

Inhibitory Effect of Wheat Germ Agglutinin on Mouse Mast Cell Adhesion to Fibronectin

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Key Words

Wheat germ agglutinin · Mouse MCP5/L mast cell line · IgE · β -Hexosaminidase release · Adhesion to fibronectin · Phorbol myristate acetate · Ionophore A23187

Abstract

Background: Mast cells play a critical role in allergic and inflammatory responses. The interactions between these cells and extracellular matrix components influence the distribution of mast cells in tissues and their biological responsiveness. It has been reported that the lectin wheat germ agglutinin (WGA) inhibits mast cell mediator release. We decided to investigate whether adhesion to fibronectin (FN), another mast cell function, which is upregulated following Fc ϵ RI cross-linking, is also inhibited by WGA. **Methods:** Mouse bone-marrow-derived mast cell line MCP5/L was used. For Fc ϵ RI-dependent mast cell activation, MCP5/L cells were sensitized with mouse IgE antibodies. WGA was added to cell suspensions simultaneously with a challenging agent and, after an appropriate incubation period, β -hexosaminidase release and adhesion to FN were determined. **Results:** Both Fc ϵ RI cross-linking-dependent mast cell adhesion to FN and mediator release were dose-dependently inhibited by WGA; however, the lectin concentrations re-

quired to induce maximum inhibition of adhesion were significantly lower. Furthermore, WGA inhibited phorbol-myristate-acetate- and A-23187-mediated mast cell adhesion to FN, i.e. processes that do not engage Fc ϵ RI. The effect of WGA on Fc ϵ RI-mediated secretion was reversed by GlcNAc. In contrast, combination of GlcNAc and NeuNAc or N,N'-diacetylchitobiose was required to reverse the inhibitory effect of WGA on mast cell adhesion. **Conclusion:** The characteristics of WGA-mediated inhibition of MCP5/L mast cell adhesion to FN suggest that mast cell integrins are targets of the inhibitory action of WGA and the sugar moieties on these receptors might be important for receptor function.

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Introduction

Mast cells play critical roles in allergic and inflammatory responses. Therefore, the tissue distribution of mast cells influences the magnitude of these events. The interactions between mast cells and their surrounding extracellular matrix components are known to have profound influences on the targeting of these cells to specific tissue locations, the distribution of mast cells in tissues and their biological responsiveness [1].

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Murine mast cells were shown to adhere to several extracellular matrix components, such as laminin [2], vitronectin [3] and fibronectin (FN) [4]. Several characteristics of murine mast cell adhesion to FN suggest that this process is mediated by integrin receptors [4, 5].

Human mast cells similarly adhere to components of extracellular matrix [1]. Isolated human skin mast cells have been shown to spontaneously adhere to FN and laminin and this adhesion is mediated by integrin receptors [6]. Despite the progress that has been made in characterizing human and mouse integrin receptors, relatively little is known concerning the precise mechanisms whereby ligand-receptor interactions lead to adhesion. It has been previously observed that adhesiveness of mast cell integrin receptors may be upregulated by a number of molecules [2–4, 7–9] and other cell surface receptors [5, 10–12]. Cross-linking of FcεRI on mouse mast cells known to trigger mediator secretion has also been shown to stimulate adhesion of these cells to FN [5, 11, 12]. Previous reports have provided evidence that both FcεRI and integrin receptors are specifically recognized by wheat germ agglutinin (WGA) [13–15], which interacts with N-acetyl-D-glucosamine, N-acetyl-D-glucosamine oligomers and N-acetyl-neuraminic acid residues [16–18]. It has been shown that WGA is able to prevent FcεRI cross-linking-dependent mediator release from rat basophilic leukemia cells [19]. The question arose whether the inhibitory action of WGA on mast cell function is limited to FcεRI-mediated mast cell mediator release. We studied the effect of WGA on mast cell adhesion to FN and found that WGA inhibits mast cell adhesion to FN.

Materials and Methods

Materials

Human FN, fetal calf serum (FCS), bovine serum albumin (BSA), ovalbumin grade V (OA), RPMI-1640, sodium pyruvate, Triton X-100, phorbol myristate acetate (PMA), A23187, glycine, *p*-nitrophenyl-N-acetyl-β-D-glucopyranoside, N-acetylglucosamine (GlcNAc), N-acetylneuraminic acid (NeuNAc), N,N'-diacetylchitobiose (Sigma Chemical Company, St. Louis, Mo., USA), L-glutamine, nonessential amino acids, penicillin/streptomycin (Gibco Ltd., Paisley, UK), HEPES (Serva Feinbiochemica, Heidelberg, Germany), 2-mercaptoethanol (Merck, Darmstadt, Germany), thymidine [6-³H], 10 Ci/mmol (NEN Life Science Products Inc., Boston, Mass., USA), and 96-well flat bottom polystyrene plates (Corning Laboratory Sciences Company, Vernon, N.Y., USA) round-bottom polystyrene plates (Nunc InterMed) were purchased from the manufacturers indicated. The MCP5/L mast cell line is a variant of the MCP5 mast cell line immortalized from a primary bone-marrow-derived culture exposed to Ad-12-SV40 virus [20].

Mast Cell Cultures

MCP5/L and CFTL15 mast cells were cultured as described previously [4], in RPMI-1640 medium supplemented with 10% heat-inactivated FCS, 10% WEHI-3 supernatant as a source of IL-3, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 4 mM L-glutamine, 25 mM HEPES, 50 μM 2-mercaptoethanol, 100 U/ml penicillin and 100 μg/ml streptomycin (complete RPMI medium). Cell number was adjusted to 2×10^5 cells/ml and cells were cultured at 37°C in a CO₂ incubator. Cultures were maintained by replacing the media on a weekly basis.

Production of Mouse Serum Containing IgE Antibodies

Female BALB/c 8-week-old mice were immunized by several subcutaneous injections of 1 μg OA absorbed on 1 mg Al(OH)₃ gel, given 2 weeks apart. One week after every antigen injection, the blood was collected for serum. The IgE titer of the pooled serum was 2,560 (mouse anti-OA IgE serum), as determined by passive cutaneous anaphylaxis in Wistar rats [21].

Sensitization of Mast Cells

MCP5/L cells suspended in complete RPMI medium at the concentration 10^6 cells/ml were incubated with mouse anti-OA IgE serum (dilution 1:10) for 18 h at 37°C in CO₂ incubator, washed 3 times and resuspended in HEPES-buffered RPMI-1640.

Mediator Release

The percentage of N-acetyl-β-D-hexosaminidase (β-Hex) activity released into the supernatants was measured as described [22], with slight modifications [5]. Briefly, 180-μl aliquots containing 8×10^4 mast cells (previously sensitized with IgE and suspended in HEPES-buffered RPMI medium without phenol red) were placed in polystyrene tubes and preincubated 5 min at 37°C. Increasing concentrations of WGA suspended in media were added to selected tubes. When the effect of WGA-specific sugars was tested, sugars were added (at the concentrations indicated) just before addition of WGA. Antigen dissolved in media (at concentrations as indicated) was then added to the assay tubes to a final volume of 200 μl and incubation was continued for 60 s. When ionophore A-23187-induced mediator secretion was tested, nonsensitized mast cells were challenged with the ionophore at a final concentration of 10^{-6} M and incubated at 37°C for 10 min. The reaction was stopped by cooling the tubes and followed by centrifugation at 4°C. Forty microliters of 1.2% Triton-X was then added to the appropriate tubes to obtain a cell lysate (total). Aliquots containing 50 μl of supernatant from control or assay tubes or 60-μl aliquots of cell lysates were transferred into the wells of 96-well plates. Ten microliters of 1.2% Triton X-100 was added to the wells containing supernatant or media (blank) in order to adjust the volume and Triton X-100 concentration. This was followed by addition of 60 μl of 0.08 M citric buffer (pH 4.5) containing 8 mM *p*-nitrophenyl-N-acetyl-β-D-glucopyranoside as a chromogenic substrate for β-Hex. The plate was then incubated at 37°C for 2 h. The reaction was stopped by the addition of 200 μl 0.2 M glycine (pH 10.7). The optical density at 410 nm was determined using the microplate reader (Dynatech MR 5000) and the percentage of enzymatic activity released into the supernatant was calculated according to the formula: β-Hex release (%) = (supernatant – blank) / (total – blank) × 100, where net β-Hex release is defined as: induced β-Hex release – spontaneous (control, nontriggered) β-Hex release.

Cell Adhesion Assay

The adhesion assay was performed as described previously [4]. Briefly, rows A and B of 96-well plates were coated with human FN for 12 h at 4°C, using FN diluted in PBS at the concentration of 10 µg/ml (100 µl of FN solution per well). Control wells were incubated with PBS alone. Binding sites were then blocked by adding 100 µl of 4% BSA in PBS to rows A and B and further incubated at 37°C for 2 h. The coating fluid was then aspirated and the wells were rinsed twice with 200 µl of PBS, which was discarded. Sensitized or nonsensitized MCP5/L cells (as indicated) were labeled with 5 µCi/ml of [³H]TdR for 12 h before addition to the assay wells. Cells were then separated from free [³H]TdR by centrifugation and resuspended in RPMI-1640 medium supplemented with 1% BSA, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 4 mM L-glutamine, 25 mM HEPES, 50 µM 2-ME, 100 U/ml penicillin and 100 µg/ml streptomycin (BSA/RPMI). Aliquots of cell suspension containing 4×10^4 cells were placed in assay wells (in rows A and B). After 5 min of preincubation, the antigen (final concentration 10 ng/ml) or PMA (final concentration 50 ng/ml) dissolved in BSA/RPMI medium were added to selected wells. Increasing concentrations of WGA were added to selected wells simultaneously with the antigen or PMA (if not otherwise stated). When the effect of WGA-specific sugars was tested, sugars were added (at indicated concentrations) just before the addition of WGA. The final volume of each sample was 100 µl. BSA/RPMI medium to rinse the assay wells was placed in rows E, F, G and H. The plates were next incubated at 37°C in a CO₂ incubator for the time indicated. After incubation, nonadherent cells were transferred from the assay wells to appropriate wells in rows C and D using a 12-channel pipette. The assay wells were then rinsed twice with the media from rows, E, F, G and H, to remove remaining nonadherent cells, and the rinsing media were returned to the original wells. Adherent cells in the wells of row A and B, and nonadherent cells in the wells of rows C to H were then harvested using Skatron cell harvester (Norway). Adherent cells from every well were harvested on one filter and nonadherent cells from two corresponding wells were harvested together on a second filter. The filter-bound radioactivity was measured in a liquid scintillation counter (Wallac 1410, LKB, Pharmacia, Sweden).

The percentage of adhesion was calculated by dividing the radioactivity associated with adherent cells by the total radioactivity associated with both nonadherent and adherent cells.

Statistical Analysis of the Data

A minimum of four experiments using independent cell cultures was done for reported data except when stated otherwise. Data shown represent the mean $\pm$ SEM of the indicated number of experiments. Statistical significance of the data was determined with a paired Student *t* test.

Results

Effect of WGA on FcεRI Cross-Linking-Induced Mast Cell Mediator Secretion and Adhesion to FN

To determine whether WGA is able to affect IgE-mediated responses of IL-3-dependent bone-marrow-derived cultured mast cell line MCP5/L, antigen-induced β-Hex release and antigen-induced mast cell adhesion to FN

were tested in parallel experiments. Sensitized MCP5/L mast cells challenged with antigen (OA, 1 µg/ml) for 60 s released β-Hex ($18.8 \pm 1.5\%$). As can be seen in figure 1a, WGA added to the cell suspensions simultaneously with antigen inhibited antigen-induced mediator release in a dose-dependent manner, reaching $86.5 \pm 3.4\%$ inhibition at the highest concentration used (20 µg/ml). Incubation of sensitized cells in FN-coated wells of flat bottom polystyrene plates in the presence of antigen (OA) resulted in a significant increase in cell adhesion to FN (fig. 1b); after 30 min of incubation of mast cells without OA $23.6 \pm 1.0\%$ of cells adhered to FN spontaneously, whereas in the presence of OA (10 ng/ml) the number of adherent cells increased to $61.6 \pm 1.6\%$. Activation of mast cells with OA in the presence of increasing concentrations of WGA resulted in significantly lower mast cell adhesion as compared to control (OA without WGA). The observed inhibitory effect of WGA on antigen-induced mast cell adhesion to FN was dose dependent. Incubation of mast cells in the presence of WGA in concentrations higher than 4 µg/ml resulted in significantly lower mast cell adhesion than that observed with nonstimulated mast cells (spontaneous adhesion) (fig. 1b). Thus, WGA inhibited IgE-mediated mast cell adhesion to FN in concentrations significantly lower than those required to inhibit mediator release.

Effect of WGA on Ionophore-A23187-Induced Mediator Release and Ionophore-A23187- or PMA-Induced Adhesion to FN

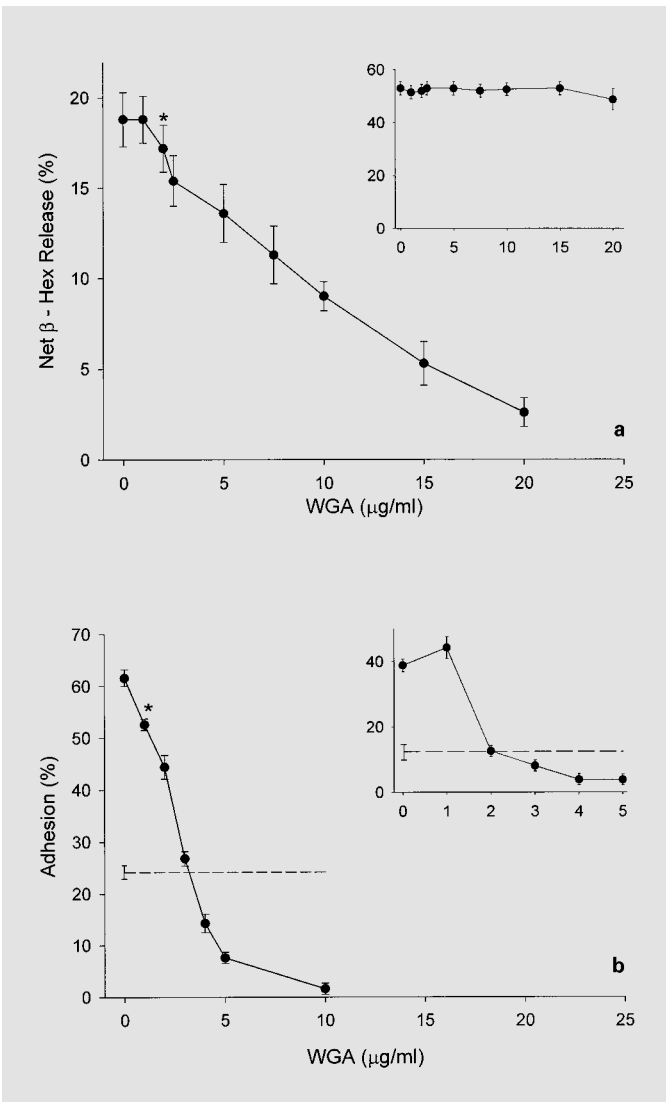
Mast cells can be activated to adhere to FN not only with IgE cross-linking, but also with stimuli that do not engage FcεRI. We next tested whether mast cell adhesion to FN and mediator release induced by stimuli other than FcεRI cross-linking are also inhibited with WGA. We measured the effect of WGA on mast cell mediator release and mast cell adhesion to FN following activation with calcium ionophore A23187. WGA in concentrations ranging from 1 to 20 µg/ml did not inhibit ionophore-induced β-Hex release (insert to fig. 1a). In contrast WGA concentrations ranging from 2 to 5 µg/ml inhibited significantly ionophore-induced adhesion to FN (insert to fig. 1b). The results show that WGA did not affect the mast cell secretory mechanism engaged in calcium-ionophore-induced β-Hex secretion, while it completely inhibited mast cell adhesion to FN induced by the same agent.

PMA has also been shown to upregulate mast cell adhesion to FN [4]. We decided to test whether PMA-mediated mast cell adhesion to FN is sensitive to WGA. As expected (fig. 2), mast cell adhesion to FN increased

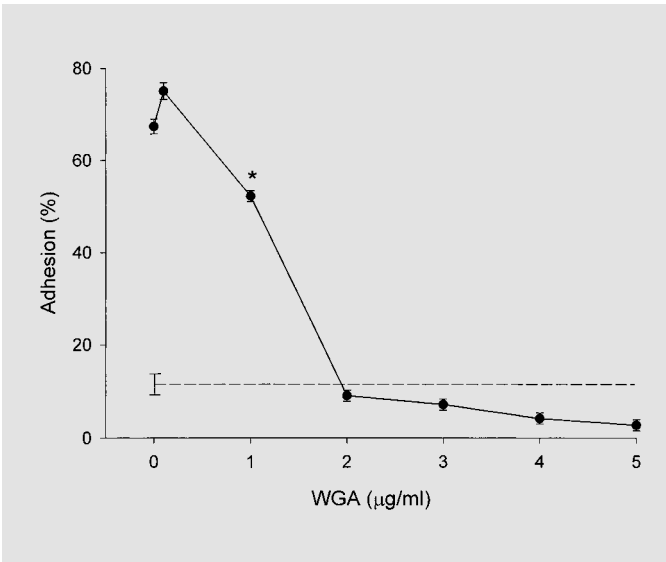
from $15.9 \pm 1.8\%$ (spontaneous adhesion) to $67.4 \pm 1.6\%$ following addition of PMA (50 ng/ml). Simultaneous addition of WGA in increasing concentrations resulted in a significant decrease in the percent of mast cells adhering to FN. There was a dose-response relationship in the inhibitory effect of WGA, with WGA concentrations of 2–5 $\mu\text{g/ml}$ decreasing the level of PMA-induced mast cell adhesion to FN below the level of adhesion observed with nonstimulated mast cells (spontaneous adhesion). The results show that WGA exhibits an inhibitory effect not only on Fc ϵ RI cross-linking-mediated mast cell adhesion, but also on adhesion induced by other stimuli. In order to see whether other mast cell lines exhibiting the ability to adhere to FN are sensitive to the inhibitory action of WGA, we performed adhesion experiments using murine IL-3-dependent mast cell line CFTL15 [23], murine mast cell line C57 [24] and human mast cell line HMC-1 [25]. CFTL15 mast cells exhibited a rate of spontaneous adhesion to FN comparable to

Fig. 1. Effect of WGA on antigen-induced mast cell β -Hex release (**a**) and adhesion to FN (**b**). **a** Sensitized mast cells were preincubated for 5 min; WGA was then added to selected tubes in increasing concentrations and the cells were challenged with OA (1 $\mu\text{g/ml}$) for 60 s. Incubation was terminated by centrifugation, and β -Hex activity was measured in supernatants. Each point represents the mean $\pm$ SEM of four independent experiments performed in triplicate. Insert: effect of WGA on A-23187-induced β -Hex release from nonsensitized MCP5/L mast cells. Each point represents the mean $\pm$ SEM of four triplicate experiments. **b** Sensitized mast cells labeled with 5 $\mu\text{Ci/ml}$ of [^3H]TdR were added to microplate wells coated with 10 $\mu\text{g/ml}$ of FN. After 5 min preincubation, the antigen (OA, final concentration 10 ng/ml) and WGA in increasing concentrations were added to appropriate wells. The plates were incubated for 30 min in a CO_2 incubator and the percentage of adherent cells was measured as described in the Methods. Each point represents the mean $\pm$ SEM of 10 independent experiments performed in duplicate. Broken line represents spontaneous adhesion of mast cells to FN. Insert: effect of WGA on A-23187-induced adhesion of nonsensitized MCP5/L mast cells to FN. Each point represents the mean $\pm$ SEM of triplicate experiment. **a**, **b** * $p < 0.05$, statistically significant difference from control.

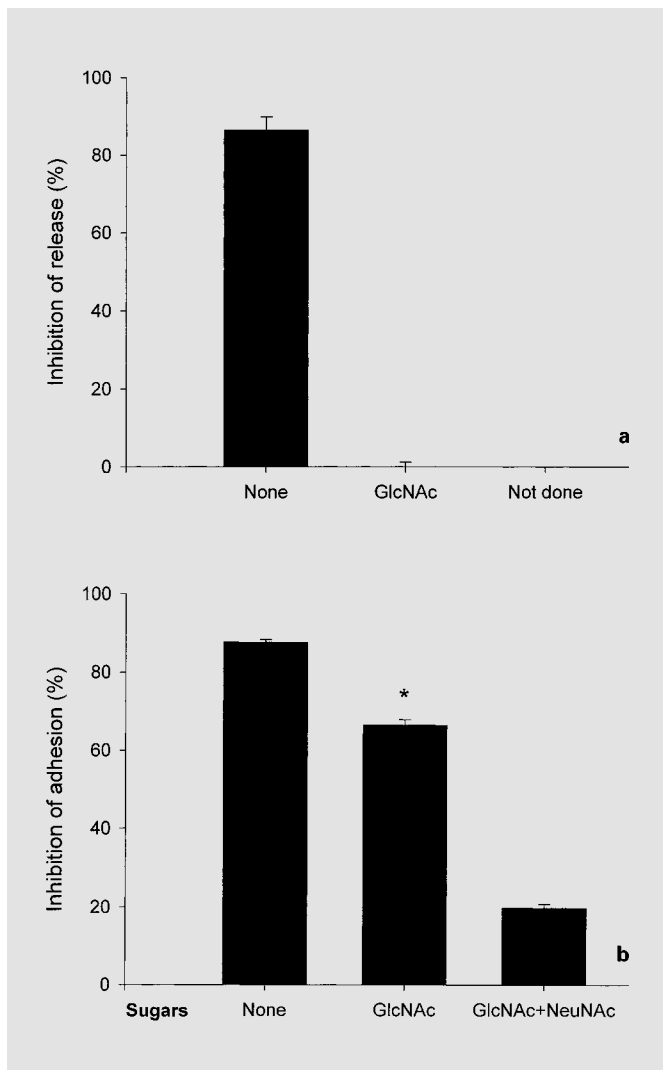
Fig. 2. Effect of WGA on PMA-induced mast cell adhesion to FN. MCP5/L mast cells labeled with 5 $\mu\text{g/ml}$ of [^3H]TdR were added to microplate wells coated with FN (10 $\mu\text{g/ml}$). After 5 min of preincubation, PMA (final concentration 50 ng/ml) and WGA in increasing concentrations were added to appropriate wells. The plates were incubated for 90 min in a CO_2 incubator and the percentage of adherent cells was measured as described in the Methods. Each point represents the mean $\pm$ SEM of the results of 6 independent experiments performed in duplicate. Broken line represents spontaneous adhesion of mast cells to FN. * $p < 0.05$, statistically significant difference from control.



1



2



MCP5/L mast cells, and this adhesion was increased significantly upon PMA-induced stimulation (table 1). WGA inhibited this adhesion dose dependently. Mast cell line C57 exhibited spontaneous and PMA-induced adhesion to FN similar to MCP5/L; however, this adhesion was only slightly inhibited by WGA (data not shown). When the interaction between HMC-1 and FN was examined, we observed that up to 80% of these cells spontaneously adhered to FN; this adhesion was not increased further when PMA was added and was not inhibited by WGA (data not shown).

WGA-Specific Sugars Abrogate Lectin-Induced Inhibition of Mast Cell Mediator Release and Adhesion to FN

We used GlcNAc and NeuNAc, sugars that are known to be recognized by WGA [14–16], to verify that WGA avidity to specific sugar moieties is involved in the observed inhibitory effect of WGA on mast cell functions.

Fig. 3. WGA-specific sugars reverse lectin-mediated inhibition of antigen-induced β -Hex release and adhesion to FN. **a** GlcNAc (final concentration 0.32 mM) was added to the mast cells just before the addition of WGA (20 μ g/ml) and the experiment of β -Hex release was performed as described in the legend to figure 1. **b** Sugars (GlcNAc or GlcNAc+NeuNAc in final concentration of every sugar 0.32 mM) were added to mast cells just before WGA (5 μ g/ml) and the adhesion experiment was performed as described in the legend to figure 1. Values represent the means $\pm$ SEM of the results of 10 independent experiments, each performed in duplicate. * $p < 0.05$, statistically significant difference from control.

Table 1. The effect of WGA on PMA-induced CFTL15 mast cell adhesion to FN

Treatment of mast cells	Adhesion, %				
	WGA, μ g/ml: 0	1	2	5	10
–	27.3 $\pm$ 0.6 ¹	n.d.	21.0 $\pm$ 1.5	20.1 $\pm$ 0.9	n.d.
PMA (50 ng/ml)	53.5 $\pm$ 4.1	42.0 $\pm$ 1.1	40.9 $\pm$ 2.2	18.3 $\pm$ 2.8	8.9 $\pm$ 0.5

CFTL 15 mast cells labeled with 5 μ g/ml of [^{3} H]TdR were added to microplate wells coated with FN (10 μ g/ml). After 5 min preincubation, PMA and WGA were added to appropriate wells in increasing concentrations. The plates were incubated for 90 min in CO₂ incubator and the percentage of adherent cells was measured as described in the Methods. Each result is the mean $\pm$ SEM of the results of two dependent triplicate experiments.

¹ Spontaneous adhesion.

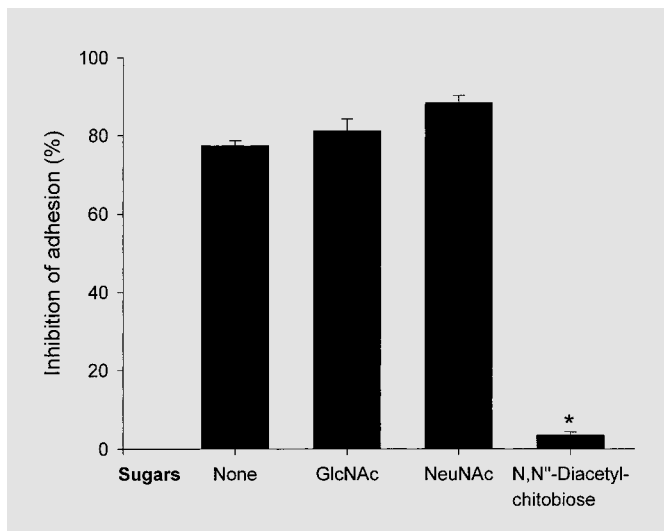


Fig. 4. N,N'-diacetylchitobiose reverses the inhibitory effect of WGA on PMA-mediated mast cell adhesion to FN. Sugars (GlcNAc and NeuNAc at a final concentration of 0.32 mM and N,N'-diacetylchitobiose at a final concentration of 1 mM) were added to the mast cells just before WGA (5 µg/ml) and the experiment of PMA-mediated mast cell adhesion to FN was performed as described in the legend to figure 2. Values represent the means $\pm$ SEM of the results of 9 independent experiments, each performed in duplicate. * $p < 0.05$, statistically significant difference from control.

Addition of GlcNAc to incubation media resulted in complete abrogation of WGA-mediated inhibition of β -Hex release from sensitized mast cells (fig. 3a). In antigen-induced adhesion experiments (fig. 3b), addition of GlcNAc to incubation media resulted in slight diminution of the inhibitory effect of WGA, whereas addition of both GlcNAc and NeuNAc resulted in complete abrogation of WGA-mediated inhibition of antigen-induced mast cell adhesion to FN. Thus, the inhibitory effect of WGA on Fc ϵ RI-mediated mast cell secretion and adhesion is sensitive to the presence of soluble lectin-specific sugars. We next verified whether WGA-induced inhibition of PMA-mediated mast cell adhesion is sensitive to the presence of specific sugars. As can be seen in figure 4, neither GlcNAc nor NeuNAc reversed the inhibitory effect of WGA, but N,N'-diacetylchitobiose, a potent inhibitor of the interaction of WGA with polysaccharides [16], abrogated the WGA-mediated inhibition. Thus, the inhibitory effect of WGA on PMA-mediated adhesion is sensitive to the presence of specific sugars, similarly to its effect on mast cell adhesion upon stimulation with antigen.

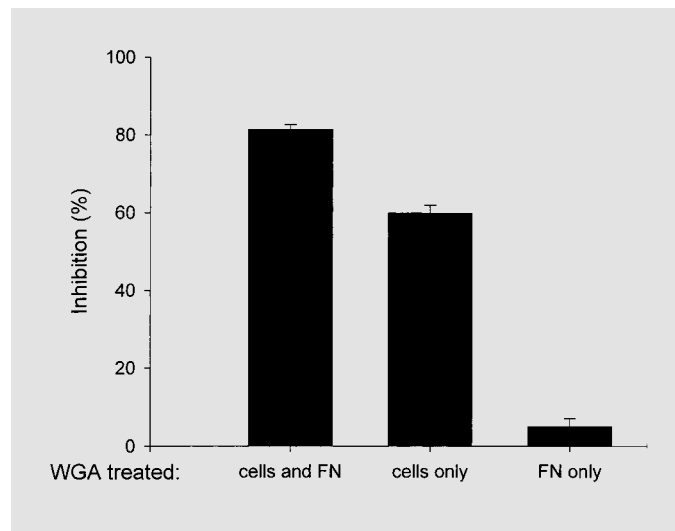


Fig. 5. Effect of WGA pretreatment of mast cells or adhesion ligand on mast cell adhesion. PMA-mediated mast cell adhesion to FN was tested in three experimental conditions: WGA-treated cells and FN: WGA at a concentration of 5 µg/ml was added simultaneously with PMA to mast cell suspension in assay wells coated with FN, as described in the legend to figure 2. WGA-treated cells only: mast cells were preincubated with WGA at final concentration of 5 µg/ml for 30 min in a CO₂ incubator, washed, resuspended in the media and then tested for PMA-mediated adhesion to FN as described in the legend to figure 2. FN only: the assay wells coated with FN were preincubated with WGA at a concentration of 5 µg/ml for 30 min in a CO₂ incubator, washed and then the adhesion assay with PMA-stimulated mast cells was performed as described in the legend to figure 2. Data represent the means $\pm$ SEM of four independent experiments, each performed in duplicate.

WGA Inhibition Depends on Its Interaction with Mast Cell Surface rather than with FN

FN is a glycoprotein and therefore it could not be ruled out that WGA inhibits mast cell adhesion interacting with adhesion substrate and not only with mast cell surface. To test this possibility, we performed adhesion experiments in which WGA was added either to the assay wells coated with FN or to the cell suspensions incubated in the test tubes. Following incubation, both cells and coated wells were washed with media to remove soluble WGA, and an adhesion experiment was performed. Pretreatment of FN-coated wells with WGA did not result in a decrease in PMA-mediated adhesion (fig. 5). In contrast, significantly less WGA-treated mast cells adhered to FN-coated wells as compared to control. This suggests that the inhibitory effect of WGA on mast cell adhesion to FN required interaction of WGA with mast cell surface rather than with adhesion ligand.

Discussion

The results presented in this paper clearly demonstrate that WGA not only inhibited antigen-induced MCP5/L mast cell mediator secretion (fig. 1a) but also antigen-mediated adhesion of these cells to FN (fig. 1b). Inhibition of mast cell mediator release may be explained by direct association of lectin with Fc ϵ RI, which prevents the antigen-mediated receptor aggregation, as has been shown previously for RBL-2H3 rat mast cells [19]. WGA inhibited mediator release in MCP5/L mast cells in concentrations similar to those reported for RBL-2H3 cells [19], and did not inhibit spontaneous mediator release (fig. 1a). In contrast, WGA inhibited antigen-mediated mast cell adhesion in significantly lower concentrations than those required to inhibit exocytosis, and inhibited spontaneous adhesion (fig. 1b). These differences suggest different mechanisms of WGA-mediated inhibition of mast cell secretion and adhesion. One possible explanation for the observed differences is interaction of WGA with mast cell receptors mediating adhesion. This is further supported by the observation that WGA strongly inhibits ionophore- and PMA-mediated mast cell adhesion to FN, processes that do not engage Fc ϵ RI (insert to fig. 1b, 2). Such explanation is also supported by analysis of sugar residues engaged in the inhibitory action of WGA on mast cell release and adhesion. Plant lectins are known for their ability to bind in a very specific fashion to mono- and oligosaccharide residues of cell membrane glycoproteins [13, 18], and WGA recognizes N-acetyl-*D*-glucosamine, N-acetyl-*D*-glucosamine oligomers and N-acetyl-neuraminic acid residues [16, 17]. While the inhibition of antigen-induced exocytosis was completely reversed by GlcNAc (fig. 3a), the inhibition of antigen-induced cell adhesion was reversed by the mixture of GlcNAc and NeuNAc (fig. 3b) and inhibition of PMA-induced adhesion was abrogated by N,N'-diacetylchitobiose (fig. 4). Thus, the inhibition of mast cell mediator release and inhibition of mast cell adhesion to FN by WGA are probably mediated by binding of WGA to different glycoproteins. While FN is also a glycoprotein and could interact with WGA, our data (fig. 5) suggest that WGA inhibited adhesion interacting with mast cell surface rather than with immobilized FN. Thus, most likely, the interaction of WGA with Fc ϵ RI is critical for inhibition of exocytosis, and WGA binding to FN receptors is critical for inhibition of adhesion.

Mast cell adhesion to FN is mediated in great part by a classical FN receptor, $\alpha_5\beta_1$ integrin [1–6, 8–12]. MCP5/L mast cell adhesion to FN, similarly to adhesion of bone-

marrow-derived mast cells, was inhibited by RGD peptides [4], suggesting that it is also mediated by this receptor. All integrins are glycoproteins [reviewed in ref. 26], and integrin FN receptor is recognized by WGA in vitro [14, 15, 27]. Although integrin-mediated adhesion to FN is based on interaction of the specific amino acid residues on α - and β -subunits with amino acid motifs on FN, the strength of binding may be modulated by various factors including glycosylation of integrin receptor [28–30]. This is consistent with our observation that WGA, which specifically recognizes carbohydrate part of the receptor, is capable of inhibiting integrin-dependent mast cell adhesion to FN.

WGA-mediated inhibition of mast cell adhesion was not limited to MCP5/L mast cells but was also observed with mouse mast cell line CFTL15 (table 1). Other mast cell lines tested (mouse mast cell line C57 and human mast cell line HMC-1) showed the lack of significant inhibition of adhesion to FN. It might be explained by possible differences in mast cell phenotype represented by these cell lines, which may include different glycosylation of adhesion receptors on cell surface.

Interaction of mast cells with extracellular matrix might be important for mast cell migration and location of these cells in tissues. It has also been shown that mast cell adhesion upregulates biological responsiveness of these cells [5, 31]. Thus, the ability of WGA to interfere with both IgE-dependent mast cell activation and integrin-mediated mast cell adhesion might suggest a potential inhibitory action of this lectin on mast-cell-mediated inflammatory processes. In addition, the ubiquity of WGA as dietary lectin may suggest the possibility of its effect on the physiology of the gastrointestinal tract [32, 33], which is rich in mast cells.

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