Tamm-Horsfall glycoprotein (THGP) and the oligosaccharide fraction liberated from THGP by hydrazinolysis inhibited tetanus toxoid-induced T cell proliferation. In fact THGP showed approximately 100-fold more inhibitory activity than the free oligosaccharides. After fractionating the oligosaccharides by anion-exchange column chromatography, the inhibitory activity could be detected in a sialidase-resistant acidic oligosaccharide fraction (fraction AR). The inhibitory activity of fraction AR was not observed when the fraction was added to the T cell culture medium 24 h after the addition of tetanus toxoid. Increased concentration of interleukin (IL-1) \( \beta \) and decreased concentration of IL-2 were observed in the T cell culture medium after the addition of fraction AR. The oligosaccharides in fraction AR also inhibited the growth of an IL-1-dependent cell line, D10-G4. These results strongly suggested that the oligosaccharides in fraction AR bind to IL-1\( \beta \) and suppress its cytokine activity. IL-1\( \beta \) actually bound to the fraction AR immobilized on an amine-bonded thin layer plate. Fractionation of the oligosaccharides indicated that only oligosaccharides containing an N-acetylgalactosamine residue and a sulfate residue bound specifically to IL-1\( \beta \). Removal of either the sulfate residue or the N-acetylgalactosamine residue from the oligosaccharides abolished both the proliferation-inhibitory and IL-1\( \beta \) binding activities. Since IL-1\( \beta \) did not bind to thyroid-stimulating hormone, which has the sulfate group at C-4 of the N-acetylgalactosamine residue in its N-linked sugar chains, the binding of IL-1\( \beta \) toward oligosaccharides in fraction AR was considered to be highly specific.

Most eukaryotic proteins occur as glycoproteins. The carbohydrate moieties of glycoproteins play important roles not only in modulation of protein properties such as stability and biological activities but also in various molecular recognition processes, including initial reaction in bacterial and viral infections, cell adhesion in inflammation and metastasis, differentiation, development, regulation, and many other intercellular communication events (1–3). Understanding the molecular mechanisms of carbohydrate recognition is therefore important for the biology of multicellular organisms. Carbohydrate-binding proteins (lectins) are widely distributed in eukaryotic cells and mediate in many specific biological functions including intercellular recognition, protein trafficking, and primitive defense reactions (4). We will describe here the novel lectin-like property of a cytokine.

Tamm-Horsfall glycoprotein (THGP)\(^1\) is a glycoprotein produced by kidney and contains approximately 30% carbohydrate. Serafini-Cessi's group (5–7) reported that THGP works as a potential suppressive agent of both the lymphocyte proliferation induced by phytohemagglutinin-L\( \gamma \) treatment and the mixed lymphocyte reaction. They also suggested that the inhibitory activity resides in the sugar moiety of the glycoprotein (6, 7). Muchmore and co-workers (8–10) found an immunosuppressive glycoprotein in the urine of pregnant women, and named it uromodulin. It inhibited T cell proliferation induced by tetanus toxoid as well as T lymphocyte proliferation induced by interleukin 1 (IL-1). They also suggested that the carbohydrate portion of uromodulin played a fundamental role in its inhibitory activity (11). That uromodulin is identical to THGP was confirmed by amino acid sequencing (12). Muchmore and Decker (13) proposed that uromodulin inhibits T cell proliferation via binding to IL-1\( \beta \) through its carbohydrate moieties and inactivates a mediator. However, Moonen et al. (14) rejected this explanation, because uromodulin interacted only with the denatured IL-1\( \beta \) adsorbed to the plastic plate but not with the native soluble IL-1\( \beta \). Therefore, the precise immunosuppressive mechanism of uromodulin has not yet been clarified.

We found that the oligosaccharide fraction, obtained by hydrazinolysis of THGP followed by N-acetylation, inhibits the T cell proliferation induced by tetanus toxoid. This paper reports the partial structural characterization of the oligosaccharides with this inhibitory activity.

### MATERIALS AND METHODS

#### Reagents

- Na\( ^{38} \)H(P), (360 mCi/mmol) and \([\text{H}]\)thymidine were purchased from NEN Life Science Products.
- \( \beta \)-N-Acetylatedhexosaminidase was purified from jack bean meal by the methods of Li and Li (15).
- Sialidase from *Arthrobacter ureafaciens* and 4-chloro-1-napthol were purchased from Nacalai Tesque, Kyoto.
- Concanavalin A (Con A) and Ficol-Hypaque were purchased from Pharmacia Biotech (Uppsala, Sweden).
- Human recombinant interleukin 1\( \beta \) (rIL-1\( \beta \)) and recombinant interleukin 2 (rIL-2) were purchased from Genzyme (Cambridge, MA).
- Anti-human IL-1\( \beta \) was from Collaborative Research Inc. (Bedford, MA).
- Biotinylated anti-rabbit immunoglobulins (G+M) antibody and *Wisteria floribunda* agglutinin-agarose were from E-Y Laboratories Inc. (Burlingame, CA).
- Avidin-biotin-peroxidase reagent (ABC reagent, Vectabtain ABC Kit) was purchased from Vector Laboratories, Inc. (Burlingame, CA).

\( ^{38} \)P Tetanus toxoid (400 limit of flocculation)

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\( ^{1-4} \) The abbreviations used are: THGP, Tamm-Horsfall glycoprotein; IL-1, interleukin 1; IL-1\( \beta \), interleukin 1\( \beta \); IL-2, interleukin 2; rIL, recombinant interleukin; BSA, bovine serum albumin; Con A, concanavalin A; \( \alpha \)-AGP, \( \alpha \)-acid glycoprotein; FCS, fetal calf serum; TSH, thyroid-stimulating hormone; PBS, phosphate-buffered saline; HPTLC, high performance thin layer chromatography.
in 50 following enzyme solutions: (i) A. ureafaciens

Cell Lines—IL-2-dependent mouse T cell line, CTLL cells, was ob-

Analytical Methods—Anion-exchange column chromatography was performed using a fast protein liquid chromatography apparatus ( Pharmacia Biotech) equipped with a Mono-Q HR5/5 column. Elution was programmed as follows: 5 mM sodium acetate, pH 4.0, for 10 min and then a linear gradient of sodium acetate, pH 4.0, to 1 M, in 10 min, at a flow rate of 0.5 ml/min at room temperature. Bio-Gel P-2 column chromatography using distilled water was performed to remove the salt or the monosaccharide from oligosaccharide fraction. Descending paper chromatography was performed using a solvent (1-butanol: ethanol:water = 4:1:1, v/v). Affinity chromatography on W. floribunda agglutinin-agarose was performed as follows. The sample dissolved in 100 μl of 10 mM phosphate-buffered saline (PBS), pH 7.4, was applied to a W. floribunda agglutinin-agarose column (2 ml) and eluted with 15 ml of the same buffer. Oligosaccharides bound to the column were eluted with 8 ml of the buffer containing 100 mM N-acetylgalactosaminate. After elution, the oligosaccharide fraction was freed from N-acetylgalactosamine by passing through a Bio-Gel P-2 column. Mild methanolysis was performed according to the procedure described previously (19).

Detection of Sulfate Ion Using Anion Chromatography—One nmol of oligosaccharides in fraction AR was heated in 6N HCl at 110 °C for 2 h. After washing with PBS again, the filter was incubated with a substrate saturated sodium bicarbonate solution containing 5% acetic anhydride and incubated at room temperature for 2 h. The filter was then washed with 1% polyvinylpyrrolidone for 1 min and then soaked in air-dried. Each oligosaccharide was dissolved in 100 μl of 0.1 M sodium acetate buffer, pH 5.0. One drop of tolune was added to the reaction mixtures to inhibit bacterial growth during incubation. After being incubated for 48 h at 37 °C, the plates were incubated with rabbit anti-human IL-1β antibodies, and incubated at room temperature for 4 h. After being washed with PBS, the plate was overlaid with 1% BSA/PBS and incubated at 4 °C for 4 h. After being washed five times with PBS, the plate was overlaid with rabbit anti-human IL-1β antibodies and incubated at 4 °C for 4 h. The filter was washed with PBS, dried, and exposed to XAR-5 x-ray film (Eastman Kodak Co.) in the dark at –80 °C for 2–5 days. The dot blot analysis was performed using a nitrocellulose filter and air-dried for several hours. The filter was washed three times with PBS, soaked in 5% BSA/PBS, and incubated at room temperature for 4 h. After being washed with PBS, the filter was soaked in 1% BSA/PBS and incubated at 4 °C for 4 h. After being washed three times with PBS, the filter was soaked in a solution containing 5% BSA/PBS, 0.1% Tween 20, and incubated at room temperature for 2 h. The filter was then washed with PBS, incubated with ABC reagent at room temperature for 30 min, and washed with PBS again. The filter was incubated with a substrate solution (10 μg/ml Tris-HCl buffer, pH 7.2, 0.3% 4-chloro-1-naphthol in methanol, 30% H2O, aqueous (5:1.0:0.1, v/v)) at room temperature for an appropriate length of time.

Binding Assays of IL-1β to Oligosaccharides—Binding assays of IL-1β to oligosaccharides were performed using a slight modification of the previously described methods (23). An amino-bonded high performance silica gel plate (HPTLC Fertigplatten NH2-Euk) was obtained from Merck (Darmstadt, Germany).

Amino-bonded high performance silica gel plate (HPTLC Fertigplatten NH2-Euk) was obtained from Merck (Darmstadt, Germany).

Cells Proliferated by Tetanus Toxoid—The amount of IL-1β in the culture medium was measured at day 3 using an enzyme-linked immunoassay kit for IL-1β (Otsuka Bioassay Research, Tokushima, Japan). The amount of IL-2 in the culture medium was measured at day 4 by counting the [3H]thymidine incorporated into CTLL cells, whose growth was dependent on IL-2. Briefly, 5 × 104 cells were incubated with 100 μl of the culture medium of RPMI 1640 containing 10% FCS at 37 °C for 18 h. The cultures were then pulsed with 0.5 μCi of [3H]thymidine overnight, and the amount of [3H]thymidine incorporated was determined as described above. For the quantitation, known amounts of rIL-2 were added to aliquots of the above assay mixture as standards.

Measurement of the Amount of Cytokine in the Culture Medium of T Cells Proliferated by Tetanus Toxoid—The amount of IL-1β in the culture medium was measured at day 3 using an enzyme-linked immunoassay kit for IL-1β (Otsuka Bioassay Research, Tokushima, Japan). The amount of IL-2 in the culture medium was measured at day 4 by counting the [3H]thymidine incorporated into CTLL cells, whose growth was dependent on IL-2. Briefly, 5 × 104 cells were incubated with 100 μl of the culture medium of RPMI 1640 containing 10% FCS at 37 °C for 18 h. The cultures were then pulsed with 0.5 μCi of [3H]thymidine overnight, and the amount of [3H]thymidine incorporated was determined as described above.

The amount of IL-1β in the culture medium was measured at day 3 using an enzyme-linked immunoassay kit for IL-1β (Otsuka Bioassay Research, Tokushima, Japan). The amount of IL-2 in the culture medium was measured at day 4 by counting the [3H]thymidine incorporated into CTLL cells, whose growth was dependent on IL-2. Briefly, 5 × 104 cells were incubated with 100 μl of the culture medium of RPMI 1640 containing 10% FCS at 37 °C for 18 h. The cultures were then pulsed with 0.5 μCi of [3H]thymidine overnight, and the amount of [3H]thymidine incorporated was determined as described above.

Tetanus Toxoid-induced T Cell Proliferation—Tetanus toxoid-induced proliferative response in human peripheral blood mononuclear cells was performed by using slight modifications of the previously described methods (22). Briefly, 10 ml of normal heparinized human blood was diluted with an equal volume of RPMI 1640 medium, and placed above 2 ml of Ficoll-Hypaque solution, and centrifuged at 800 × g for 15 min at room temperature. Monocyte-enriched population located between upper and lower layers was carefully collected. The cells were washed twice with RPMI 1640 containing 10% autologous plasma by alternate centrifugation and resuspension. The viable cells (2 × 105) in 10% autologous plasma were incubated in 96-well microtiter plates with tetanus toxoid and various amounts of each oligosaccharide in a final volume of 200 μl. After incubation for 6 days, the cultures were pulsed with 0.5 μCi of [3H]thymidine for 6 h, and the amount of [3H]thymidine incorporated into the cells was determined.

Tetanus toxoid-in-duced proliferation of normal human peripheral blood mononuclear cells was performed by using slight modifications of the previously described methods (22). Briefly, 10 ml of normal heparinized human blood was diluted with an equal volume of RPMI 1640 medium, and placed above 2 ml of Ficoll-Hypaque solution, and centrifuged at 800 × g for 15 min at room temperature. Monocyte-enriched population located between upper and lower layers was carefully collected. The cells were washed twice with RPMI 1640 containing 10% autologous plasma by alternate centrifugation and resuspension. The viable cells (2 × 105) in 10% autologous plasma were incubated in 96-well microtiter plates with tetanus toxoid and various amounts of each oligosaccharide in a final volume of 200 μl. After incubation for 6 days, the cultures were pulsed with 0.5 μCi of [3H]thymidine for 6 h, and the amount of [3H]thymidine incorporated into the cells was determined.
RESULTS

N-Linked Sugar Chains Obtained from THGP Inhibited Tetanus Toxoid-induced T Cell Proliferation—The oligosaccharide fractions obtained from THGP by hydrazinolysis followed by N-acetylation were added to the culture medium of tetanus toxoid-specific T cell proliferation. The effect of these oligosaccharides on the proliferation reaction is shown in Fig. 1. The concentration required for 50% inhibition of tetanus toxoid-induced proliferation was around 10 \( \mu \text{M} \). At this concentration, neither the oligosaccharides liberated from \( \alpha_1 \)AGP nor those from RNase B inhibited tetanus toxoid-induced T cell proliferation (data not shown). In order to characterize the oligosaccharide showing the inhibitory activity, the oligosaccharide mixture obtained from THGP was fractionated. To facilitate detection of the oligosaccharides in further fractionation procedures, a small amount of tritium-labeled oligosaccharide mixture from THGP was added.

The oligosaccharide mixture was subjected to anion-exchange column chromatography with a Mono-Q HR5/5 column. As shown in Fig. 2A, oligosaccharides were separated into a neutral fraction (N) that eluted with 5 mM sodium acetate, pH 4.0, and an acidic fraction (A) that eluted with 500 mM sodium acetate, pH 4.0. When fraction A was exhaustively incubated with \( A. ureafaciens \) sialidase, 67% of it was converted to neutral components (named fraction AN), and the remainder (named fraction AR) was resistant to this enzymatic treatment (Fig. 2B). Approximately 80% of oligosaccharides in fraction AR were converted to neutral components (named fraction ARN), and the remainder (named fraction ARR) was resistant to this enzymatic treatment (Fig. 2C). The sulfate ion in the above reaction mixture was detected as shown in Fig. 3. This result indicates that the acidic nature of sialidase-resistant oligosaccharides is due to the sulfate residue. Based on the analytical data, about 3 nmol of sulfate ion was released from 1 nmol of oligosaccharide mixture in fraction AR under these experimental conditions. Remaining acidic oligosaccharides in fraction ARR in Fig. 2C were converted to the neutral components by heating in 0.1 N CF\(_3\COOH\) at 80 °C for 2 h. However, the acidic nature of oligosaccharides in fraction ARR could not be determined due to the limited amounts of sample. Based on the radioactivities, the molar ratio of oligosaccharides in each fraction was calculated as follows: fraction N (10%), fraction A (90%), fraction AN (60%), fraction AR (30%), fraction ARN (25%), and fraction ARR (5%).

On the other hand, when fraction A was directly subjected to mild methanolysis in order to determine the ratio of oligosaccharides carrying only the sulfate residue, a very small part of the fraction was converted to a neutral oligosaccharide mixture (named fraction AMN), and most of the oligosaccharides remained acidic as indicated by the fraction AMR peak in Fig. 2D. The molar ratio of the oligosaccharides in the two fractions was calculated on the basis of their radioactivities, fraction AMN (3%) and fraction AMR (87%). These results indicate that 60% of the THGP oligosaccharides contain only sialic acid residues,
3% contain only sulfate residues, and 27% contain both sialic acid and sulfate residues as their acidic components.

Inhibition Activities of the Oligosaccharide Fractions—The inhibitory activity of each oligosaccharide fraction thus obtained on the T cell proliferation was examined, and the results are summarized in Fig. 4. Oligosaccharides in fraction A and fraction AR showed strong inhibitory activity. On the other hand, oligosaccharides in fraction ARN, which was converted to a neutral fraction by mild methanolysis treatment as described above, showed no inhibitory activity. These results indicate that the sulfate residue of oligosaccharides in fraction AR was essential for the expression of inhibitory activities. This interpretation is supported by the fact that fractions ARR, AMN, and AMR showed no inhibitory activity (Fig. 4). It is noteworthy that fraction AR showed only slightly more potent inhibitory activity than fraction A, whereas fraction A was separated into bioactive fraction AR and inactive fraction AN after sialidase treatment as described above. The reason for this has not yet been determined, although a possible explanation is that the presence of the sialic acid residues, which is not essential for proliferation-inhibitory activity, on the sugar chains in fraction A may enhance the inhibitory activity of the sulfated oligosaccharides. As compared with other inactive fractions, the neutral fraction N, which was reported to contain only high mannose-type oligosaccharides (24, 25), had a slight inhibitory effect on the T cell proliferation at a concentration of ~100 μM.

Inhibitory Mechanism of Oligosaccharides on T Cell Proliferation—To clarify the mechanisms of inhibitory activity of oligosaccharides in fraction AR, we changed the time of oligosaccharide addition. When added 24 h or more after the addition of tetanus toxoid, oligosaccharides in the fraction AR showed no inhibitory activity at all (Fig. 5). This result suggests that sulfated oligosaccharides in fraction AR acted at an early stage of antigen-specific T cell proliferation.

Therefore, we quantified the amount of IL-1β and IL-2 molecules in the culture medium of tetanus toxoid-activated T cells under the effect of various amounts of fraction AR, since these soluble mediators are known to play important roles at the early stage of antigen-specific T cell proliferation. As shown in Fig. 6, the amount of IL-1β in the T cell culture medium increased in parallel with the amount of fraction AR added. In contrast, the amount of IL-2 decreased on increasing the concentration of oligosaccharides. A possible explanation for these interesting results is that fraction AR inhibited the binding of IL-1β to its receptor on the surface of T cells. As a result, the decline of signal transduction induced by IL-1β in T cells would reduce IL-2 production. This inhibition can be induced by bind-
ing of the oligosaccharides to either IL-1β molecules or IL-1 receptors.

In order to find out whether the oligosaccharides in fraction AR can directly act on the IL-1β molecule or not, we examined the effect of fraction AR on the culture of an IL-1-responsive D10-G4 cell line (17, 18). As shown in Fig. 7, both total oligosaccharides liberated from THGP (W in the figure) and fraction AR inhibited the proliferation of D10-G4 cells. However, oligosaccharides derived from other glycoproteins, α1AGP and RNase B, did not show any effect at all (Fig. 7). These results suggest that the inhibition of antigen-specific T cell proliferation might be due to interaction of the unique oligosaccharides of THGP with IL-1β.

Binding of IL-1β to Immobilized Oligosaccharides on an NH₂
HPTLC Plate—We then examined whether IL-1β binds directly to the oligosaccharides of THGP by a thin layer overlay method as described previously (23). As shown in Fig. 8A, IL-1β bound to oligosaccharides in fractions A and AR but not to those in fractions AN, ARN, ARR, AMR, and AMN. These results are consistent with the data that only fractions AR and A could inhibit T cell proliferation (see Fig. 4). IL-1β also did not bind to the oligosaccharide fractions from thyroglobulin and α1AGP, which did not inhibit the T cell proliferation (Fig. 8A). The radioactivities retained by immobilized oligosaccharides in Fig. 8A were quantified densitometrically, and the data are shown in Fig. 8B. The data clearly indicated that IL-1β bound only to the oligosaccharides in fractions A and AR derived from THGP. It must be stressed here that IL-1β very weakly bound to the porcine thyroglobulin oligosaccharides, which included sulfated biantennary sugar chains (26). The results indicate that oligosaccharides with not only sulfate residues but also additional structural features are recognized by the IL-1β molecule.

In order to elucidate the structure of the IL-1β ligand, fraction AR was applied to a W. floribunda agglutinin-agarose column, which is known to recognize a peripheral β-linked N-acetylgalactosamine (27), and separated into an unbound fraction (fraction I in Fig. 9A) and a bound fraction subsequently eluted with 100 mM N-acetylgalactosamine (fraction II in Fig. 9A). The percent molar ratio of these two frac-
Fig. 10. Coexistence of the inhibitory activity and IL-1β binding activity in fraction AR and fraction II obtained by W. floribunda agglutinin column chromatography. A, effect of addition of the oligosaccharides fractionated by the W. floribunda agglutinin-agarose column on tetanus toxoid-induced lymphocyte proliferation. Fractions I and II are the same as in Fig. 9A. B, binding of IL-1β to each oligosaccharide fraction in A. The amount of IL-1β bound to each oligosaccharide fraction was determined by densitometry of the NH₂-HPTLC plate as described in Fig. 8. Detailed procedures are described under "Materials and Methods."

Fig. 11. Binding of IL-1β to glycoproteins (A) and glycosphingolipids (B). One nanomole of each glycosphingolipid was spotted onto the plate. Detailed procedures are described under "Materials and Methods."

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The inhibitory activity of IL-1β and IL-1γ binding activity in fraction AR and fraction II obtained by W. floribunda agglutinin column chromatography. A, effect of addition of the oligosaccharides fractionated by the W. floribunda agglutinin-agarose column on tetanus toxoid-induced lymphocyte proliferation. Fractions I and II are the same as in Fig. 9A. B, binding of IL-1β to each oligosaccharide fraction in A. The amount of IL-1β bound to each oligosaccharide fraction was determined by densitometry of the NH₂-HPTLC plate as described in Fig. 8. Detailed procedures are described under "Materials and Methods."

Binding of Glycoproteins and Glycosphingolipids with IL-1β—In an attempt to clarify the relationship between the sulfate and N-acetylgalactosamine residues, we examined the binding of IL-1β to TSH because the SO₄-4GalNAc group was present in the sugar chains of TSH (28). When we studied the interaction of THGP and TSH with IL-1β, only THGP could be stained with IL-1β under the experimental conditions used (Fig. 11A). This suggests that IL-1β could not bind the SO₄-4GalNAc group. Next, we examined the reactivity of IL-1β with various glycolipids containing sulfate and β-N-acetylgalactosamine residues. As shown in Fig. 11B, IL-1β did not bind to sulfatide at all. This is consistent with the results that the oligosaccharides of thyroglobulin did not react with IL-1β as shown in Fig. 8, because sulfatide contains only the SO₄-3Gal group similar to thyroglobulin (26, 29). On the other hand, it is quite interesting that IL-1β can bind weakly to SM2 but not to GM2 at all, although SM2 and GM2 have similar peripheral structures: the GalNAcβ1→4(R-3)Galβ1→ in which R is SO₄ for SM2 and is sialic acid for GM2 (30). Therefore, the GalNAcβ1→4SO₄-3Galβ1→ group could be at least a part of the ligand for IL-1β molecule.

DISCUSSION

Muchmore’s group (25, 31) reported that the glycopeptides containing high mannose type sugar chains derived from THGP inhibited antigen-specific T cell proliferation by 50% in the concentration range 0.2–2 μM. Dall’Olio et al. (7) reported that glycopeptides containing the complex type or high mannose type sugar chains obtained by Pronase digestion of THGP inhibited the lymphocyte proliferation induced by mixed lymphocyte reaction. Conflicting evidence is that the glycopeptides obtained from ovalbumin by Pronase digestion, which should contain a series of high mannose type sugar chains together with a series of hybrid type sugar chains (2), did not show any inhibitory activity (7). Our data demonstrate, however, that oligosaccharides in fraction N, which contains high mannose type sugar chains (24, 25), showed very little inhibitory activity even at the concentration of 100 μM (Fig. 4). In contrast, oligosaccharides in fraction AR inhibited T cell proliferation by 50% at the concentration of 2 μM.

We propose in this report that the immunosuppressive properties of THGP are expressed by the interaction of its carbohydrate moieties with IL-1β. Our results demonstrate that oligosaccharides in fraction AR inhibited the proliferation of D10-G4 cells, which shows IL-1-dependent growth (17, 18), and that IL-1β interacted specifically with the oligosaccharides in...
fraction AR immobilized on the NH₂ HPTLC plate. By further fractionation of the oligosaccharides in fraction AR, it was found that the oligosaccharides containing β-N-acetylgalactosamine and sulfate residues specifically bind to IL-1β. Two conflicting data were reported relating to our findings. Muchmore and Decker (13) reported that the N-linked sugar chains of uromodulin interacted with IL-1, although Moonen et al. (14) reported that THGP did not interact with soluble native cytokines and could only bind to denatured cytokines at low pH. Additionally, Fukushima et al. (32) demonstrated that IL-1β interacts with the glycosylphosphatidylinositol anchor. We recently found that IL-1β molecules radiolabeled with 125I by Bolton-Hunter reagent, which reacted with N-terminal amino acid and lysine residues of the peptide portion (33), could no longer bind to THGP oligosaccharides. This result suggests that N-terminal amino acid and/or lysine residues of IL-1β may play a role as the carbohydrate-binding site. In contrast, it was reported that even after the 125I-labeling IL-1β molecules can bind to their receptors on the cell surface (34). Therefore, care must be taken when using 125I-labeled IL-1β as a probe to investigate the lectin-like activity of IL-1β. The possibility that the inhibitory oligosaccharides bind to tetanus toxoid and consequently inhibit the T cell proliferation will be ruled out because toxoid-independent T cell proliferation induced by combination between phytohemagglutinin-L4 and IL-1β was also inhibited by oligosaccharides in fraction AR (data not shown).

THGP has been found to contain more than 150 N-linked sugar chains, and the structures of only 30 oligosaccharides have been determined (35, 36). Among them, two types of terminal sulfated elements were found, 4-O-sulfated GalNAc and 3-O-sulfated Gal (36). None of these are considered to be the actual ligand of IL-1β from our current study. These results indicate that not only the presence of a sulfate residue and a β-N-acetylgalactosamine residue but also a specific linkage(s) between the two groups is required for the ligand of IL-1β. Based on the reactivity with glycosphingolipids, the GalNAcβ1→4(SO₄⁻⁻)₃Galβ1→ group could be considered as a part of an epitope for binding to the IL-1β molecule. Therefore, structural determination of the remaining oligosaccharides of THGP, especially sulfated and β-N-acetylgalactosamine-containing N-linked sugar chains, will be required in order to elucidate the inhibitory mechanism of the IL-1β molecule and the physiological roles of the lectin-like property. Acknowledgments—We thank Professor Jiro Tatsuno, National Defense Medical College, for encouraging this work and Professor Ineishizuka, Teikyo University School of Medicine, for the kind gift of SM2.

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