29 pmole unlabeled-rhuIL-1β. Scatchard analysis revealed that YT cells have approximately 500 IL-1 receptors/cell (see Figure 2). The dissociation constant for ^{125}I-rhuIL-1β binding to YT cells was approximately 0.2 nM, the concentration of IL-1 required to saturate 1/2 the available binding sites. Results are from duplicate samples from a single representative experiment.

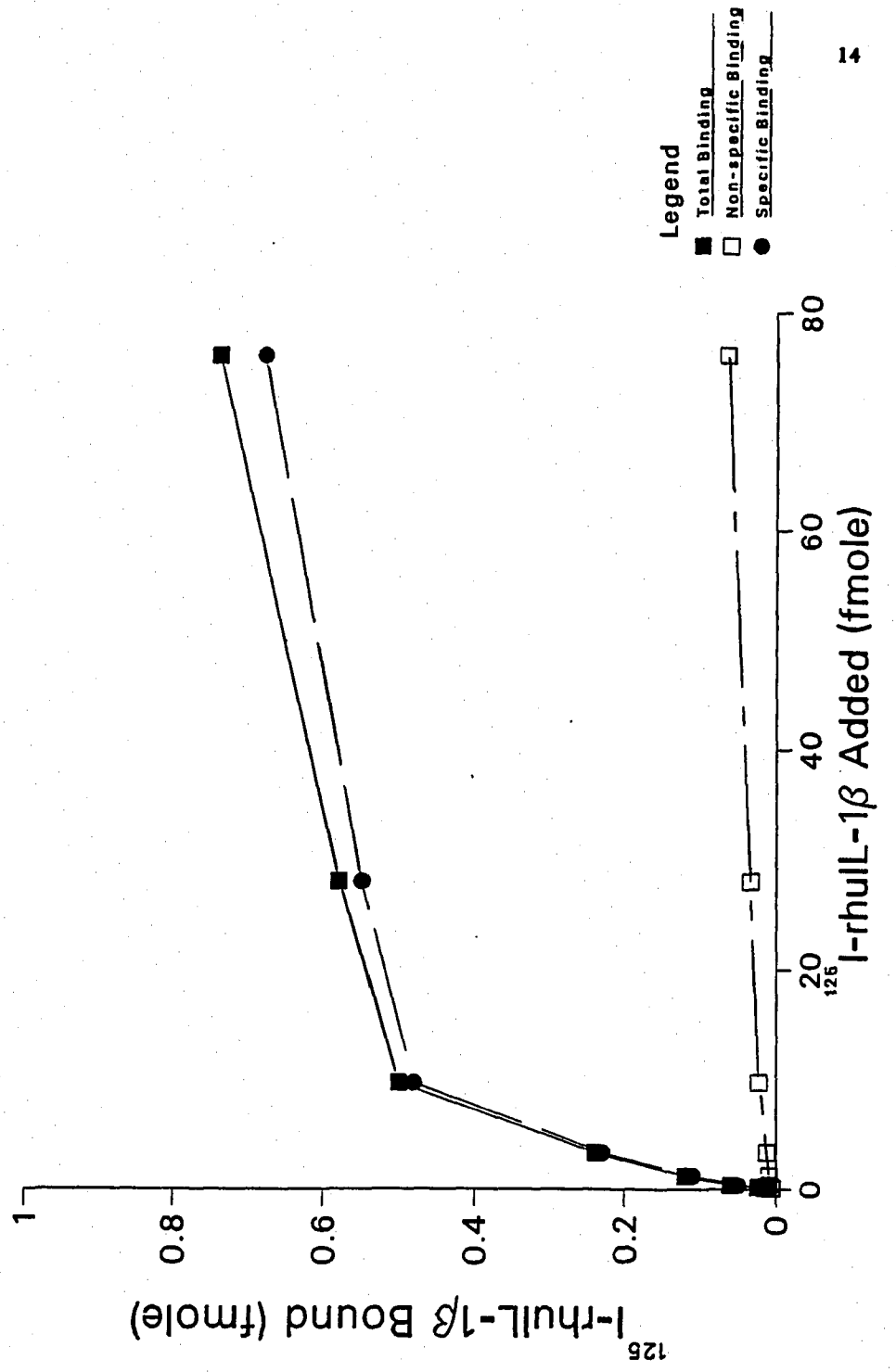

Figure 1. **Binding of ^{125}I-rhuIL-1β to YT Cells**

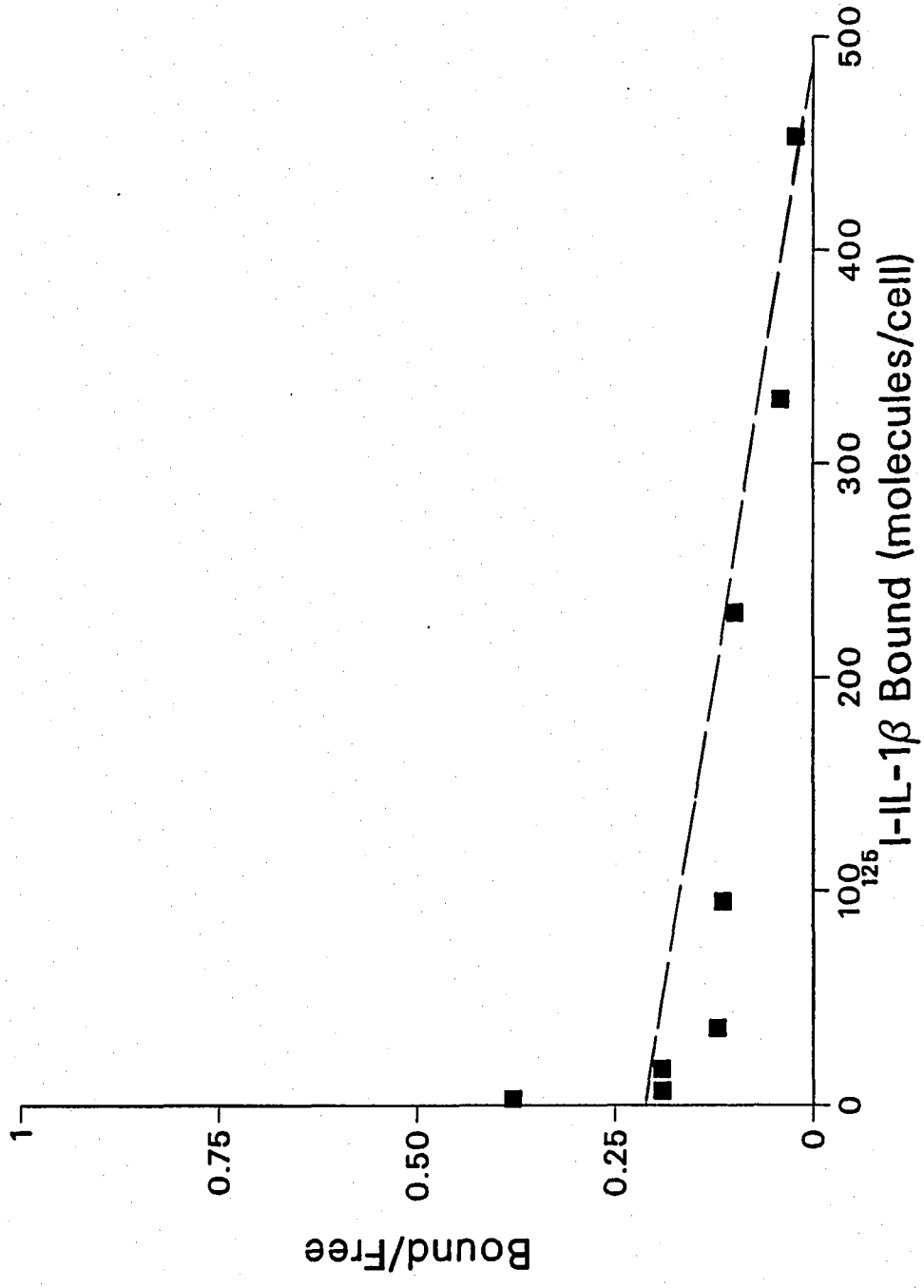

Figure 2. Binding of ^{125}I-rhuIL-1β to YT Cells: Scatchard Analysis

Inhibition of Binding of ^{125}I-rhuIL-1β to YT Cells by Unlabeled rhuIL-1β

As shown in Figure 3, YT cells (2 x 10^7/ml) were incubated with a saturating concentration (11-28 fmole/tube; specific activities 1641-1761 Ci/mmole) of ^{125}I-rhuIL-1β and varying concentrations of unlabeled-rhuIL-1β (0.003-3 nM) in 0.2 ml binding media for 60 minutes at room temperature. Data represent the mean ± SEM from 4-5 experiments in which duplicate samples were run. This titration of rhuIL-1β against a fixed amount of ^{125}-I-rhuIL-1β enabled determination of the concentration of IL-1β which would reduce maximum binding of iodinated-IL-1β to YT cells by 50% (IC_{50}). The IC_{50} was 3 ng/ml rhuIL-1β.

Effect of Lectins on Binding of ^{125}I-rhuIL-1β to YT Cells

YT cells (2 x 10^7/ml) were incubated with a saturating concentration of ^{125}I-rhuIL-1β (10-35 fmole; specific activities 1313-2210 Ci/mmole) and various concentrations (200, 40 or 8 μg/ml) of lectin in 0.2 ml binding media for 60 minutes at room temperature. Non-specific background binding (NSB) was determined by incubation of YT cells with 500 ng/ml unlabeled-rhuIL-1β. Data represent the mean ± SEM of 1-3 experiments. As seen in Table 2, WGA and Con A demonstrated significant inhibition of binding at 200 μg/ml, PWM exhibited some inhibition, while PHA had no effect on binding. In subsequent experiments several other lectins were tested. The data shown in Table 3 indicate that SBA, LCH, MGA, UEA and WFA did not inhibit binding of ^{125}I-rhuIL-1β to YT cells. The lentil lectin,

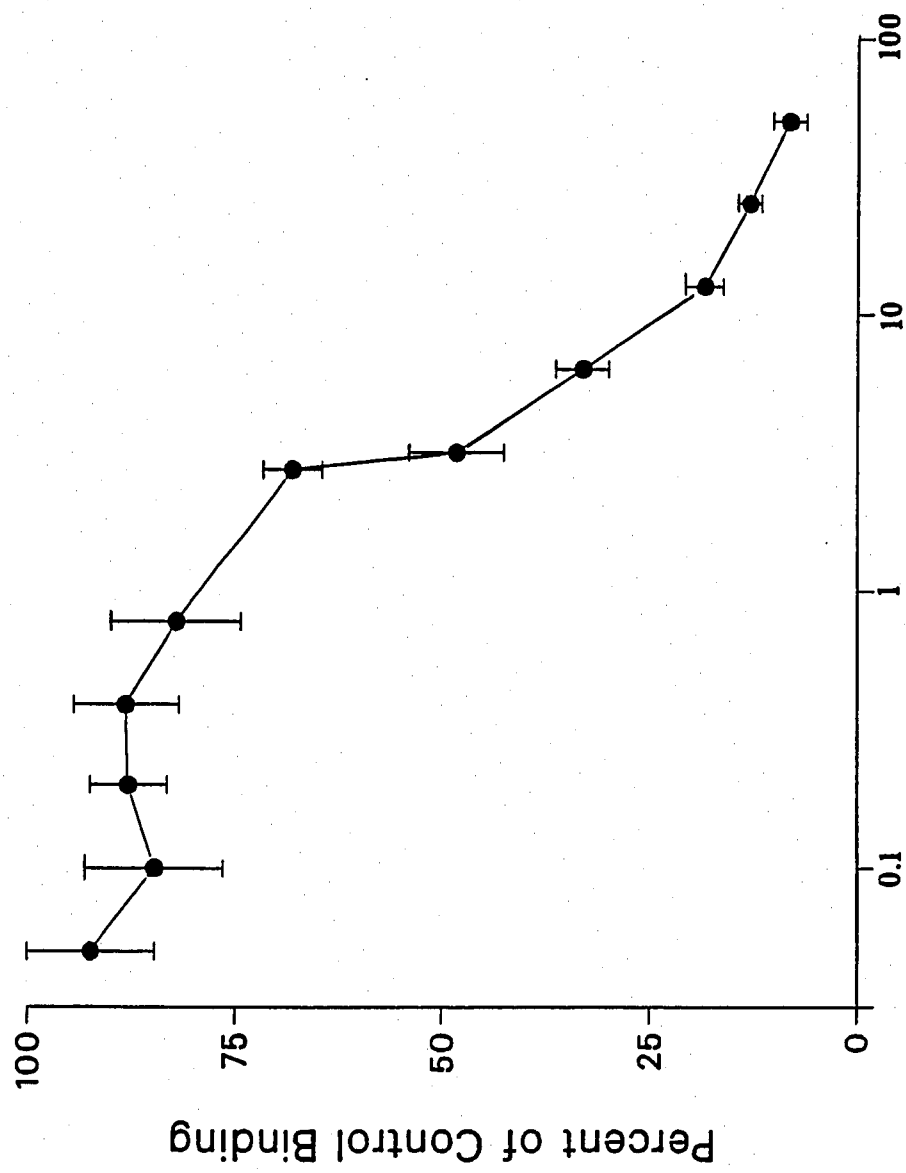

Figure 3. Inhibition of Binding of ^{125}I-rhuIL-1β to YT Cells by Unlabeled-rhuIL-1β

LCH, and the Mycoplasma lectin, MGA, produced some enhancement of binding at 40-200 μg/ml.

Inhibition of IL-1 Binding by rhuIL-1β or Wheat Germ Agglutinin

Results are shown in Figure 4 in which YT cells (2 x 10^7/ml) were incubated in the presence of rhuIL-1β (0.1-500 ng/ml) or WGA (7.8-1000 μg/ml) and a saturating concentration of ^{125}I-rhuIL-1β (20 fmole/tube; specific activity 1593 Ci/mmole) in 0.2 ml binding media for 60 minutes at room temperature. Data represent the mean ± SEM of 2 experiments in which duplicate samples were assayed. As illustrated in the plot in Figure 4, there was an almost 30,000-fold difference between rhuIL-1β and WGA in terms of ability to antagonize ^{125}I-rhuIL-1β binding to YT cells. The IC_{50} for rhuIL-1β was 1.4 ± 0.4 ng/ml, while the IC_{50} for WGA was 31 ± 10.5 μg/ml.

Effect of Sugar Moieties on Inhibition of IL-1 Binding by Lectins

Various concentrations of lectin (250, 50, 10 μg/ml) were pre-incubated with excess sugar (2.5mg/ml; 0.01-.02 M) for 30 minutes at room temperature prior to addition of YT cells (2 x 10^7/ml), ^{125}I-rhuIL-1β (10-35 fmole/tube; specific activities 1313-2210 Ci/mmole) and incubation 60 minutes at room temperature. Total volume was 0.2 ml in binding media. Data represent the mean of 2-3 experiments in which duplicate samples were run. As shown in Figures 5 & 6, sugars alone did not cause inhibition or significant enhancement of IL-1 binding to YT cells. In

Table 2

Effect of Lectins on Binding of ^{125}I-rulL-1β to YT Cells

Sample	Concentration μM	^{125}I-rulL-1β Bound[a] cpm	% Control
Control	--	2173 ± 160	100
WGA	5.6	1080 ± 174[b]	50
	1.1	1728 ± 253	80
	0.2	2320 ± 187	107
PHA	3.1	2734 ± 171	109
	1.6	2195 ± 164	101
	0.3	2080 ± 148	96
PWM	6.3	1495 ± 116	69
	1.3	1809 ± 189	83
	0.3	1933 ± 273	89
Con A	2.0	1339 ± 178[b]	62
	0.4	2186 ± 280	101
	0.1	2146 ± 223	99
NSB[c]	--	136 ± 13[b]	6

[a] YT cells ($2x10^7$/ml) were incubated with a saturating concentration of ^{125}I-rulL-1β (35 fmole; specific activity 1313 Ci/mmole) and various concentrations (200, 40 or 8 μg/ml) of lectin in 0.2 ml binding media for 60 minutes at room temperature. Data represent the mean ± SEM of 2-3 experiments.

[b] $p \leq 0.01$, as compared to Control binding

[c] NSB = non-specific binding

Table 3

Effect of Lectins on Binding of ^{125}I-ruIL-1β to YT Cells

Sample	Concentration μM	^{125}I-ruIL-1β Bound[a] cpm	% Control
Control	--	2508 ± 4	100
SBA	1.8	2569 ± 37	102
	0.4	2506 ± 18	100
	0.1	2446 ± 14	98
LCH	4.1	2837 ± 65[b]	113
	0.8	2714 ± 2[b]	108
	0.2	2451 ± 51	98
MGA	200[c]	2910 ± 58[b]	116
	40	2582 ± 56	103
	8	2452 ± 20	98
UEA	1.2	2443 ± 53	97
	0.2	2484 ± 56	99
	0.1	2260 ± 110	90
WFA	2.9	2577 ± 99	103
	0.6	2415 ± 25	96
	0.1	2388 ± 88	95
NSB[d]	--	116 ± 4	5

[a] YT cells (2x10^7/ml) were incubated with a saturating concentration of ^{125}I-ruIL-1β (10 fmole; specific activity 2210 Ci/mmole) and various concentrations (200, 40 or 8 μg/ml) of lectin in 0.2 ml binding media for 60 minutes at room temperature. Data represent the mean ± SEM of duplicate samples from a single representative experiment.

[b] $p \leq 0.01$, as compared to Control binding

[c] Concentration expressed in μg/ml; molecular weight undetermined

[d] NSB = non-specific binding

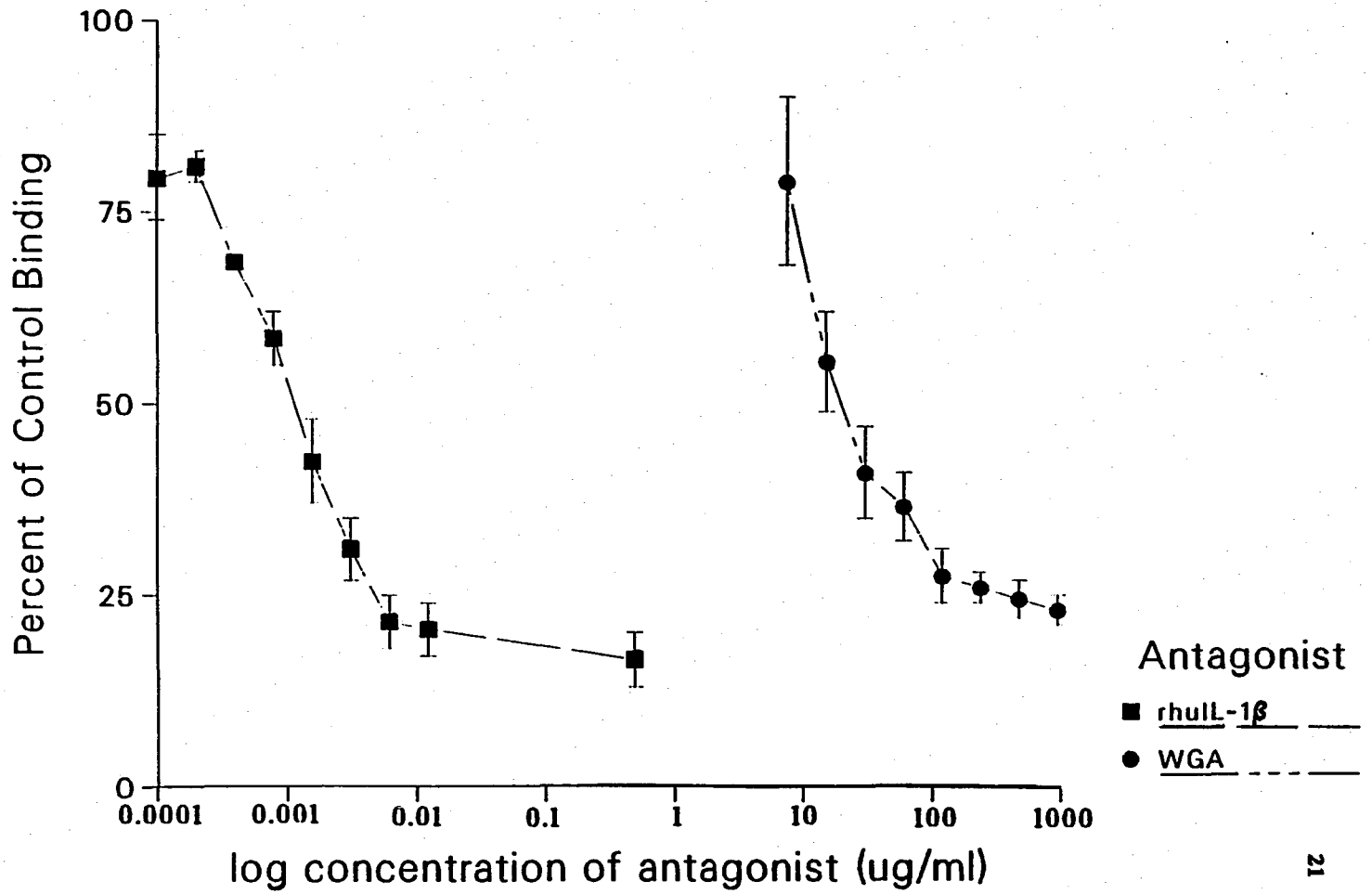

Figure 4. **Inhibition of IL-1 Binding by rhuIL-1β or Wheat Germ Agglutinin**

addition, lower concentrations of sugars (0.1-2 mg/ml) had no effect on binding (data not shown). As seen in Figure 5, the glucoside MG effectively prevented Con A inhibition of IL-1 binding, while other sugars incubated with Con A had no effect. Also shown in Figure 5, only NAGlu significantly abrogated WGA inhibition of IL-1 binding; other sugars were ineffective. PHA had a slight enhancing effect on IL-1 binding, as seen previously (Table 2), and the addition of NAGlu did not appreciably alter that effect (Figure 5). However, NAGlu did abolish the inhibition of IL-1 binding caused by PWM (Figure 5). Sugars other than NAGlu were not tested with the lectins PHA or PWM. As shown in Figure 6 none of the sugars tested had any effect on binding, alone or in combination with the lectins, LCH, MGA, SBA, UEA, or WFA.

Effect of Tunicamycin Treatment on Binding of ^{125}I-rhuIL-1β to YT Cells

YT cells (4 x 10^5/ml) were incubated with various concentrations of tunicamycin (0.01-20 μg/ml) in culture media for 24 hours at 37°C. The cells were washed once and re-suspended in binding media (2 x 10^7/ml), then incubated with a saturating concentration of ^{125}I-rhuIL-1β (10 fmole/tube; specific activity 2210 Ci/mmole) in 0.2 ml binding media for 60 minutes at room temperature. Data represent the mean ± SEM of 2 experiments in which quadruplicate samples were tested. There was no significant change in cell viability which ranged from 88% for untreated cells to 82-97% for tunicamycin treatment. As shown in Figure 7,

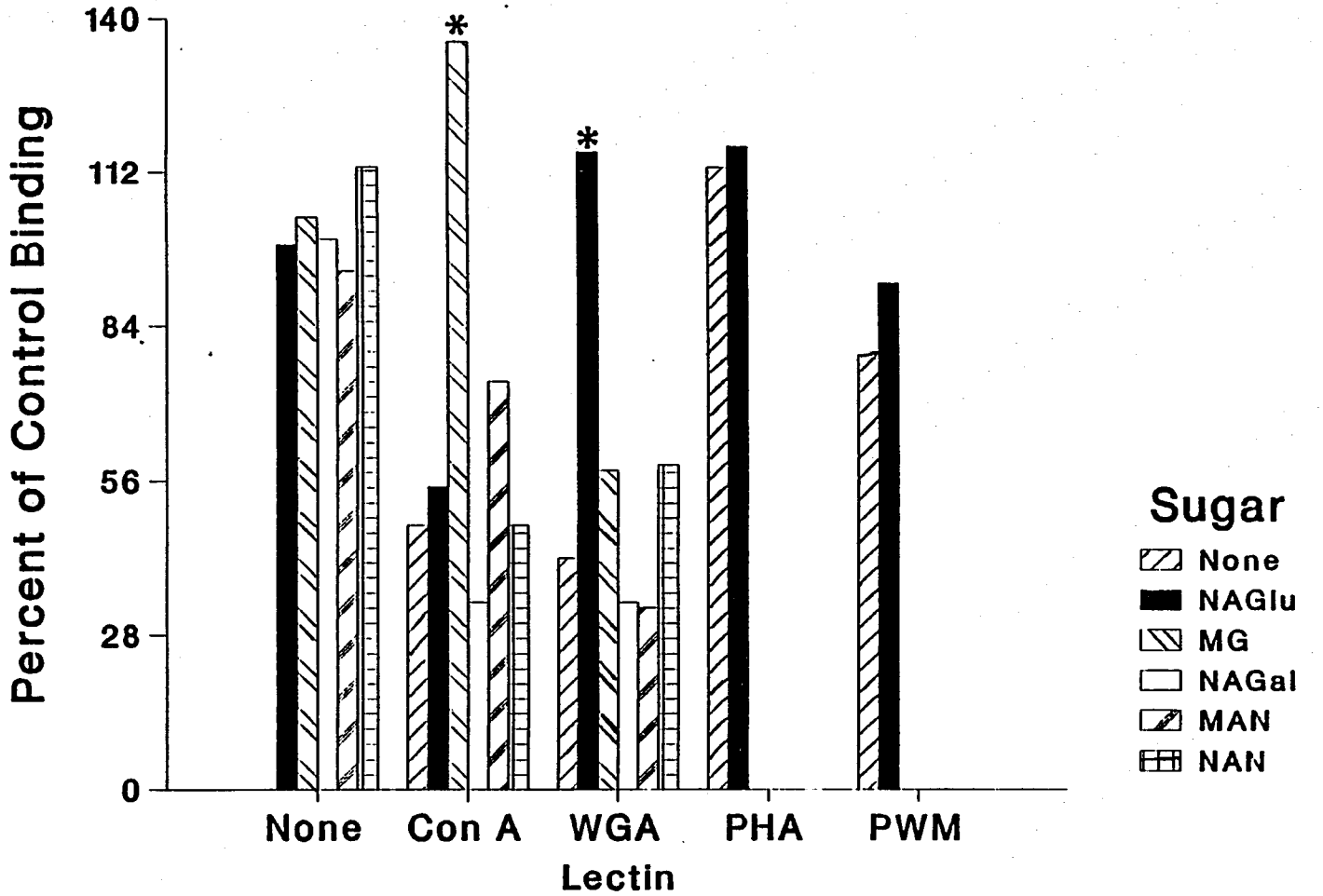

Figure 5. Effect of Sugar Moieties on Inhibition of IL-1 Binding by Lectins

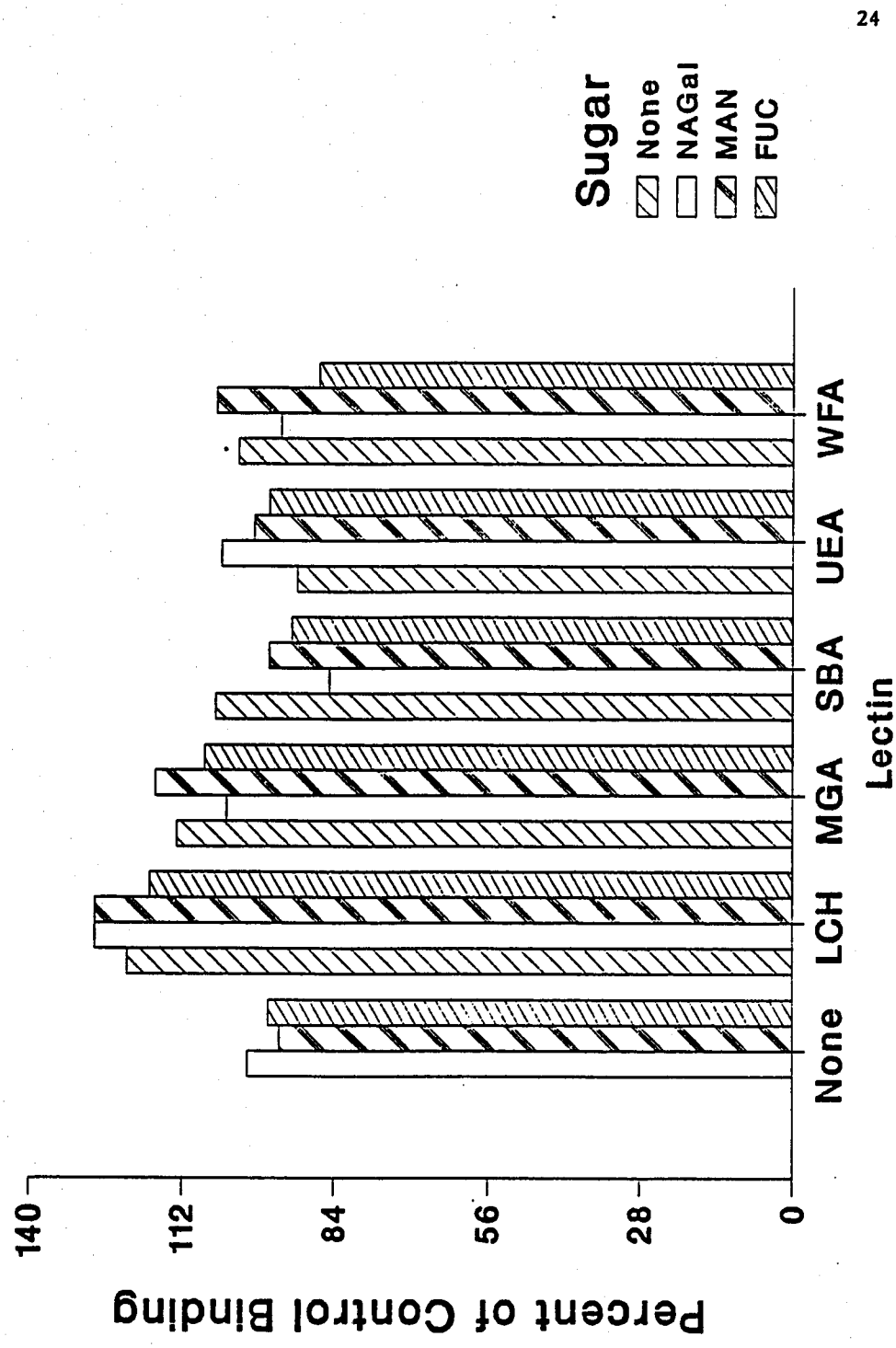

Figure 6. Effect of Sugar Moieties on Inhibition of IL-1 Binding by Lectins

treatment of YT cells with tunicamycin caused a dose-related inhibition of binding of ^{125}I-rhuIL-1β. YT treatment with 5 μg/ml tunicamycin over 24 hours caused a 61% decrease in binding of ^{125}I-rhuIL-1β, while 10-20 μg/ml tunicamycin further inhibited binding by 76% during that same period of incubation.

Kinetics of Tunicamycin Effect on Binding of ^{125}I-rhuIL-1β to YT Cells

YT cells (2-4 x 10^5/ml) were incubated with 2.5 μg/ml tunicamycin in culture media for various lengths of time (0-48 hours) at 37°C. The cells were washed once, re-suspended in binding media (2 x 10^7/ml), and then incubated with a saturating concentration of ^{125}I-rhuIL-1β (8-10 fmole/tube; specific activity 2210 Ci/mmole) in 0.2 ml binding media for 60 minutes at room temperature. Data represent the mean of quadruplicate samples from a single experiment. Tunicamycin treatment did not alter YT cell viability, until it had been present for 48 hours, at which time the cells were only 50% viable. As seen in Figure 8, tunicamycin treatment affected a time-related inhibition of ^{125}I-rhuIL-1β binding to YT cells with maximum inhibition occurring at 24 hours.

Flow Cytometric Analysis of WGA-FITC Binding Following Tunicamycin Treatment of YT Cells

YT cells (3 x 10^5/ml) were incubated with 5 μg/ml tunicamycin in culture media for 24 hours at 37°C. The cells were washed twice, re-suspended in FL media (1 x 10^7/ml) and incubated with WGA-FITC (0.1

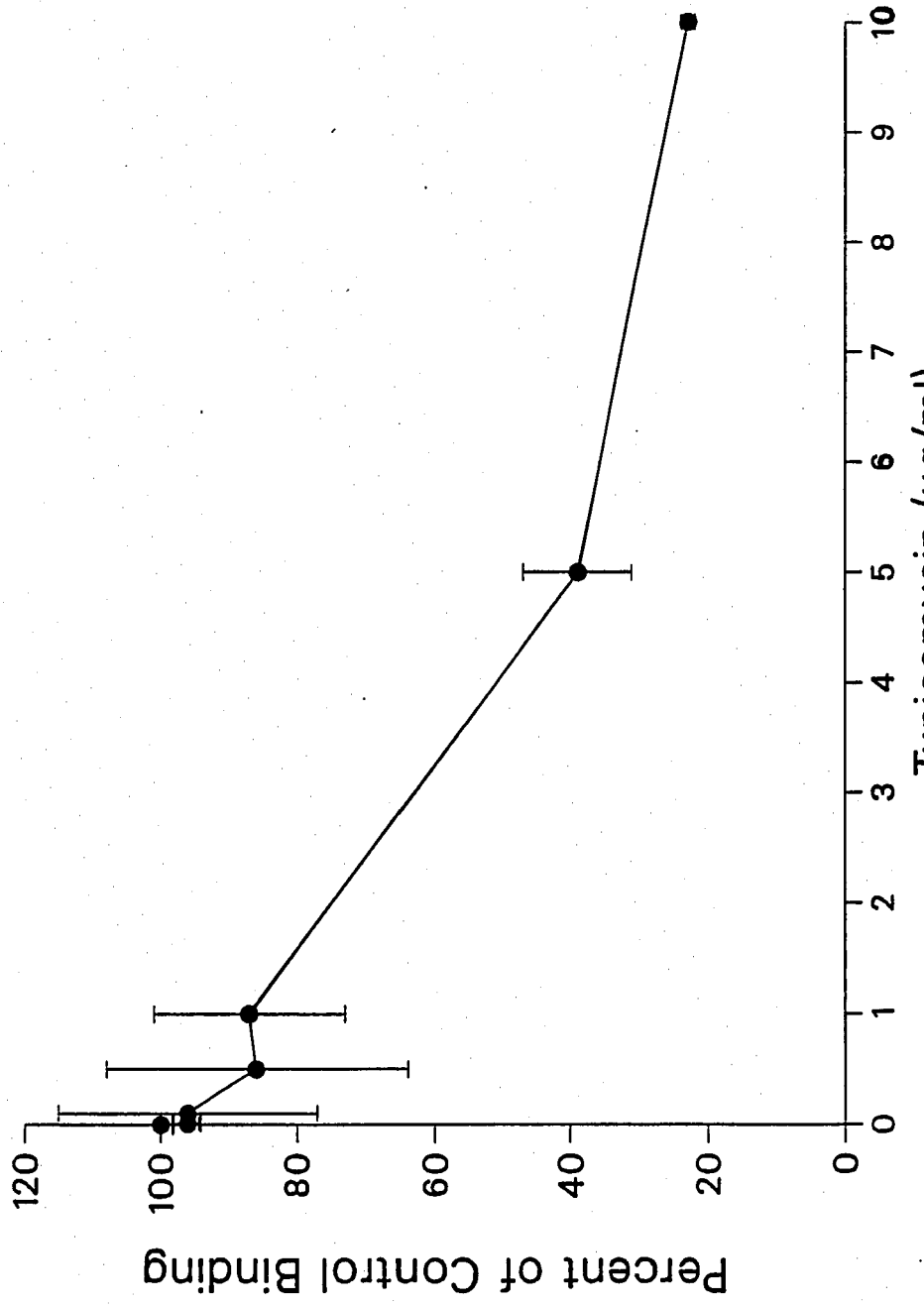

Figure 7. Effect of Tunicamycin Treatment on Binding of ^{125}I-rhuIL-1β to YT Cells

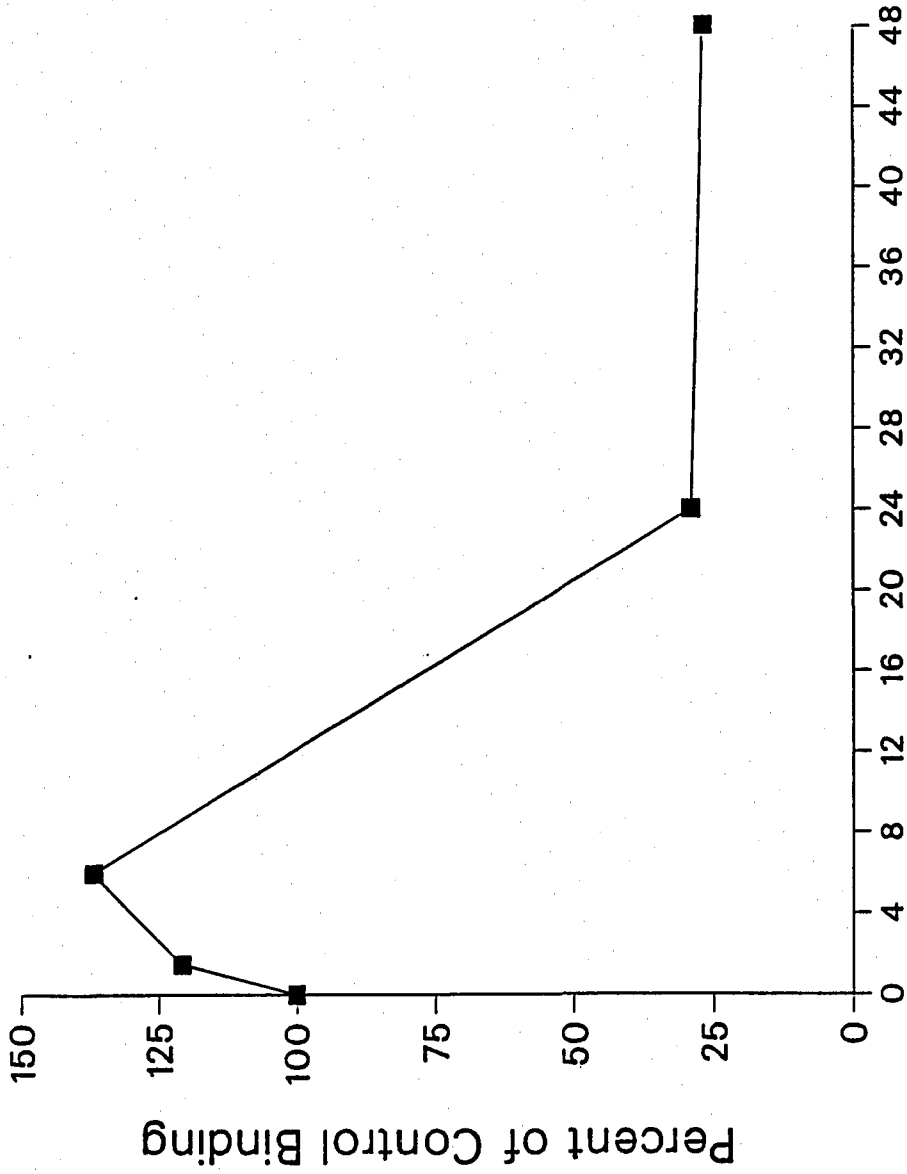

Figure 8. **Kinetics of Tunicamycin Effect on Binding of ^{125}I-rhuIL-1β to YT Cells**

mg/ml) for 60 minutes at 4°C. Fluorescence of fixed cells was measured via flow cytometry. Data are representative of a single experiment in which fluorescence of 20,000 individual cells per sample were evaluated. Background autofluorescence was negligible (2-2.3%). Data is expressed as relative cell number versus relative fluorescence intensity (Figure 9). Peak fluorescence of WGA-FITC bound to YT cells occurred at channel #171 for untreated cells (mean channel = 177.9 ± 22.3), while peak fluorescence for tunicamycin treated cells was at channel #157 (mean channel = 164.9 ± 24.8). This shift in mean fluorescence channels, however, was not statistically significant. The percentage of fluorescent cells was 99.3% for untreated cells and 99.5% for tunicamycin-treated YT cells.

Binding of ^{125}I-rhuIL-1β to YT Cells Following Removal of Tunicamycin

YT cells (4 x 10^5/ml) were incubated with 5 μg/ml tunicamycin in culture media for 24 hours at 37°C, then washed twice, re-suspended in sterile culture media (2 x 10^5/ml) and incubated at 37°C for various periods of time (0-72 hours). The cells were then washed once, re-suspended in binding media (2 x 10^7/ml), and incubated with a saturating concentration of ^{125}I-rhuIL-1β (9-12 fmole/tube; specific activity 2611 Ci/mmole) in 0.2 ml binding media for 60 minutes at room temperature. Data represent the mean of duplicate samples from a single experiment. Viability of untreated YT cells averaged 85% over the entire length of the

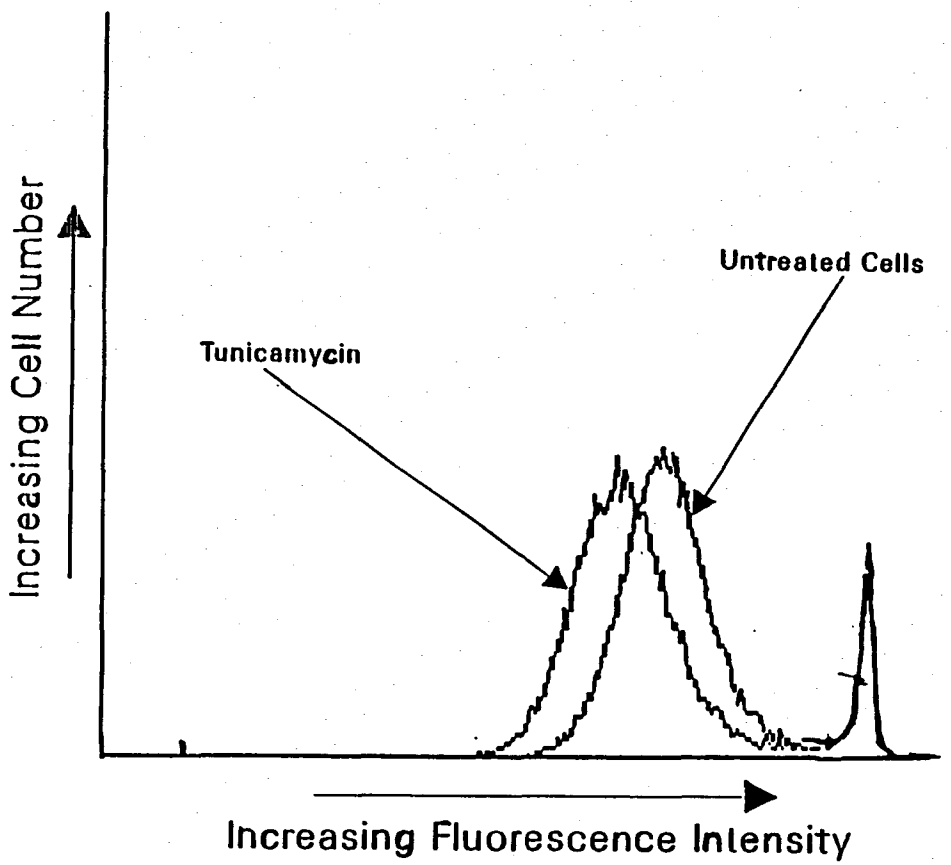

Figure 9. **Flow Cytometric Analysis of WGA-FITC Binding Following Tunicamycin Treatment of YT Cells**

experiment. Viability of tunicamycin treated cells was 82% following the initial 24 hour incubation. After removal of tunicamycin and re-culturing, cell viability of tunicamycin-treated cells was between 60-63% for the next 6-72 hours, during which cell division occurred at a much slower rate than normal. Recovery of IL-1 binding was not seen until 48 hours following removal of tunicamycin, as shown in Figure 10. A large increase in binding of ^{125}I-rhuIL-1β to YT cells (170% Control) occurred at 72 hours.

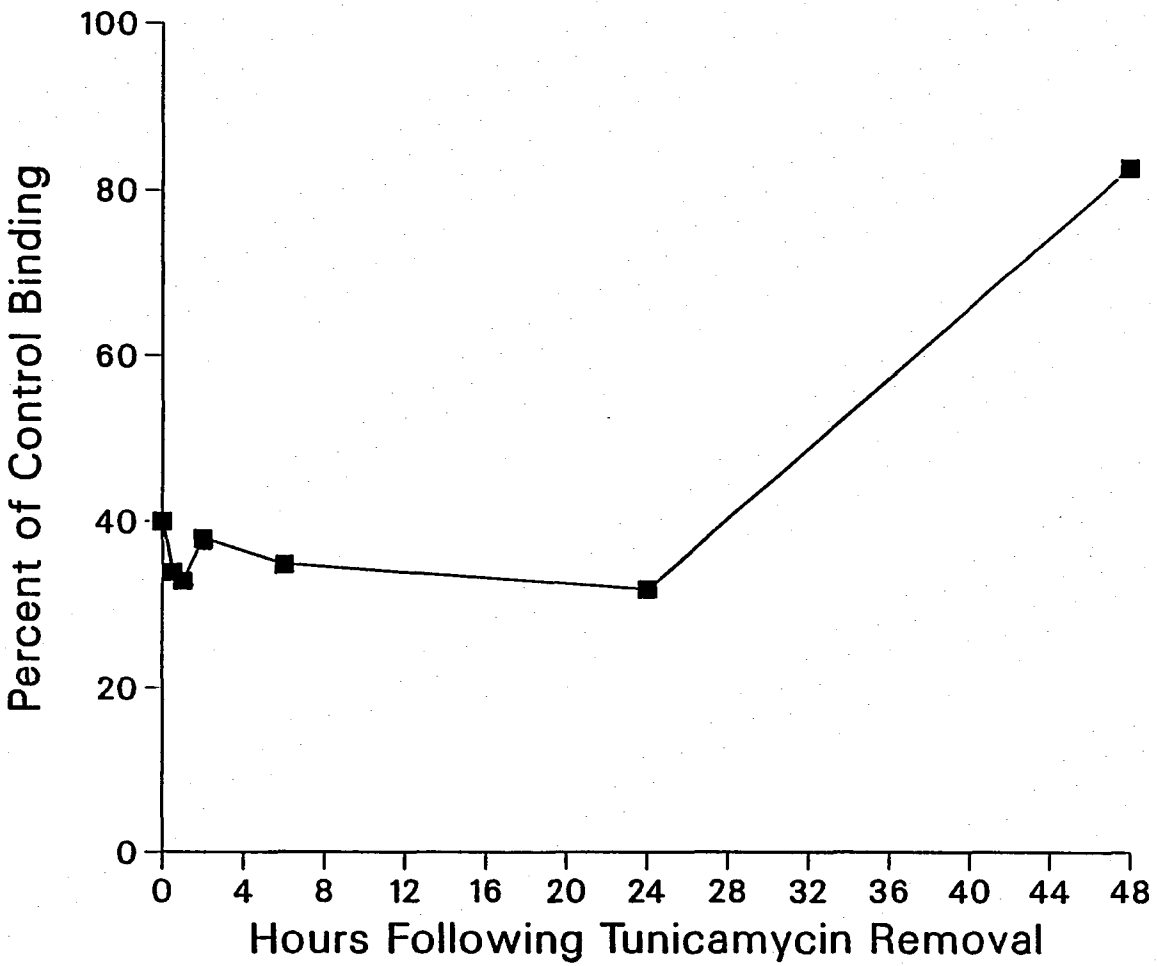

Figure 10. **Binding of ^{125}I-rhuIL-1β to YT Cells Following Removal of Tunicamycin**

DISCUSSION

Iodinated-IL-1β displayed a high degree of specific binding to intact YT cells. Binding data were generated under equilibrium conditions, previously determined (Matsushima et al., 1986a), and included the presence of sodium azide which would prevent receptor-ligand internalization or degradation. Binding appeared to be saturable, as well as dependent on ligand concentration. The kinetics of IL-1 binding appear to be commensurate with that of IL-1 biological responses (Dower et al., 1985, Paslay, 1987). Therefore, these data are consistent with the presence of specific IL-1 receptors on YT cells. In addition, scatchard plots were linear, suggesting a simple unimolecular interaction of ligand with a single class of binding sites. The dissociation constant for this binding was similar to Kd's reported by other investigators (Matsushima et al., 1986a; Matsushima et al., 1986b; Dower et al., 1985). However, the number of IL-1 receptors reported by Matsushima et al. (1986b) was considerably higher than that observed here, although these cells were obtained from Matsushima's cultures at the National Cancer Institute. It is possible that YT cells grown in Kalamazoo have lost the ability to express as high a proportion of IL-1 receptors as they once had.

Unlabeled recombinant IL-1β was capable of competing, in a concentration-dependent fashion, with iodinated IL-1β for binding to YT cells. The IC_{50} for this inhibition was consistent with the Kd revealed by scatchard analysis, suggesting stoichiometric interaction.

The plant lectins WGA and Con A had a statisticly significant inhibitory effect on IL-1 binding to intact YT cells. PWM also inhibited IL-1 binding, but in a less consistent manner than WGA or Con A. Fairly large quantities of lectin (40-200 µg/ml) were required to demonstrate inhibition of IL-1 binding. Since there are so few IL-1 receptors on YT cells, the data suggests that these lectins may be binding to other sites on YT cells. In fact other investigators have reported 39-46 million binding sites for WGA on baby hamster kidney fibroblasts (Monsigny et al., 1980) and 300 million binding sites for WGA and Con A on isolated rat fat cells with an apparent association constant of 80 nM (Cuatrecasas, 1973). Saturation of that many sites would require at least 6-16 µg/ml of WGA or Con A. These lectins have long been used to agglutinate and/or mitogenically stimulate lymphocytes in a wide variety of in vitro immunological protocols with optimal concentrations in the µg/ml range (Gillis & Mizel, 1981; Lloyd & Panush, 1977; Middleton & Bullock, 1984). Data with fluorescein-labeled WGA show a large number of YT cells that are very brightly fluorescent, suggesting that YT cells possess large numbers of lectin binding sites. Scatchard analyses performed by Cuatrecasas (1973) and Monsigny et al. (1980) demonstrated that lectin binding is quite complex with multiple binding sites of varying affinities involved. These authors quoted general affinity constants, but indicated that discrete binding constants for each site were impossible to assign. Therefore, estimating the amount of lectin required to inhibit IL-1 binding is difficult. The data shown here, particularly for WGA, would suggest

that inhibition of IL-1 binding can be realized if large quantities of lectin are added, perhaps 10-100 times more than estimates of lectin binding sites would suggest. Other investigators (Carpenter & Cohen, 1977; Costlow & Gallagher, 1977) reported results similar to these in which μg quantities of lectin are required to inhibit binding. Finally, sugars present in the binding media could impede lectin binding and, therefore, increase the amount of lectin required to inhibit IL-1 binding. Internalization of lectin or cell surface receptors should not be an issue here, because all binding experiments were performed in the presence of sodium azide, an anti-metabolite.

Certain sugars act as "haptenic inhibitors" (Hedo, 1984) of lectin interactions with cells. This sugar specificity was apparent in the data described here, in which WGA and Con A inhibition of IL-1 binding was completely abrogated by previous exposure of WGA with NAGlu or Con A with MG, while exposure of these lectins to irrelevant sugars or sugars alone had no significant effect. These results suggest that WGA and Con A can effectively compete with IL-1 for specific moieties on or close to the IL-1 receptor.

Although lectin binding to glycoconjugates is reversible (Hedo, 1984), sugars had to be pre-incubated with lectin in order to prevent lectin-inhibition of IL-1 binding in these experiments. Perhaps, a higher temperature, longer incubation time, or higher sugar concentration would have enabled sugars to effect reversal of the lectin-inhibition, if

sugar and lectin were added simultaneously with IL-1. However, those experiments were not attempted here.

Lentil lectin (LCH), which shares the same sugar specificities as Con A, did not inhibit IL-1 binding in these studies. Although Hedo, Harrison and Roth (1981) have reported that Con A and LCH are equally capable of inhibiting insulin binding to placental receptors, other investigators have observed that Con A is much more effective than LCH in blocking epidermal growth factor binding (Carpenter & Cohen, 1977) or prolactin binding (Costlow & Gallagher, 1977) to human fibroblasts. Con A, which is tetravalent, may be able to cross-link receptors more effectively than LCH, which is divalent, and thereby inhibit IL-1 binding. However, Carpenter and Cohen (1977) suggest that the differences may also result from other aspects of lectin binding, such as the 100-fold lower association constant of LCH for sugar residues, as compared to Con A.

The lectin and sugar specificity demonstrated in these studies suggests that N-acetyl-glucosamine and/or glucosides may be found on or in close proximity to the IL-1 receptor. These data, along with the inhibitory effects of tunicamycin treatment, imply that the IL-1 receptor is glycosylated. Since sialic acid- and fucose-specific lectins did not inhibit IL-1 binding, this could indicate that the carbohydrate moieties on the IL-1 receptor protein may not be complex sugars or that processing in the golgi does not result in a highly modified carbohydrate structure for the IL-1 membrane receptor.

The lectin specificity observed in these experiments may aid in the selection of a lectin for isolation and purification of the IL-1 receptor by lectin chromatography. It should be noted that inhibition of binding by a free lectin, does not necessarily mean that an immobilized derivative of that lectin will bind and retain the receptor in question (Hedo, 1984). In fact the lack of effect of a lectin on receptor binding does not mean that it cannot be used in affinity chromatography, since there are examples of purification of the insulin receptor with ricin-agarose, even though free ricin does not alter insulin binding (Hedo et al., 1981). WGA and Con A have been used extensively in purification of a variety of receptors (Hedo, 1984), and, since both lectins interfere with IL-1 binding, it is quite possible that immobilized-WGA or Con A would retain the IL-1 receptor. Data presented here further suggest that specific simple sugars could be used to elute bound IL-1 receptor from affinity columns.

The results observed following incubation of YT cells with tunicamycin, an antibiotic inhibitor of glycosylation, suggest that the IL-1 receptor may be glycosylated and that glycosylation is important for ligand binding. Alternatively, tunicamycin treatment may have effected IL-1 receptor expression levels by having a detrimental effect on proteins involved mechanistically in receptor synthesis, transport, membrane localization, function and/or membrane turnover. Tunicamycin has been shown to selectively block glycoprotein synthesis by inhibition of NAGlu derivatives, without significant effect on overall protein synthesis (Chatterjee et al., 1979; Rosen, Chia, Fung & Rubin, 1979). I observed that

during a 24 hour incubation with 5 μg/ml tunicamycin, cell viability was unchanged. However, the ability of YT cells to bind WGA-FITC was only somewhat decreased when these cells were tunicamycin-treated, suggesting only a partiai inhibition of glycosylation or slow turnover of lectin binding sites. Tunicamycin's effect on IL-1 receptor binding was related to both time and concentration, and was reversible upon removal of tunicamycin from the culture medium. Restoration of ligand binding following removal of tunicamycin was a slow process, suggesting that receptor turnover may not be rapid. Recently, Mizel, Dayer, Krane and Mergenhagen (1981) reported a turnover rate of approximately 11 hours for IL-1 receptors on murine EL4 T cell lymphoma cells.

CONCLUSION

I have demonstrated specific IL-1 receptors on human YT cells by using iodinated IL-1β of high specific activity in a rapid, intact cell binding protocol. IL-1 binding was antagonized by the plant lectins WGA and Con A in a concentration-dependent manner. This inhibition could be completely abolished if WGA and Con A had been pre-incubated with the lectin-specific sugars, NAGlu or MG. Incubation with tunicamycin, the antibiotic inhibitor of glycosylation, caused a reversible, time- and concentration-dependent decrease in IL-1 binding.

These data suggest that glycoconjugate moieties on or near the IL-1 receptor site are important for optimal receptor binding in intact YT cells. In addition, isolation and purification of IL-1 receptors may be realized through the use of immobilized-WGA or Con A deriviatives by affinity chromatographic methods.

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