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Isolation and characterization of the potential receptor for wheat germ agglutinin from human neutrophils

Carlos Solórzano · Stéphane Bouquelet · M. Ali Pereyra · Francisco Blanco-Favela · Marie-Christine Slomianny · Raúl Chavez · Ricardo Lascurain · Edgar Zenteno · Concepción Agundis

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Abstract Neutrophils participate in host protection and central to this process is the regulation of oxidative mechanisms. We purified by affinity chromatography the receptor for the GlcNAc-specific WGA from CD14+ CD16+ cell lysates (WGAr). The receptor is a 141 kDa glycoprotein constituted by two subunits of 78 and 63 kDa. It is mainly composed of Ser, Asx, and Gly, and, in a minor proportion, His, Cys, and Pro. Its glycan portion contains GlcNAc, Gal, and Man; NeuAc and GalNAc were identified in a minor proportion. The amino acid sequence of the WGA receptor was predicted from tryptic peptides by MALDI-TOF, both subunits showed homology with cytokeratin type II (26 and 29% for the 78 and 63 kDa subunits, respectively); the 78 kDa subunit showed also homology with the human transferrin receptor (24%). Antibodies against WGAr induce higher oxidative burst than WGA, determined by NBT reduction; however, this effect was inhibited ($p < 0.05$) with GlcNAc suggesting that WGAr participates as mediator in signal transduction in neutrophils.

C. Solórzano
Departamento de Bioquímica, Instituto Nacional de Enfermedades Respiratorias, Tlalpan, 14080, Mexico

S. Bouquelet · M.-C. Slomianny
Laboratoire de Chimie Biologique de la Université des Sciences et Technologies de Lille, UMR du CNRS n° 8576, Villeneuve d'Ascq, 59655 France, Cedex

M. A. Pereyra · F. Blanco-Favela
Unidad de Investigación, Centro Médico Nacional Siglo XXI, IMSS, 10230 Mexico

R. Chavez · R. Lascurain · E. Zenteno · C. Agundis (✉)
Laboratorio de Inmunología, Departamento de Bioquímica, Facultad de Medicina, UNAM, PO Box 70159. 04510 Mexico, D F, Mexico
e-mail: agundis@bq.unam.mx

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Abbreviations

BSA	bovine serum albumin
WGA	wheat germ agglutinin
WGAr	wheat germ agglutinin neutrophils receptor
PMA	phorbol 12-myristate 13-acetate
FITC	fluorescein isothiocyanate
NBT	nitroblue tetrazolium
GlcNAc	N-acetyl-D-glucosamine
GalNAc	N-acetyl-D-galactosamine
Gal	galactose
Man	Mannose
Fuc	Fucose
NeuAc	sialic acid; *N*-acetyl-neuraminic acid

Introduction

Lectins are excellent tools for oligosaccharide characterization as well as for isolation of cellular populations. A number of studies have shown that lectin binding to cell surface carbohydrates triggers various biological effects [1]. Some lectins, such as phytohemagglutinin and concanavalin A, are able to stimulate T lymphocyte proliferation. These lectins bind to the carbohydrate portion of the T cell receptor, leading to its crosslinking, activation, and stimulation of lymphokine production and T cell proliferation [1,2]. Lectins have been widely used in fractionation of cellular subpopulations. By selective agglutination with peanut agglutinin (*Arachis hypogaea*), it is possible to purify cortical immature thymocytes [3]; T and B splenocytes have been fractionated with the

lectin from *Glycine max* [4]. *Helix pomatia* can be employed for the identification and isolation of T cells [5], and *Vicia villosa* agglutinin recognizes specifically lymphocytes bearing the CD8+ (cytotoxic) phenotype [6]. Moreover, some of these lectins are currently used to evaluate the immune status of patients [1].

The wheat germ agglutinin (WGA) contains two identical chains, both of which have high affinity for GlcNAc; moreover, the dimer $(GlcNAc)_2$ and trimer $(GlcNAc)_3$ containing oligosaccharides having 13 and 3700 times higher affinity, respectively, than the monomer. WGA binds with high affinity to internal GlcNAc residues in large oligosaccharides containing repeat sequences of Gal ß1-4GlcNAc ß1-3; sialic acid is involved only in low-affinity interactions with WGA [7,8]. Diverse studies confirmed that WGA possesses four hevein domains, which confer the chitin-binding property to several plant proteins, such as the class I endochitinases [9]; besides, those domains seem to be responsible for the serological cross-reactivity observed in serum of patients with latex or fruit allergies [10,11]. WGA induces NADPH-oxidase activity in human neutrophils by interacting with mobilizable receptors [12]. Recent findings indicate that *Helicobacter pylori* [13] and the lectin hevein [14], as well as the adhesin from members of the Pasteurellacea group [15], are potent activators of oxidative burst in neutrophils through specific interaction with sialic acid and GlcNAc-containing receptors, respectively, and this activity could induce pro-inflammatory processes that result in an immune response imbalance producing different pathologies [12,16]. These results might indicate that the WGA receptor (WGAr) could participate in activation of the oxidative burst process via cell surface glycosylated receptors [15,17]. Based on the aforementioned, this work was aimed at purifying a receptor for WGA in human neutrophils to understand better the role of neutrophil receptors in the regulation of neutrophils' biological activity.

Material and methods

Reagents

Polymorphprep density 1.113 (axis shield), red blood cell lysis buffer, RPMI-1640 culture medium, BSA fraction V, all sugars used in this study, WGA, PMA, Zymosan-A, FITC, NBT grade III crystalline, sodium azide, dimethyl sulfoxide, and octyl phenoxy polyethoxyethanol (Triton X-100) were purchased from Sigma Chemical Co. (St Louis, MO, USA); R-phycoerythrin (RPE)-conjugated mouse anti-human CD14 and CD16 monoclonal antibodies were from Serotec Ltd (Oxford, England); methanol and salts were acquired from J.T. Baker, Inc. (Phillipsburg, NJ, USA). WGA (3 mg) in 0.1 M carbonate buffer, pH 8.5, was conjugated to FITC. After 2 h of incubation at room temperature (22 ± 3°C), FITC-WGA was dialyzed against phosphate buffered saline (PBS: 0.01 M sodium phosphate, 0.14 M sodium chloride, pH 7.2) [18]. Final sample concentration was adjusted to 1 mg/ml, which was fractionated in aliquots and stored at −20°C until used.

Neutrophils

The cells were isolated from heparinized human peripheral blood by Polymorphprep density gradient centrifugation, 450 × *g* for 30 min, 18°C [19]. The granulocytes were treated with lysis buffer for 3 min at 37°C to remove red blood cells that were washed by centrifugation and the cellular pellet was suspended at a cell concentration of 4×10^6 cells/ml of RPMI-1640 culture medium. Cell population purity was assessed by flow cytometry.

Flow cytometry

The WGA-positive granulocytes were enumerated by Flow Cytometry Analysis in a FACS Excalibur, Becton Dickinson Co. (Mountain View, CA, USA). Suspended granulocytes (1×10^6 in 1 ml PBS) were incubated separately in FITC-WGA at concentrations of 0.1 to 10 μg, for 15 min at 4°C. Double stains were also performed through 30 min of incubation with 10 μl of FITC-WGA (diluted 1:1000) and, simultaneously, with 10 μl of RPE-mouse anti-human CD16 monoclonal antibodies (diluted 1:100) in PBS supplemented with 0.2% BSA and 0.2% sodium azide (PBS-BSA-A). After incubation at room temperature, stained granulocytes were washed twice in PBS-BSA-A. The cells were suspended in 1 ml of FACS flow solution and analyzed through flow cytometry, 10,000 cells were counted. The number of WGA positive granulocytes was determined in three different experiments using FITC-WGA.

Receptor purification

Purified neutrophils (10^8) were lysed in a solution of PBS (1 ml) containing 1 μg/ml aprotinin-A, 1 μg/ml pepstatin, 2 μg/ml leupeptin, 2 mM phenylmethylsulfonyl fluoride, and 1.0% (v/v) Triton X-100, for 30 min at 4°C under shaking. Nuclei, cell debris, and mitochondria were removed by centrifugation, first for 10 min at 250 × *g*, then for 30 min at 18,000 × *g*. Pellets were eliminated and the clear supernatant was loaded on a WGA-Sepharose 4B-CL (Pharmacia, Uppsala, Sweden) column (3 × 1 cm), equilibrated previously with 0.1% Triton X-100 in PBS (PBS-T) at 4°C. The unretained material was eluted with PBS-T and the fraction corresponding to the bound protein was eluted with 200 mM GlcNAc in PBS-T. The collected fractions were dialyzed against PBS and their absorbance was determined at A_{280}. Finally,

GlcNAc-eluted fractions were pooled, dialyzed against distilled water, and freeze-dried for further analysis.

Polyacrylamide gel electrophoresis

The molecular mass of the WGA receptor from human neutrophils was evaluated by 10% polyacrylamide gel electrophoresis (SDS-PAGE) using the Laemmli discontinuous buffer system [20]. Before electrophoresis, samples were treated with 50 μM 2-mercaptoethanol, boiled for 5 min, and gels were stained with 0.1% Coomassie brilliant blue G250 (Sigma). The molecular mass of the native purified receptor was determined by gel filtration chromatography on a column (100 $\times$ 1.6 cm) containing Sephacryl S-300 (Pharmacia), equilibrated with PBS at a flow rate of 12 ml/h. A_{280} was determined in 1-ml collected fractions. Relative molecular mass of the lectin was obtained by comparing the elution profile with molecular weight standards (Sigma).

Analytical methods

Protein was determined with the DC-protein assay kit according to manufacturer's instructions (Bio-Rad Laboratories, Hercules, CA, USA). Carbohydrate composition of the purified receptor was determined with the heptafluorobutyrate derivatives of methyl glycosides, obtained after methanolysis in 0.5 M methanol-HCl for 24 h at 80°C; lysine (Sigma) was used as internal standard. The samples were analyzed by gas-chromatography using a capillary column (25 $\times$ 0.32 mm) of 5% Silicone OV 210, (Applied Science Lab, Buffalo, NY, USA), in a Varian 2100 gas chromatograph (Orsay, France), as described by Zanetta et al. [21]. The amino acid analysis was performed in a 100 μg sample that was hydrolyzed under vacuum with 2 ml of 6 M HCl at 110°C in sealed tubes for 24, 48, and 72 h. The samples were analyzed on an automatic amino acid analyzer Durrum 500, according to Bidlingmeyer et al. [22], using norleucine as internal standard.

Peptide mass fingerprint

Peptide mass fingerprint of the WGAr was determined by matrix-assisted laser desorption ionization mass spectrometry-time of flight (MALDI-TOF) after trypsin digestion of the subunits identified through SDS-PAGE. After electrophoresis of the purified lectin receptor, the subunits were excised with a scalpel, and each band containing 200 pmoles of protein was digested with 0.5 μg trypsin (Promega sequencing grade, Uppsala, Sweden) in 100 μl ammonium bicarbonate, pH 8.0, at 37°C, for 24 h. The peptides were extracted with 60% acetonitrile containing 5% formic acid. The enzyme digest was then evaporated to dryness and re-hydrated with 10 μl of 0.1% trifluoroacetic acid (TFA). Peptides were desalted on Zip-Tip (Millipore) and eluted with HCCA matrix solution (10 mg/ml, α-cyano-4-hydroxy-trans-cinnamic acid, in 60% acetonitrile + 0.1% TFA) allowing the mixture to crystallize at room temperature. Positive ions of the peptides were measured by MALDI-TOF on a PerSeptive Biosystems Voyager Elite reflectron mass spectrometer (PerSeptive Biosystems, Framingham, MA, USA), equipped with a 337 nm UV laser. Mass spectra were acquired in reflectron mode under 20 kV acceleration voltage and positive detection. Control assays were performed using trypsin alone to identify self-digested peptide mass and with angiotensin I as standard (Mr 1296.7) [23,24]. The mass of the $[M + H]^+$ ions from peptides produced by tryptic digestion was compared with those obtained from the NCBInr (ProFound 2004/01/26) database.

Antibodies against WGAr

Antibodies against the purified WGA neutrophils receptor were obtained from immunized 8 weeks old CD-1 female mice (25 to 30 g weight), immunization was performed intraperitoneally with 200 μg of the purified receptor in 0.1 ml Freund's complete adjuvant (Sigma) and two boosters, through the same route, with 100 μg/0.1 ml incomplete adjuvant were applied at 10 days intervals. Mice were bled and the serum pooled until needed.

Granulocytes oxidative burst

Oxidative burst was tested in polymorphprep granulocytes, using an NBT-Zymosan solution, as follows: 0.2% NBT in isotonic saline solution was filtered through 0.45-μm pore Millipore membrane (Molsheim, France) and incubated with 0.1% Zymosan-A during 2 h at 37°C in water bath under gentle shaking. The assay was performed according to [25]. In brief, 100 μl suspended granulocytes (4×10^5 cells) and 100 μl culture medium were placed into each well of a 96-well polystyrene microtiter plate (Nunc Inc, Roskilde, Denmark) and incubated for 30 min at 37°C in a 5% CO_2 atmosphere to form a monolayer of adherent cells; 100 μl NBT-Zymosan per well were added. PMA (1 mg/ml), WGA, or antibodies against WGAr, at different concentrations in 5 μl/culture medium, was added to each well individually and incubated during 60 min at 37°C in a 5% CO_2 atmosphere. The supernatant was then removed and wells were washed two times thoroughly with 200 μl of 70% methanol and dried at room temperature to achieve total evaporation of methanol. Then, 120 μl 2 M KOH/well was added and incubated for 60 min more under gentle shaking followed by the addition of dimethyl sulfoxide at 140 μl/well. The content of the wells was mixed immediately and optical density from the developed color was read at 630_{nm} on an automatic ELISA reader (Labsystems, Multiskan MS, Finland), using as blank a well

without cells but containing Zymosan-NBT solution, and revealed as indicated. Control experiments were performed using 5 μl BSA (1 mg/ml). The specificity of the effect induced by the lectin was confirmed by using the WGA that had been preincubated for 30 min with different monosaccharides such as 200 mM NeuAc, Gal, Man, GalNAc, Glc, L-Fuc, D-Fuc or GlcNAc. Mann-Whitney U-test determined significant differences.

Results

WGA specificity for neutrophils

Double staining assays showed that those cells that bound WGA were also stained by anti-CD16 antibodies, indicating that they were neutrophils. Double fluorescence analysis showed that 75% of WGA-positive granulocytes were also CD16+ cells, confirming that they correspond to neutrophil polymorphonuclear cells (Figure 1).

Receptor purification

Neutrophils (10^8) purified by density gradient, rendered 1.67 mg of soluble protein after lysis. The glycoprotein recognized by WGA was purified from the neutrophils cell lysate by affinity chromatography. The WGA binding protein was eluted with 200 mM GlcNAc (Figure 2). The amount of purified protein was 50 μg, which corresponds to 2.6% of the cell lysate protein concentration. Lower than 200 mM GlcNAc concentrations or 0.5 M NaCl failed to elute the receptor from the WGA-Sepharose column, confirming the specificity in the interaction of the potential neutrophils receptor with the lectin (not shown).

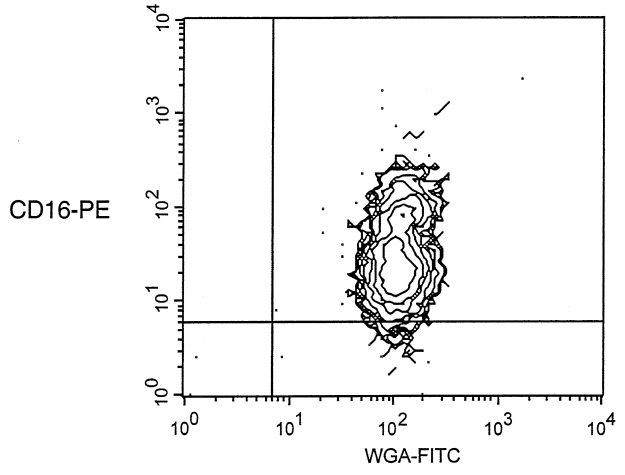

Fig. 1 Flow cytometric analysis of human peripheral blood granulocytes stained with FITC- WGA-FITC and CD-16.

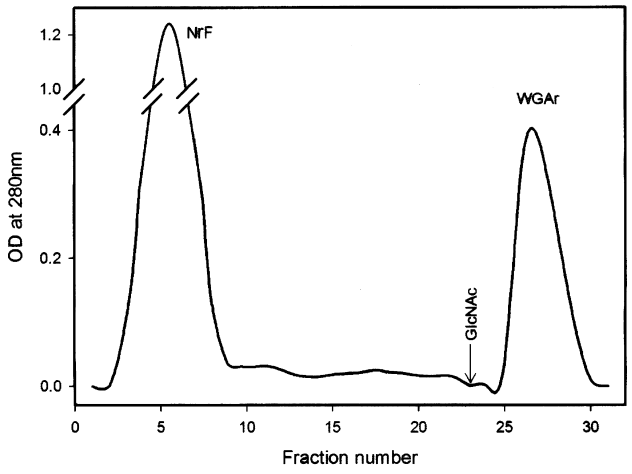

Fig. 2 Purification of the potential receptor for WGA from human neutrophils. Leukocytes lysate was purified on a WGA-agarose column. The unretained fraction (Nrf) was eluted with PBS-T (1% TritonX-100) and the affinity purified receptor (WGAr) was eluted by adding 200 mM GlcNAc in PBS-T. Optical density A_{280} was determined in fractions dialyzed previously against PBS.

Chemical characterization

Electrophoretic analysis of the affinity purified WGA-receptor from human neutrophils showed two bands of 78 and 63 kDa (Figure 3). Control assays using WGA, showed a

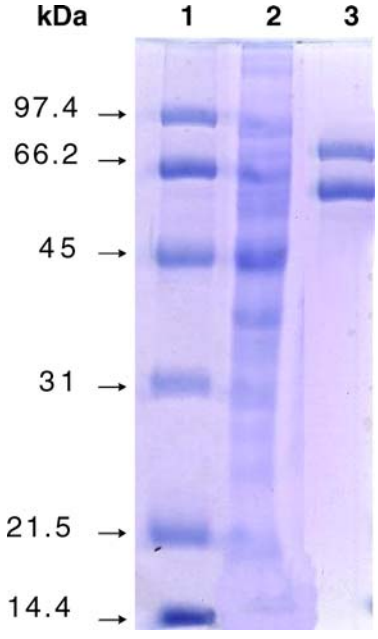

Fig. 3 SDS-PAGE of the purified WGA-neutrophils receptor. Lane 1, molecular weight markers. Lane 2, 50 μg of neutrophils cell lysate. Lane 3, 10 μg of purified WGA-neutrophils receptor eluted with 200 mM GlcNAc. The molecular weight markers are: myosin (205 kDa); ß-galactosidase *Escherichia coli* (116 kDa); phosphorylase B (97 kDa); fructose-6-phosphate kinase (84 kDa); bovine serum albumin (66 kDa); glutamic dehydrogenase (55 kDa); ovoalbumin (45 kDa); glyceraldehyde-3-phosphate dehydrogenase (36 kDa).

Table 1 Amino acid composition of the potential human neutrophils receptor for wheat germ agglutinin

Amino acid	Residues per molecule*
Aspartic acid	106.4
Glutamic acid	84.3
Serine	280.0
Glycine	92.8
Histidine	12.0
Arginine	13.0
Threonine	72.4
Alanine	92.6
Proline	81.8
Tyrosine	19.1
Valine	48.1
Methionine	18.0
Cysteine	5.7
Isoleucine	72.2
Leucine	62.3
Phenylalanine	64.5
Lysine	48.6
Total	1173.8

*Considering 141 kDa and that the potential receptor contained 10% sugar by weight.

protein band of 28 kDa (not shown), confirming that the 78 and 63 kDa subunits correspond to the WGA-receptor. The purified receptor is a 141 kDa protein as determined by gel filtration chromatography on Sephacryl S-300. The WGA-receptor contained 10% of sugars by weight, it is composed mainly by Ser, Asx, and Gly; His, Cys, and Pro are present in a minor proportion (Table 1). Its glycan portion contains GlcNAc, Gal, and Man; while NeuAc, and GalNAc were found in minor proportions (Table 2).

Table 2 Carbohydrate composition of the potential neutrophils receptor for wheat germ agglutinin

Carbohydrate	Residues per molecule*
Man	23.4
Gal	19.5
GlcNAc	19.1
GalNAc	3.1
NeuAc	8.6
Total	73.7

*Considering 141 kDa and that the potential receptor contained 10% sugar by weight.

Peptide mass fingerprint

Tryptic digestion of the purified receptor yielded 81 and 74 peptidic fractions for the 78 and 63 kDa subunits, respectively. The m/z of the identified fractions ranged from 791.3 to 3478.3 for the 78 kDa subunit and from 714.2 to 3655.9 for the 63 kDa subunit of the WGA-neutrophil receptor. The molecular mass of the $[M^+H]^+$ ions from the MALDI-TOF spectrum of digested fractions was compared with the relative values obtained from the NCBInr (ProFound 2004/01/26) database. The identified peptides of the 78 kDa WGA-receptor subunit covered 26% of the amino acid sequence of the cytokeratin type II (Access number gi|1346343|sp|P04264|K2C1) and 24% of the human transferrin receptor, and the 63 kDa subunit covered 29% of the amino acid sequence of cytokeratin type II (Table 3 an 4).

Table 3 Predicted amino acid sequence from tryptic peptides of the 78 kDa subunit of the potential WGA-neutrophils receptor determined by MALDI-TOF.

The molecular ions from the LSIMS spectrum of the tryptic digest were identified by comparing their relative values with those obtained from the NCBInr (ProFound 2004/01/26) database. Matched peptides with the highest score corresponded to human cytokeratin type II (Access number gi|1346343|sp|P04264|K2C1) (26% homology) and human transferrin receptor (24%).

m/z	Residue	No	Sequence
831.485	75	82	SISISVAR
1032.509	484	492	TLLEGEESR
1178.594	377	386	YEELQITAGR
1276.707	473	483	LALDLEIATYR
1301.709	344	355	SLDLDSIIAEVK
1301.709	393	403	NSKIEISELNR
1474.752	212	223	WELLQQVDTSTR
1474.752	200	211	FLEQQNQVLQTK
1637.843	186	199	SLNNQFASFIDKVR
1656.778	13	29	SGGGFSSGSAGIINYQR
1992.943	224	239	THNLEPYFESFINNLR
2020.913	625	644	SSGGSSSVRFVSTTYSGVTR
2149.043	224	240	THNLEPYFESFINNLRR
2285.099	367	386	AEAESLYQSKYEELQITAGR
2382.901	519	549	GGGGGGYGSGGSSYGSGGGSYGSGGGGGGGR
2931.476	200	223	FLEQQNQVLQTKWELLQQVDTSTR

Table 4 Predicted amino acid sequence from tryptic peptides of the 63 kDa subunit of the potential WGA-neutrophils receptor determined by MALDI-TOF

m/z	Residue	No	Sequence
1059.564	225	233	TLLDIDNTR
1156.591	251	261	QGVDADINGLR
1231.595	14	29	SGGGGGGGLGSGGSIR
1234.568	47	59	FSSSSGYGGGSSR
1790.728	491	513	GGSGGSYGGGGSGGGYGGGSGSR
1836.970	375	390	HGVQELEIELQSQLSK
1850.934	322	336	TLNDMRQEYEQLIAK
2704.124	64	95	GGGGSFGYSYGGGSGGGFSASSLGGGFGGGSR
2901.430	200	224	NYSPYYNTIDDLKDQIVDLTVGNNK

The molecular ions from the LSIMS spectrum of the tryptic digest were identified by comparing their relative values with those obtained from the NCBInr (ProFound 2004/01/26) database. Matched peptides with the highest score corresponded to human cytokeratin type II (Access number gi|1346343|sp|P04264|K2C1) (29% homology).

Granulocytes oxidative burst

Granulocytes were stimulated with 1 μg WGA per 1×10^5 cells, as optimal dose, and serum from mice immunized with the WGA-neutrophils receptor. WGA, Anti-WGAr and PMA produced 1.5 and 2.1 times more oxidative response than granulocytes stimulated with WGA; however, production of NBTr was significantly higher ($p < 0.01$) than in neutrophils stimulated with BSA, as control (Table 5). The sugar specificity of WGA was tested by inhibiting the production of NBTr by neutrophils stimulated with WGA preincubated with monosaccharides and only 200 mM GlcNAc showed significant effect when compared with WGA-stimulated cells. The effect of GlcNAc on WGA was not statistically different from that obtained in neutrophils incubated in the presence of BSA (Table 5). Other sugars at identical concentrations, such as NeuAc, Gal, Man, GalNAc, Glc, L-Fuc and D-Fuc, showed no significant effect on WGA or anti-WGA antibodies' capacity to activate neutrophils oxidative burst.

Table 5 Nitroblue tetrazolium reduction (NBTr) by human peripheral blood granulocytes

Stimulation	Concentration (μg/4 $\times$ 10^5 cells)	nmoles NBTr
BSA	18	1.7 ± 0.7
PMA	5	5.9 ± 0.8*
WGA	1	4.7 ± 1.1*
WGA+ GlcNAc	1	2.4 ± 0.4
WGA+ WGAr	1	1.6 ± 0.5
Anti-WGA	dil 1:128	5.4 ± 0.2
Anti-WGA+GlcNAc	dil 1:128	3.2 ± 0.4*

Numerical values represent the mean $\pm$ SD of four experiments.
*Significant difference ($p < 0.05$) determined by Mann-Whitney U-test as compared with BSA-treated cells. Phorbol myristate acetate (PMA) was used as a control of positive stimulation. GlcNAc at 200 mM concentration. Sugars that showed no effect on WGA or anti-WGA antibodies at 200 mM were: NeuAc, Gal, Man, GalNAc, Glc, L-Fuc, and D-Fuc; moreover, none of these carbohydrates showed any effect on the WGA or the antibodies against WGAr induced activation.

Discussion

WGA recognizes neutrophil polymorphonuclear cells ($CD16^+$). Our studies indicate that WGA-mediated activation of oxidative burst is exerted by the interaction with specific membrane receptors through non-opsonic mechanisms. This biological property was dependent on the specific recognition of GlcNAc receptors, as demonstrated by the reversal of the WGA effect on neutrophils by treatment with GlcNAc. WGA recognition of neutrophils can be explained by the fact that neutrophils have high density of non-reducing terminal GlcNAc residues [16]. It has been shown that WGA binds tightly to cytochrome *b* via the $gp91^{phox}$ glycan, WGA treatment induces changes in membrane cardiolipin, increasing permeability transition and favoring mitochondrial changes through intracellular trafficking signals; although, the initiation mechanisms responsible for this activation are not fully understood [26].

We purified by affinity chromatography the potential receptor for WGA from CD16+ cell lysates. The purified receptor (WGAr) corresponded to 2.6 % of the cell lysate protein. The WGAr is a glycoprotein of 141 kDa, composed of two subunits of 78 and 63 kDa. It is mainly composed of Ser, Asx, and Gly; in minor proportion His, Cys and Pro; its glycan portion, which comprises 10% of the total weight, contained GlcNAc, Gal, and Man, and in minor proportions, NeuAc and GalNAc. Peptide mass fingerprint of the WGAr was determined from tryptic peptides by MALDI-TOF, peptides from 78 and 63 kDa subunits matched with cytokeratin type II (26 and 29%, respectively) and the 78 kDa subunit peptides also matched with the human transferrin receptor (24%). Cytokeratin type II subgroup of the intermediate filament proteins family has been shown to modulate specific

signal transduction pathways involved in the control of relevant functions, such as epithelial cell growth and apoptosis [27]. The transferrin receptor is a 90 kDa type II membrane protein that is expressed as a homodimer, Parker *et al*. [28] reported that canine parvovirus and feline panleukopenia virus bind to the human and feline transferrin receptors that are used to infect cells. Although studies on the mechanisms that induce the oxidative burst in granulocytes by WGA are still in progress, previous reports have suggested that the oxidative burst induced by stimulation of the receptor for WGA is dependent on microfilaments and serine protease functions [29] similarly to PMA (a membrane perturbating agent).

The antibodies against the WGA-receptor induced oxidative burst in neutrophils, this effect was higher than that obtained with WGA, suggesting the presence of GlcNAc-containing receptors in neutrophils, probably due to higher affinity constants, since antibodies showed higher affinity for specific ligands than for lectins. The modification of proteins by GlcNAc has been suggested to play a role in the regulation of a variety of signal transduction pathways in neutrophils, such as enhanced motility and directional migration induced by chemo-attractants. This sugar residue seems to modulate the activities of signaling intermediates known to regulate neutrophils movement. It has been shown that WGA is internalized via a coated small-pit vesicle pathway and reaches vacuoles and endosomes [30]. This could indicate that the proposed WGA receptor participates in activation of the oxidative burst process via cell surface molecules by intracellular signaling [31].

Considering that several authors suggested that GlcNAcylation of membrane proteins is an important signaling element in neutrophils and that WGA interacts with specific GlcNAc-containing receptors in neutrophils, it could be inferred that GlcNAc is expressed on the surface of these cells in a particular activation or maturation state, since earlier works demonstrated by Western-blot analysis that WGA binds to several proteins in the membrane of activated neutrophils [12]. Modification of proteins by GlcNAc is suggested to play a role in the regulation of a variety of signal transduction pathways in neutrophils [31]; besides, GlcNAcylation of membrane proteins is an important signaling element to regulate neutrophil movement and activation to release pro-inflammatory molecules [32]. Our results indicate that WGA induced activation of neutrophils' oxidative burst similarly to hevein, the allergenic lectin present in rubber latex, offering a model to study such a unique and previously untested pathway that includes neutrophils in allergic diseases [9–11,14].

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