PURIFICATION AND CHARACTERIZATION OF A MANNOSE-CONTAINING DISACCHARIDE OBTAINED FROM HUMAN PREGNANCY URINE

A New Immunoregulatory Saccharide

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Essentially all of the cellular immune elements and processes that have been identified can be stimulated in vitro by the addition of exogenous plant lectins. Various lectins have been shown to induce T cell proliferation; T cell, K cell, polymorphonuclear leukocyte, and macrophage cytotoxic effector function; lymphokine secretion; helper activity; suppressor activity; B cell proliferation; and differentiation of B cells for immunoglobulin secretion (1–6).

Lectins bind to specific carbohydrate sequences and are thought to activate cells by interaction with the carbohydrate portion of endogenous glycoprotein and glycolipid receptors on the cell membrane, which are critical for cellular activation. Such activation by plant lectins can be prevented when large excesses of an appropriate saccharide are added to compete with lectin binding to these cell surface glycolipid and glycoprotein receptors.

Lectin-like molecules are not restricted to the plant kingdom. Invertebrate hemolymph is a rich source for such carbohydrate-specific molecules (7–8). Increasing evidence is accumulating which demonstrates that lectins or sugar-binding receptor structures are present on many types of vertebrate cells as well. For example, fibroblasts express receptors with specificity for mannose-6-phosphate and hepatocytes have a receptor for galactose (9, 10). Endogenous lectins have also been shown to modulate a number of in vitro immunologic reactions. For example, the lymphokine migration inhibitory factor (MIF) binds to a fucosidase-sensitive receptor on the macrophage surface (11). The activity of the cytokines SISS-T and SISS-B (soluble immune suppressor substances of T and B cells, respectively) is blocked by their interaction with specific monosaccharides, *N*-acetyl-D-glucosamine and L-rhamnose, respectively, and suppressor T cells that are activated during infectious mononucleosis are inhibited by D-mannose and related sugars (12, 13).

D-Mannose also has a number of other immunoregulatory effects in vitro: it inhibits spontaneous monocyte cytotoxicity toward certain erythrocyte targets, it blocks natural cytotoxicity (NC)[1] but not alloimmune cytotoxicity, and it inhibits natural killer activity but not antibody-dependent cytotoxicity toward

[1] *Abbreviations used in this paper:* Con A, concanavalin A; α-D-Man*p* 1-6-D-Man, α-D-mannopyranoside 1-6-D-mannose; NC, natural cytotoxic; TLC, thin-layer chromatography.

identical targets (14–16). D-Mannose has also been shown to inhibit antigen-induced human T lymphocyte proliferative responses in vitro (17). Interestingly, a mannose polymer from yeast mannan also is inhibitory to human T cell proliferation, and an immunosuppressive substance found in the serum of patients with chronic mucocutaneous candidiasis has been identified as this fungal product (18).

Even from these early studies with defined sugars, it appears that their effects are almost as protean as those of plant lectins. However, studies using sugars like D-mannose suffer from several major disadvantages. First, in vitro effects are only seen using very large molar concentrations of sugar (i.e., 10–50 mM) and, second, some sugars like L-rhamnose have very interesting in vitro effects but are not found on mammalian glycoproteins. To address these problems, we sought a source of readily available saccharides that would be amenable to purification and might be more specific in their actions.

Human pregnancy urine is known to contain immunoregulatory factors (19). Furthermore, urine is a rich source of complex saccharides. As part of a larger study characterizing the immunoregulatory effects of human pregnancy urine, we noted that low molecular weight fractions of human pregnancy urine were immunosuppressive in vitro. We have characterized one of these factors and found that it is an α-D-mannopyranoside 1-6-D-mannose (α-D-Man*p* 1-6-D-Man) dimer capable of blocking early events necessary for an antigen-specific T cell response. Furthermore, this mannose dimer is in excess of 100 times more potent than its parent D-mannose.

Materials and Methods

Source of Human Pregnancy Urine. Freshly obtained, first-void morning samples from individuals between 20 and 40 wk of gestation were frozen at −20°C before use.

Reagents. Concanavalin A (Con A) Sepharose was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). RPMI 1640 and balanced salt solution were purchased from Microbiological Associates (Bethesda, MD). Tetanus toxoid was purchased from the Massachusetts Department of Public Health, Boston. Fractogel 40S (a rigid molecular sieving gel) was purchased from EM Science (Gibbstown, NJ).

Antigen-specific Proliferative Assays. Briefly, heparinized peripheral blood (preservative-free heparin, ~10U/cc; O'Neal, Jones and Feldman, St. Louis, MO) was centrifuged for 5 min at 100 *g* and the buffy coat was collected. The buffy coat was washed once with balanced salt solution and then 2×10^5 cells in 0.15 cc RPMI 1640 supplemented with 10% autologous plasma and 2 mM L-glutamine were added to flat-bottomed, 96-well tissue culture plates (Costar, Data Packaging, Cambridge, MA). 0.05 cc of an appropriate dilution of tetanus toxoid or tissue culture media was added and the plates were allowed to incubate in a humidified 5% CO_2 incubator at 37°C for 6 d. These cells were pulsed with 0.5 μCi tritiated thymidine, allowed to incubate a further 4.5 h, and then harvested on filter paper disks and counted for tritiated thymidine incorporation.

Analytical Procedures. Sugar and methylation analyses were carried out as previously described (20–22). Gas liquid chromatography/mass spectrometry was carried out on a Finnigan 4021 chromatograph (Finnigan MAT, San Jose, CA) equipped with an SE-30 wall-coated open tubular (W.C.O.T.) capillary column (25 m × 0.2 mm). Mass spectra were recorded at 70 eV with an ionization current of 0.3 mA and an ion source temperature of 200°C. Spectra were processed on a Nova 3 on-line computer system (Data General Corp., Westboro, MA).

Source of Sugars. D-Mannose and methyl-α-D-mannopyranoside were purchased from Sigma Chemical Co., (St. Louis, MO). Most lots of methyl-α-D-mannopyranoside were

shown to be homogeneous on high performance thin-layer chromatography (TLC); occasionally, trace contaminants with the migration characteristics of D-mannose could be detected on overloaded plates.

TLC was performed using 10- × 20-cm, high performance silica gel plates with a preabsorbant loading area (Whatman Laboratory Products Inc., Clifton, NJ). Plates were developed with Orcinol spray reagent (Sigma Chemical Co.).

Results

Initial screening experiments suggested that immobilized Con A was capable of retaining immunoregulatory factors found in human pregnancy urine. Thus, our purification scheme was biased toward mannose-containing compounds.

15 d of first-void morning urine (~3 liters) from individual donors was passed over Con A Sepharose columns (35 cc bed volume, 500 ml urine) at room temperature. The columns were washed with two-bed volumes of phosphate-buffered saline containing 1 mM $MnCl_2$; they were then eluted with two-bed volumes of phosphate-buffered saline containing 250 mM methyl-α-D-mannoside. This material was pooled and lyophilized, and the majority of the methyl-α-D-mannoside was removed on a 5 × 100 cm Sephadex G-10 column eluted with distilled water and monitored for both OD 280 and change in refractive index. The protein-rich fraction was pooled, lyophilized, and redissolved in 0.01 M ammonium acetate buffer, pH 4.75. This material was placed on a 2.6 × 90 cm Sephadex G-75 column and eluted with acetate buffer. The elution pattern of this purification step resulted in four distinct peaks (Fig. 1). Each of these peaks was collected and lyophilized to dryness to remove volatile ammonium acetate (which is nonspecifically toxic to lymphocyte function in vitro). Each peak was then reconstituted to an equal volume in distilled water and tested for its effects on antigen-specific lymphocyte proliferation (Fig. 1). Peak IV, which contained

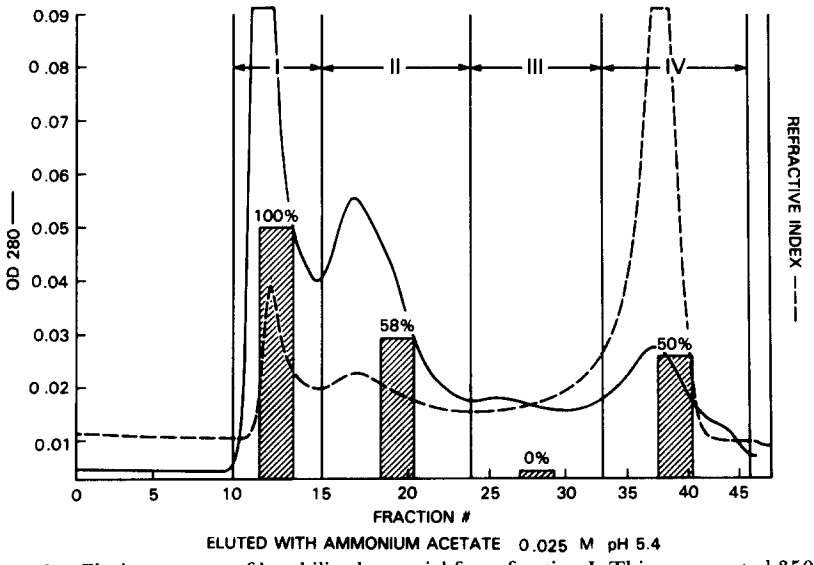

FIGURE 1. Elution pattern of lyophilized material from fraction I. This represented 350 mg of protein. Stippled bars show relative inhibition of each of the pooled fractions as described in the text.

smaller molecular weight components, was consistently immunosuppressive and was further characterized.

Concentrated material from peak IV was placed on a 1.5 × 165 cm Fractogel 40S column (a recently available molecular sieving chromatography reagent based on rigid porous polyvinyl beads) and eluted with distilled water. The elution profile of this peak IV material was complex and varied from donor to donor. A representative pattern is shown in Fig. 2. Each fraction was concentrated by lyophilization and tested for its ability to inhibit T cell proliferation

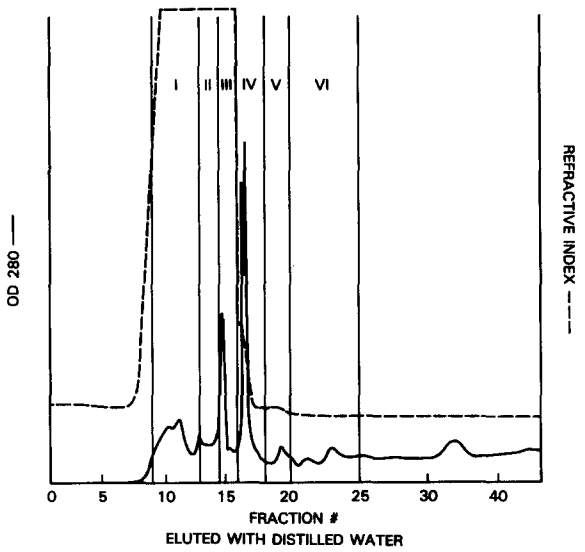

FIGURE 2. Elution pattern of fraction IV (3 cc containing 10.2 mg sugar and 3.6 mg protein) from Fig. 1 run over a 1.5 × 165 cm Fractogel 40S column eluted with distilled water. Each fraction was lyophilized and reconstituted with distilled water to 1 cc. The sugar concentrations were: I, 2.8 mg; II, 2.2 mg; III, 1.2 mg; IV, 0.4 mg; V, 0 mg; VI, 0 mg.

TABLE I

Bioactivity of Fractogel 40S Fractions

Fraction	Concentration	cpm	Percent inhibition
	mg/ml		
I	0.014	8,474	35
II	0.011	7,884	40
III	0.006	9,963	25
IV	0.002	17,589	<0
V	0.0	10,270	22
VI	0.0	13,100	0

Fractions from Fig. 2 were pooled and lyophilized to complete dryness and then reconstituted with distilled water. The sugar concentration of each was determined using the cysteine sulfuric acid method and is expressed as the final concentration in the culture supernatant. These cultures were stimulated with tetanus toxoid and allowed to incubate for 6 d. Results represent the mean of triplicate determinations and are expressed as counts per minute (cpm). Cultures without added sugar gave 13,114 cpm; those without added antigen gave 148 cpm.

induced by the antigen tetanus toxoid. Table I depicts representative data from one of three similar experiments. Fractions I–III reliably inhibited tetanus toxoid–induced proliferation, with fraction II showing the most inhibitory activity. Experiments with other individual urine donors had shown activity in a similar area and with a similar TLC pattern, so fraction II was further characterized. Fig. 3 shows a high performance thin-layer chromatogram of fraction II run on silica in ethanol/butanol/water, 40:40:20. An unstained TLC plate run in parallel was divided into four fractions as shown in Fig. 3 and the fractions were eluted with a mixture of water/ethanol, 75:25, and lyophilized to dryness. Each fraction was tested for bioactivity in the tetanus toxoid proliferation assay (Table II). The material in fractions I and II showed the most activity. Fraction II was then rechromatographed and shown to be homogeneous on high-performance TLC with an R_f of 0.49 (Fig. 4). Homogeneous material with identical migratory characteristics was obtained and subjected to structural analysis.

Sugar analysis showed D-mannose as the monosaccharide present. The material was reduced with $NaBD_4$, permethylated, and analyzed by gas chromatography/mass spectrometry. The mass spectrum is shown in Fig. 5. The primary and secondary fragments m/z 219, m/z 187, and m/z 155 are characteristic for a nonreducing terminal hexose residue. The ion m/z 236 shows an $NaBD_4$-reduced

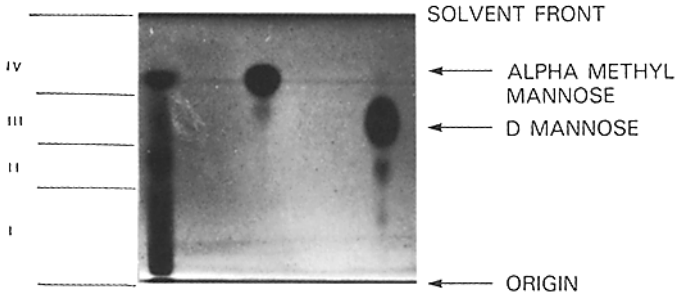

FIGURE 3. High performance thin-layer chromatography of fraction II from Fig. 2 run with butanol/ethanol/water, 40:40:20, and developed with Orcinol.

TABLE II
Bioactivity of TLC Fractions

Area	Volume added	
	1 μl	0.2 μl
	%	
I	67	73
II	17	41
III	41	87
IV	106	96

An unstained TLC was run in parallel with Fig. 4, starting with a total of 100 μg of fraction II from Fig. 3. The chromatogram was developed with ethanol/butanol/water, 40:40:20, and each fraction was scraped, eluted, and lyophilized to dryness. The fractions were reconstituted to 50 μl, 1 μl, and 1 μl of a 1:5 dilution and were added to the standard antigen proliferation assay. Results are expressed as a percent of the untreated cultures.

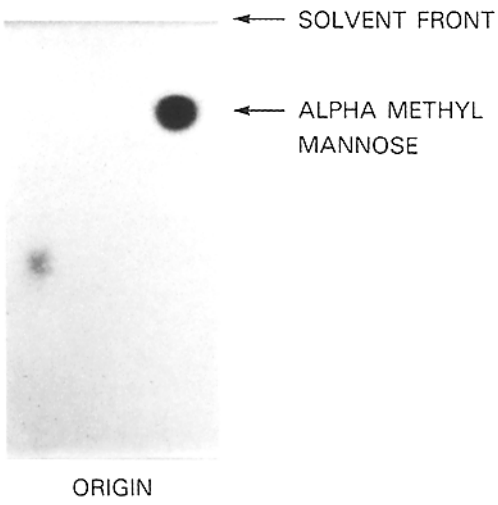

FIGURE 4. Rechromatographed sample of fraction II from Fig. 3 run under similar conditions.

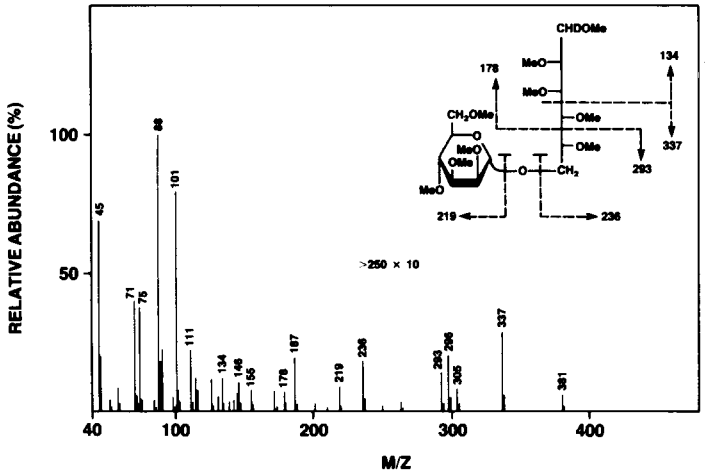

FIGURE 5. Mass spectrum of the disaccharide alditol from fraction II. Primary fragments are indicated.

hexose. Fragments formed by cleavage within the alditol m/z 134, m/z 178, m/z 293, m/z 337, and m/z 381 show that the reduced terminal is substituted in the 6 position. Thus, two D-mannose derivatives were observed, representing a nonreducing moiety and a 6-*O*-substituted D-mannitol. The anomeric configuration must be α, since the compound bound to Con A Sepharose. The deduced structure must therefore be α-D-Man*p* 1-6-D-Man.

As an independent approach to determining whether this mannose disaccharide isolated from urine was indeed an immunosuppressive molecule, this compound, isolated and purified from an entirely different source, was tested for bioactivity in the T cell proliferative assay. α-D-Man*p* 1-6-D-Man was purified from yeast cell wall extracts as previously described (23). Fig. 6 compares the in

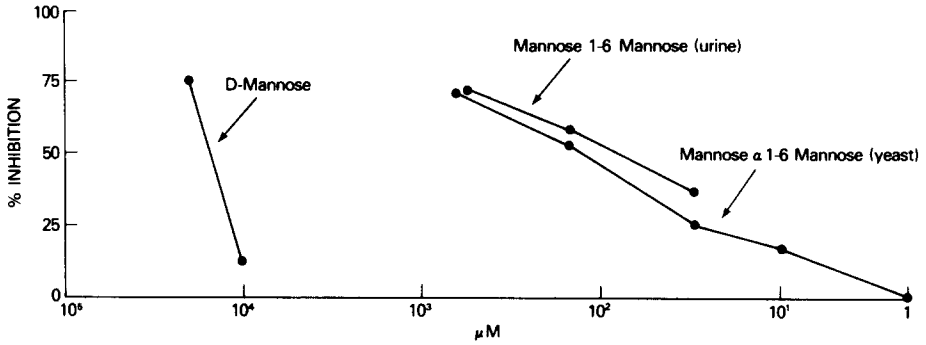

FIGURE 6. Relative inhibition of the urinary mannose 1-6 mannose compared with the yeast-derived material. For comparison, D-mannose is also shown. Results are expressed as percent inhibition compared with the untreated controls in each experiment. The yeast values represent the average of four separate experiments; the urine-derived material represents the average of two experiments.

vitro effects on T cell proliferation of these two independently purified mannose disaccharides.

Thus, we have shown that α-D-Man*p* 1-6-D-Man is capable of modulating in vitro T cell responsiveness to the recall antigen tetanus toxoid. A series of experiments was then undertaken to further characterize the cellular specificity of this inhibition. In data not shown, inhibition by α-D-Man*p* 1-6-D-Man failed to demonstrate any apparent antigen specificity, since in vitro responsiveness to the antigens tetanus toxoid, candida, and streptokinase-streptodornase was inhibited to a similar extent. However, this mannose dimer had dramatically different effects on assays measuring monocyte or B cell reactivity. These results are summarized in Table III. Part I of Table III examines the in vitro induction of spontaneous monocyte-mediated cytotoxicity (24, 25). This assay of monocyte cytotoxicity is exquisitely sensitive to a variety of exogenous stimuli, including sugars, chemotherapeutic agents, mitogens, and anti-inflammatory drugs (16, 26–28). At the concentrations we employed, D-Man*p* 1-6-D-Man failed to affect monocyte cytotoxicity in either a negative or positive fashion. Part II of Table III examines the effect of this mannose dimer on the expression of reverse hemolytic plaque-forming cells using pokeweed mitogen as a stimulus (4). Interestingly, α-D-Man*p* 1-6-D-Man had no inhibitory effect in this assay as well. The effect of α-D-Man*p* 1-6-D-Man on pokeweed mitogen–induced cellular proliferation was examined in a companion series of experiments. In contrast to the B cell assay, this mannose dimer was inhibitory for cellular proliferation induced with pokeweed mitogen.

Finally, experiments were performed to further characterize the inhibition of antigen-specific T cell proliferation seen with this 1-6 dimer of mannose and to exclude toxicity as a trivial explanation of our results. First, no differences were seen in cell viability, whether or not α-D-Man*p* 1-6-D-Man was added for 24 h to the cultures (data not shown). More importantly, kinetic studies of the timing of α-D-Man*p* 1-6-D-Man addition to these cultures strongly argue against simple metabolic or toxic effects. Thus, Table IV shows that when this sugar is added to mononuclear cell cultures after 24 h of a 144 h culture period, no inhibitory

TABLE III
Effect of Dimer on Monocyte and B Cell Function

		Concentration of D-Man*p* 1-6-D-Man		
		375 μM	75 μM	None
(I)	Spontaneous monocyte-mediated cytotoxicity Percent ^{51}Cr release:	76	76	71
(II)	Total reverse hemolytic plaque-forming cells	750 μM	375 μM	None
	Plaque-forming cells/culture:	19,700	19,600	20,500
(III)	Proliferation to pokewood mitogen	750 μM	None	
	cpm (5×10^4 MNL):	54,200	79,200	
	cpm (2.5×10^4 MNL):	18,600	35,500	

Effect of 1-6 mannose dimer on various in vitro assays. Spontaneous monocyte-mediated cytotoxicity was performed exactly as previously described (24). Briefly, 7×10^6 Ficoll-Hypaque-separated peripheral blood mononuclear cells are incubated for 7 d in NCTC 109 medium with 2% added fetal calf serum. The inhibitory disaccharide was added at the initiation of culture. Cytotoxic monocytes are harvested on the 7th d and assayed for spontaneous cytotoxicity against chicken red blood cell targets. Results represent the mean of triplicate determinations and are expressed as percent ^{51}Cr release. Total reverse hemolytic plaque-forming cells were enumerated after 6 d of stimulation with pokeweed mitogen as previously described (4). This assay measures polyclonal stimulation by pokeweed mitogen. The cultures consisted of 2×10^5 human mononuclear cells on flat-bottomed microtiter dishes with added pokeweed mitogen and 10% fetal calf serum. Results are expressed as plaque-forming cells per culture and represent the mean of triplicate cultures. Proliferation to pokeweed mitogen was measured in the same way that tetanus toxoid proliferation was measured except that (*a*) the polyclonal mitogen was added instead of specific antigen, and (*b*) fewer responding mononuclear lymphocytes (MNL) were used because the response to pokeweed mitogen was suboptimal at 2×10^5 cells per microtiter well in a 6-d assay.

TABLE IV
Functional Identity of Urinary and Yeast-derived Disaccharides

Source	No addition	Day 0	Day 1
Urine	19,708	4,562	18,615
Yeast	12,237	3,433	11,282

Results of two independent experiments examining the kinetics of inhibition by α-D-Man*p* 1-6-D-Man. Two sources were used, the first purified from human pregnancy urine and the second independently purified from yeast cell walls. In each experiment, human mononuclear cells were stimulated with tetanus toxoid antigen and the dimer was added either at time zero or after 24 h of culture (final concentration, 750 μM).

activity is observed. This observation not only makes toxicity unlikely, but also demonstrates that this disaccharide must be interfering with a very early step required for normal antigen-specific T cell proliferation to occur. Thus, by 24 h of culture, when accelerated DNA synthesis has not yet become apparent, addition of sugar is no longer capable of inhibiting T cell proliferation.

Finally, Table V suggests that this early inhibition requires cellular collaboration. Interestingly, preincubation of either enriched monocytes or highly purified lymphocytes with dimer for 24 h has no effect on their subsequent ability to

TABLE V
Effect of Preincubation with 1-6 Mannose Dimer

Source of cells	Thymidine incorporation	
	Experiment 1	Experiment 2
	cpm	
Unseparated (media)*	85,261	11,739
Unseparated (dimer)	19,982	4,470
Lymphocytes (media) + monocytes (dimer)	80,895	12,335
Lymphocytes (media) + monocytes (media)	69,998	11,296
Lymphocytes (dimer) + monocytes (media)	72,545	13,937
Lymphocytes (dimer) + monocytes (dimer)	74,468	ND

Peripheral blood mononuclear cells were separated into monocyte-enriched and monocyte-depleted preparations using adherence to plastic dishes and subsequent passage over Sephadex G-10 columns as previously described (27). The lymphocyte-enriched populations had <1% monocytes and did not respond to soluble antigen without the addition of monocytes (<300 cpm for experiments 1 and 2). These various cell populations were then preincubated in either media or 750 μM of the mannose dimer for 20 h.

* Experiment 1 preincubated unseparated cells, whereas experiment 2 used a mixture of 20% monocytes, 80% lymphocytes instead of unseparated cells to control for potential removal of a subpopulation of cells. The pretreatment is noted in parentheses. After preincubation, the various cell populations were washed twice with media and resuspended in fresh media without further addition of dimer. The cell populations were then mixed as noted and assayed for tetanus toxoid induced thymidine incorporation after 6 d. Results are expressed as counts per minute of quadruplicate (experiment 1) or triplicate (experiment 2) determinations. Background in the absence of added antigen was <200 cpm.

either present antigen or proliferate after being washed free of dimer. However, preincubation of these two subpopulations together with dimer renders them incapable of mounting a normal antigen-specific proliferative response.

Discussion

Human pregnancy urine is a rich and complex source of both large and small molecular weight saccharides and glycoproteins with immunoregulatory activity. We have purified and characterized an α-D-Man*p* 1-6-D-Man disaccharide from human pregnancy urine capable of blocking early events required for an in vitro proliferative response to specific antigen. Material with an R_f of 0.48–0.51 (in ethanol/butanol/water, 40:40:20) was purified from three separate individual donors and in each instance it was immunosuppressive in vitro. However, α-D-Man*p* 1-6-D-Man is not the only immunoregulatory, small molecular weight sugar found in human pregnancy urine, and each of three individual donors exhibited different patterns of sugar excretion. Several low molecular weight saccharides, as well as at least two larger molecular weight glycoproteins, affected our bioassay. Because of the complex bioactivity of even partially purified pregnancy urine, it is not possible to calculate a starting specific activity or an estimate of overall yield, since contaminants other than α-D-Man*p* 1-6-D-Man can also affect the bioassay system. Furthermore, the variability in the patterns of sugar recovery that we observed suggests that α-D-Man*p* 1-6-D-Man may represent a by-product derived from a more complex glycoprotein or glycolipid; however, our data

conclusively argue that α-D-Man*p* 1-6-D-Man may have important effects on in vitro antigen-specific proliferation. Fig. 6 demonstrates that α-D-Man*p* 1-6-D-Man, compared with D-mannose, has a much broader dose-response range and therefore the inhibitory curve has a different slope. Because of this quite different rate of change, estimates of relative potency are somewhat arbitrary. If one accepts 50% inhibition as a comparative point, then mannose 1-6 mannose is ~100 times more potent than D-mannose; if, however, one compares 30% inhibition, then α-D-Man*p* 1-6-D-Man is almost 500 times more potent.

Mannose-α-1-6 linkages are quite common in human mannose-rich glycoproteins; however, they tend to be proximal with respect to the protein moiety. A number of mammalian glycoproteins, including serotransferrin, lactotransferrin, fetuin, prothrombin, α_1-acid glycoprotein, and thyroglobulin, will yield α-D-Man*p* 1-6-D-Man on degradation. Furthermore, accumulation of this dimer has been reported in the storage disease fucosidosis and G.M. gangliosidosis (29). The exact cellular mechanism by which α-D-Man*p* 1-6-D-Man acts to suppress antigen-specific proliferation remains to be determined, however. The kinetics of inhibition of α-D-Man*p* 1-6-D-Man are different from those seen using D-mannose. Addition of D-mannose inhibits T cell proliferation throughout the culture period, whereas α-D-Man*p* 1-6-D-Man inhibits only when added at the initiation of culture. These data suggest that a requisite monocyte/T cell interaction or, alternatively, a recruiting or amplifying factor is being blocked. Furthermore, since mannose 1-6 mannose has very little effect when added late during culture, these kinetics suggest that the mannose 1-6 dimer is unlikely to be acting as a general metabolic inhibitor.

In fact, data in Table III suggest that α-D-Man*p* 1-6-D-Man may have a preferential effect on T cell proliferation. Thus, the dimer had no apparent effect on the in vitro induction of human spontaneous monocyte cytotoxicity. This assay is quite sensitive to the addition of exogenous agents that can affect monocyte function. For example, pokeweed mitogen and antisera to Dr antigens dramatically inhibit this assay while *cis* platinum, X irradiation, and phenylalanine mustard via different mechanisms actually enhance cytotoxicity (26–28, 30, 31). Not only did this mannose dimer fail to inhibit the generation of cytotoxic monocytes, but it also failed to inhibit the generation of antibody-secreting cells as assayed with a reverse hemolytic plaquing assay using pokeweed mitogen as a stimulant. Interestingly, the dimer did inhibit cellular proliferation induced by pokeweed mitogen. Since pokeweed mitogen is both a T cell and a B cell mitogen, the fact that it inhibits proliferation without reducing the number of observed plaque-forming cells suggests that α-D-Man*p* 1-6-D-Man may have a preferential effect on T cell recruitment and proliferation, while having little or no effect on B cell proliferation and function. In an effort to dissect this effect on T cells further, mixture experiments were performed.

Table V shows that mannose 1-6 mannose does not act on either monocytes or T cells individually, but requires that both cell types be present. Thus, preincubation of whole mononuclear cell populations with mannose 1-6 mannose for 20 h, followed by washing of the cells and reconstitution in fresh media, results in suppression of a 6-d antigen proliferation assay. Interestingly, preincubation of either monocytes by themselves or highly purified lymphocytes with

α-D-Manp 1-6-D-Man fails to inhibit the activity of either cell population to support normal antigen proliferation, even when the two pretreated cell types are remixed after 24 h. Thus, the first 24 h of the assay are critical; both monocytes and lymphocytes must be physically together, and the mechanism of action does not appear to be trivial or a general metabolic phenomenon.

The fact that suppression is seen at micromolar concentrations argues for some specificity in the ability of α-D-Manp 1-6-D-Man to alter in vitro immunologic reactions. However, it is important to point out that several other fractions from human pregnancy urine are also inhibitory. We have not studied normal urine for similar immunoregulatory molecules, but we suspect that it, too, will contain molecules of interest.

D-Mannose has been previously shown to interfere with a variety of in vitro reactions. For example, D-mannose blocks receptor-mediated uptake of certain enzymes (9). Furthermore, D-mannose has been reported to block NC, to inhibit antigen-specific proliferation, to block spontaneous monocyte-mediated cytotoxicity, and to reverse inhibition of in vitro immunoglobulin synthesis mediated by T cells found in the circulation of patients with infectious mononucleosis (13). Yeast mannan is also known to inhibit antigen-specific proliferation, and it has been proposed that certain patients with chronic mucocutaneous candidiasis fail to respond normally to antigen stimulation because of circulating mannan (18). In this regard, it is interesting to note that α-D-Manp 1-6-D-Man is a major linkage found in many mannans. Furthermore, we have preliminary evidence that complex mannose oligosaccharides composed primarily of D-mannose-α-1-6 linkages are dramatically more inhibitory than the simple dimer, and these compounds seem to share similar kinetics with the dimer. This implies that multivalency may be very important.

In conclusion, we believe that plant lectins are able to activate the immune system by binding to physiologic glycoprotein and glycolipid receptors. In the same way that exogenous lectins have been widely employed to activate various immunologic reactions, we propose that careful study of the effects of defined sugars will be equally illuminating. The 1-6 dimer of D-mannose that we describe already shows an affinity and immunologic specificity not exhibited by its parent, D-mannose. Thus, sugars like α-D-Manp 1-6-D-Man may begin to be used as probes to interfere with the function of endogenous lectin like activity in an effort to further dissect cell-mediated immune responses in vitro.

Summary

Endogenous mammalian lectin-like sugar-binding molecules have been previously described that have immunoregulatory properties. Further, the addition of defined simple saccharides to lymphocyte cultures has been shown to inhibit a variety of in vitro lymphocyte functions, presumably because these sugars are able to compete with the binding of endogenous lectins to critical membrane receptors. In this report, we describe the isolation and characterization of a D-mannose-containing disaccharide in human pregnancy urine that inhibits the proliferative response of human T lymphocytes. The inhibitory disaccharide was purified to homogeneity by sequential steps including affinity chromatography on immobilized concanavalin A and molecular sizing on Sephadex G-75 and

then Fractogel 40S columns, with final purification on high-performance thin-layer chromatography. By mass spectrometry of the purified material as its permethylated derivative, the deduced structure of this compound was α-D-Man*p* 1-6-D-Man. To confirm that this disaccharide was in fact immunosuppressive, an identical disaccharide was prepared by sequential digestion of yeast cell wall polysaccharide. The urinary and yeast disaccharides had identical immunosuppressive properties. It has been previously reported that D-mannose is inhibitory for antigen-specific proliferative assays in the range of 10–50 mM. The purified α-D-Man*p* 1-6-D-Man disaccharide was inhibitory at 100-fold-lower concentrations. Further, while D-mannose inhibits T cell proliferation when added at anytime up to 24 h before harvest of a 6-d lymphocyte culture, α-D-Man*p* 1-6-D-Man disaccharide was inhibitory only if added at the initiation of culture and had no inhibitory effect if added just 24 h later. These data support the concept that simple sugar compounds can exhibit marked immunoregulatory activity in vitro. The impact of these molecules on the regulation of immune responses in vivo is unknown, as is their precise mechanism of action, but structural and chemical identification should now permit a detailed analysis of these issues.

We wish to thank Dr. Clinton E. Ballou for the very generous gift of purified 1-6 mannose dimer obtained from yeast mannoprotein and for very helpful discussion.

Received for publication 7 May 1984 and in revised form 13 August 1984.

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