GLYCOPROTEIN RECEPTORS TO WHEAT GERM AGGLUTININ ON THE SURFACE OF MURINE MACROPHAGES

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Peritoneal macrophages elicited in various ways exhibited marked wheat germ agglutinin (WGA)-dependent cytolytic activity against MM46 cells [nearly 100% lysis at effector to target ratio (E/T) = 40], while macrophage cell lines J774.1 and WEHI-3 cells showed less activity (−25% at E/T = 0.63–2.5) and no cytolysis (E/T = 40), respectively. Studies on the kinetics of WGA-binding showed that peritoneal macrophages had an association constant (Kₐ) of 0.09–0.18 × 10⁶ M⁻¹ and the number of their binding sites (N) was 2–7.7 × 10⁷/cell, while the Kₐ and N values of J774.1 and WEHI-3 cells were 1.2 × 10⁶ M⁻¹ and 4.1 × 10⁷/cell and 0.6 × 10⁸ M⁻¹ and 5.4 × 10⁷/cell, respectively. On the other hand, cytolytic target MM46 cells showed values of 0.27 × 10⁶ M⁻¹ and 1.8 × 10⁷/cell. Lower affinities and moderate numbers of binding sites coincided with the efficient WGA-dependent cytolytic activities of the effector cells.

The surface of peritoneal macrophages and J774.1 and MM46 cells was iodinated, the cells lysed, and the lysates fractionated on a WGA-Sepharose column and the WGA-binding surface-glycoproteins in GlcNAc-specific fractions of the eluate were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Peritoneal macrophages gave three major bands of 153 kilo dalton (kDa), 140 and 90 kDa proteins, whereas J774.1 and MM46 cells both gave 6 bands of very similar molecular weights and intensities that were quite unlike those of peritoneal macrophages.

These results suggest that the three major WGA-binding glycoproteins on peritoneal macrophages are involved in adherence and the lytic process in WGA-dependent macrophage-mediated cytolysis.

Keywords — wheat germ agglutinin (WGA); macrophage; cytolysis; receptor

INTRODUCTION

Monocyte-macrophage lineage cells are thought to constitute one of the components of the host defense mechanism against cancer. We have investigated mediator-dependent macrophage-mediated tumor cell lysis in vitro.¹⁻³ An antibody specific to tumor-associated antigen or some selected lectins, such as wheat germ agglutinin (WGA), can act as a mediator. An essential step in lectin-dependent macrophage-mediated cytolysis (LDMC) is sugar-specific recognition by lectins of glycoconjugates on both tumor cells and macrophages and the subsequent binding between both cells results in tumor cell lysis.¹) However, these molecules on the cell surface have not been identified and the cytolytic mechanism of LDMC is still not clear.

There have been many studies on the interactions of lectins with animal cells, including immunocompetent cells. The lectin-binding molecules on cell surfaces, which probably transfer signals across the membrane have been characterized in attempts to elucidate the molecular mechanism of transmembrane control of cell functions.⁴⁻⁶ In LDMC, the lectin-initiated binding of macrophages to tumor cells is an essential step. Moreover, the triggering of surface molecules, presumably involved in the lytic mechanism, by appropriate lectins such as WGA is essential for inducing the cytolytic function of macrophages. Thus, characterization of the WGA-binding molecules on macrophage should be helpful in clarifying the mechanism of LDMC.

In this report, we compared the activities in WGA-dependent tumor cell lysis of various elicited peritoneal macrophage preparations with that of murine macrophage-line cells, studied the binding kinetics of WGA to these cells and characterized the WGA-binding surface glycoproteins. The possibility that these glycoproteins are responsible for the cytolytic action of macrophages is discussed.
MATERIALS AND METHODS

Animals and Cell Lines — Male C3H/He mice were purchased from Shizuoka Experimental Animal Farm (Shizuoka, Japan). Mice were used at 8–11 weeks of age. The macrophage cell lines J774.1 and WEHI-3, which were originally derived from BALB/c mice, were grown in RPMI-1640 medium (Nissui Seiyaku Co., Tokyo) supplemented with 100 units/ml of penicillin (Banyu Pharmaceutical Co., Tokyo), 100 units/ml of streptomycin (Meiji Seika Co., Tokyo) and 10% fetal calf serum (FCS, Flow Laboratories, Stanmore, N. S. W., Australia).

Tumor Cells — MM46, a transplantable ascites tumor from a spontaneous mammary carcinoma in a C3H/He mouse, was passaged weekly in the peritoneal cavity of syngeneic C3H/He male mice and was used 6–8 d after transplantation.

Preparation of Peritoneal Exudate Cells — Mice were treated intraperitoneally (i.p.) 4 d, with 1 ml of 10% proteose peptone (DIFCO Laboratories, Detroit, MI, U.S.A.) in saline, 1 ml of thioglycolate broth (DIFCO Laboratories, Detroit) or 0.2 ml of 0.5 mg/ml of Bacillus Calmette Guerin (BCG, Japan BCG Laboratory, Tokyo) in saline, 24 h with 0.4 ml of 1% glycogen (Wako Pure Chemicals, Osaka) in saline, or 6 h with 2 ml of 12% sodium casein (Tokyo Chemicals, Tokyo) in saline and the peritoneal exudate cells (PEC) obtained were designated PP-PEC, TG-PEC, BCG-PEC, glycogen-PEC and casein-PEC, respectively. Unless otherwise indicated, the PEC were recovered after an intraperitoneal injection of 5 ml of cold RPMI-1640 medium and the cells were washed several times with RPMI-1640 medium by centrifugation at 300 × g for 5 min. The washed PEC were cytocentrifuged and stained with Giemsa solution to determine the percentages of macrophages among nucleate cells. The percentages were 80 to 90% for PP-PEC, TG-PEC, BCG-PEC and glycogen-PEC but 20% for casein-PEC.

Lectin and Affinity Absorbent — WGA obtained from E-Y Laboratories (San Mateo, CA, U.S.A.) was dissolved in phosphate-buffered saline (PBS, 137 mM NaCl, 2.6 mM KCl, 8.1 mM Na₂HPO₄ and 1.5 mM KH₂PO₄, pH 7.4) and stored at −80 °C until use. WGA-Sepharose 6 MB (about 5 mg lectin per 1 ml of gel) was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden).

Cytolysis Assay — LDLC was assayed by measuring ⁵¹Cr release from labeled target MM46 tumor cells, as described previously.¹ PE C suspended in RPMI-1640 medium supplemented with 1% FCS (FCS-RPMI) and 5 × 10³ ⁵¹Cr-labeled MM46 tumor cells were mixed in wells of flat-bottomed 96-wells microtiter plates (Nunc, OK4000 Roskilde, Denmark). After addition of a lectin solution, the mixtures were incubated in 0.25 ml of FCS-RPMI for 15 h at 37 °C under an atmosphere of 5% CO₂ in air. Then the plates were centrifuged at 450 × g for 5 min and the radioactivity of the supernatant fluids was measured. Cytotoxic activity was expressed as follows:

\[
\% \text{ cytolysis} = \left( \frac{\text{experimental cpm} - \text{control cpm}}{\text{maximum releasable cpm} - \text{control cpm}} \right) \times 100
\]

The maximum release of ⁵¹Cr, measured after freezing and thawing labeled tumor cells four times and then subjecting the cells to hypotonic treatment, was equivalent to 70 to 80% of the total cell-associated radioactivity. The control count was measured as the radioactivity released from labeled cells alone with or without the presence of a lectin.

Iodination of WGA — WGA was iodinated with Na¹²⁵I (New England Nuclear, Boston, MA., U.S.A.), using chloramine-T by the method of Hubbard and Cohn² with slight modifications.¹²⁵I-Labeled WGA was separated from free ¹²⁵Iiodine by gel filtration in PBS. The ¹²⁵I-labeled WGA solution obtained, with a specific radioactivity of 1.2 × 10⁶ cpm/μg protein, was stored at −20 °C until use. An experiment for dose-related response revealed that ¹²⁵I-labeled WGA retained more than the half of LDLC activity of the untreated lectin.

WGA-Binding Assay — Binding reactions were carried out in plastic counting tubes that had been soaked for at least 2 h in 2 ml of 2 mg/ml bovine serum albumin. The reaction mixture contained approximately 1 × 10⁶ cells in 0.5 ml of FCS-RPMI supplemented with 10 mM Na₂-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES, pH 7.4) in the presence of various concentrations of ¹²⁵I-labeled WGA with or without 0.2 M N-acetylglucosamine (GlcNAc). After incubation for 2–3 h in an ice
bath, the mixture was centrifuged and 50 μl of the supernatant fluid were reserved for measurement of the free 125I-labeled WGA concentration, while the cells were washed 4 times with 2 ml of cold PBS. The amount of cell-bound radioactivity was calculated from the radioactivity of the precipitated cells in the tube minus the background count of the tube after removal of the cells by washing. In each case, experiments were carried out in triplicate. Specific binding of radioactivity was calculated as the cell-bound radioactivity in the absence of GlcNAc minus that in the presence of GlcNAc.

**Labeling and Solubilization of Cells** — Cells were radiiodinated by the method of Hubbard and Cohn3 with slight modifications. A suspension of 4 to 10 × 10⁷ cells in 1 to 2 ml of PBS was mixed with 1 unit of lactoperoxidase (Sigma Chemical Co., St. Louis, MO., U.S.A.) and 200 μCi of Na¹²⁵I. Then 2 units of glucose oxidase (grade II; Boehringer-Mannheim GmbH, W. Germany) and 5 μmol of glucose were added. The mixture was incubated for 30 min at 37°C with continuous shaking and then 5 to 10 ml of cold PBS containing 0.5 mg Na₂S₂O₃ and 20 mg KI were added to stop the reaction. The labeled cells were then washed 5 times with cold PBS by centrifugation at 3000 × g for 5 min, and resuspended in 1 ml of 10 mM tris(hydroxymethyl)-amino methane buffer (Tris, pH 7.0) containing 0.2% Triton X-100 and 1 mM phenylmethanesulfonyl fluoride (PMSF). The cell suspension was mixed vigorously and incubated for 1 to 2 h at 4°C. The extract was obtained by centrifuging the mixture at 15000 × g for 15 min at 4°C.

**Isolation of WGA-Binding Proteins** — WGA-binding proteins were isolated by a method similar to that described by Iwata et al.5 Briefly, a Triton X-100 extract was applied to a WGA-Sepharose 6 MB column (0.9 × 7 cm) equilibrated with 10 mM Tris–HCl (pH 7.0) buffer containing 0.2% Triton X-100. The first peak was eluted with the same buffer at room temperature and when the radioactivity of the eluate had decreased to the background level, the elution buffer was changed to a buffer containing 0.5 M glucose, as a nonhaptenic sugar, and then to a buffer containing 0.5 M GlcNAc, as the haptenic sugar. The fractions eluted with the haptenic sugar were pooled and dialyzed alternately against absolute ethanol and distilled water several times at room temperature in Spectrapor membranes (Spectrum Medical Industries, Inc., Los Angeles, CA., U.S.A.) to remove the detergent. After extensive dialysis, the pooled fractions were evaporated and analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

**SDS-PAGE** — The evaporated samples were dissolved by boiling for 3 min in 125 mM Tris–HCl (pH 6.8) containing 5% SDS, 10% glycerol, 2 mM PMSF and 5% 2-mercaptoethanol and then promptly subjected to electrophoresis by the method of Laemmli8 on 2 mm thick 10% polyacrylamide separating gel with 4% stacking gel. After electrophoresis, gels were stained with Coomassie Brilliant Blue R-250. The dried gels containing radiiodinated samples were exposed to Kodak ARX-5 X-ray film with an intensifying screen and developed by a commercial X-ray film process. Molecular weights were determined by comparison with standard proteins (Pharmacia Fine Chemicals, Uppsala, Sweden) which were subjected to electrophoresis simultaneously with samples.

**Protein Assay** — Protein was measured by the method of Lowry et al.9 with bovine serum albumin as the standard.

**RESULTS**

**WGA-Dependent MM46-Cytolysis by Various Macrophage Preparations**

We studied the WGA-dependent tumor cytolitic activities of variously elicited peritoneal macrophages and macrophage cell lines by in vitro assay of ⁵¹Cr release using MM46 mammary cells as target cells (Figs. 1 and 2). Four different peritoneal macrophage preparations, glycogen-PEC, PP-PEC, TG-PEC and BCG-PEC, exhibited similar cytolytic activities; maximal cytolsis (90–100% cytolsis) was achieved when the effector to target ratio (E/T) was increased to 40–160. The casein-PEC preparation had less cytolytic activity, causing 11% cytolsis at an E/T of 160 (Fig. 1). In the absence of WGA, a slight direct cytolsis was observed with BCG-PEC but not with other preparations, even at an E/T of 160 (Fig. 1). The adherent cell fractions for all the above PEC preparations except for casein-PEC exhibited similar potentials for WGA-dependent cytolsis as shown in Fig. 1 (data not shown).

On the contrary, the macrophage cell lines, J774.1 and WEHI-3, both exhibited restricted
cytolysis of MM46 target cells (Fig. 2). At 10 μg/ml of WGA, maximal cytolysis (about 20%) was obtained at a low E/T ratio of 1 to 5, while a higher E/T ratio of 40 repressed cytolysis completely. The reason for repression of cytolysis at a high E/T ratio is unknown but the results show that both J774.1 and WEHI-3 cells have only a limited ability to mediate WGA-dependent cytolysis of MM46 tumor cells.

**WGA-Binding Assay**

In LDMC, the binding of effector cells to target cells through recognition of the surface sugar moiety by WGA is an essential step in cytolysis.1 We studied the characteristics of the binding of WGA to the cells concerned. The results of binding experiments as values for specific binding of WGA to cells are shown in Fig. 3. At a WGA concentration of 2.8 × 10⁻⁷ M (10 μg/ml), which can effectively induce the LDMC reaction (cf. Fig. 1), the amount of specific binding of WGA varied with the cells, 1.9, 1.3, 1.1, 0.5 and 0.2–0.3 × 10⁻¹⁷ mol WGA/cell for MM46, J774.1, WEHI-3, casein-PEC and four peritoneal macrophage preparations, respectively.

**FIG. 1. WGA-Dependent Cytolytic Activity of Various Elicited PECs**

Glycogen-PEC (○ , ●), PP-PEC (▽ , ▼), TG-PEC (□ , ■), BCG-PEC (△ , ▲) and casein-PEC (◇ , ◆) were obtained as described in Materials and Methods and their cytolytic activity on MM46 tumor cells was assayed in the presence (open symbols) or absence (closed symbols) of WGA (10 μg/ml).

**FIG. 2. WGA-Dependent Cytolytic Activity of Macrophage Line Cells**

The cytolytic activity of J774.1 cells (□ , ■) and WEHI-3 cells (△ , ▲) on MM46 tumor cells was assayed in the presence (open symbols) or absence (closed symbols) of WGA (10 μg/ml). Data for glycogen-PEC (○ , ●) are shown for comparison.

**FIG. 3. Binding of WGA to Cell Surfaces**

The amounts of GlcNAc-specific binding of WGA to MM46 cells (▲), J774.1 (○), WEHI-3 (●), casein-PEC (△), TG-PEC (▽), BCG-PEC (▼), PP-PEC (□) and glycogen-PEC (■) were measured as described in Materials and Methods.
### TABLE I. Scatchard Analysis of WGA-Binding to Cells

<table>
<thead>
<tr>
<th>Class</th>
<th>$K_a$ ($\times 10^6$ M(^{-1}))(^a)</th>
<th>N ($\times 10^8$/cell)(^b)</th>
<th>LDMC(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Glycogen-PEC (macrophage)</td>
<td>0.18</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>PP-PEC (macrophage)</td>
<td>0.10</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>TG-PEC (macrophage)</td>
<td>0.12</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>BCG-PEC (macrophage)</td>
<td>0.09</td>
<td>0.77</td>
</tr>
<tr>
<td>II</td>
<td>MM46</td>
<td>0.27</td>
<td>1.8</td>
</tr>
<tr>
<td>III</td>
<td>J774.1</td>
<td>1.2</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>WEHI-3</td>
<td>0.6</td>
<td>0.54</td>
</tr>
<tr>
<td>IV</td>
<td>Casein-PEC (PMN)</td>
<td>1.4</td>
<td>0.14</td>
</tr>
</tbody>
</table>

*Values were calculated from Scatchard plots of the data in Fig. 3.*

\(^a)\) Affinity constant.  
\(^b)\) Number of binding sites.  
\(^c)\) Activity of LDMC scored from the results in Figs. 1 and 2.  
\(^d)\) Not determined.

The binding data are shown in Fig. 3 and, by calculation from Scatchard plots of these data, the number of binding sites per cell and the apparent association constant for WGA-binding are summarized in Table I. Because \(^{125}\)I-labeled WGA did not fully retain LDMC activity of the untreated lectin (more than the half), the iodination must affect the binding profile of WGA molecule. Therefore, the data are approximate or relative estimates. From these kinetic analyses, these PEC preparations and cell lines could be classified into four distinct classes. Class I consists of glycogen-PEC, PP-PEC, TG-PEC and BCG-PEC which showing low apparent affinity and a moderate number of binding sites for WGA with slight differences between preparations. Class II consists of MM46 cells which have similar apparent affinity for WGA as those of class I cells but with more than three times the binding sites. Class III consists of macrophage cell lines J774.1 and WEHI-3 with 3- to 10-fold greater apparent affinity for WGA than class I but with a similar number of binding sites. Class IV consists of casein-PEC which contained 80% of polymorphonuclear cells showing unique WGA-binding kinetics with the same high affinity of class III but with the same or fewer number of binding sites as class I cells.

**Comparison of WGA-Binding Surface Glycoproteins**

Lectin-induced transmembrane control of cell function is thought to be mediated by a glycoprotein(s) integrated externally into the cell membrane that is recognized by the lectin. Although some glycolipids may also have a WGA-binding capacity, the functional sites recognized by WGA are probably glycoprotein(s) on the cell surface.

Therefore, we next analyzed and compared specific binding of WGA to cell surface proteins on PP-PEC and J774.1 cells, as LDMC effector cells of classes I and III, respectively, because they could be obtained in good yields, and on MM46 cells (class II) as target cells. Casein-PEC, which consists mainly of polymorphonuclear cells with few macrophages was not examined.

![Affinity Chromatography on WGA-Sepharose](image)

**FIG. 4.** Affinity Chromatography on WGA-Sepharose of the Triton X-100-Solubilized Supernatant Fluid of Radioiodinated PP-PEC

Details of the conditions for chromatography are described in Materials and Methods. Arrows indicate the points of buffer change. Fractions indicated with a bar were collected for analysis by SDS-PAGE.
125I-Surface labeled intact cells were solubilized with Triton X-100 and the cell lysates were separated from cell debris by centrifugation. The recoveries of radioactivity in the lysates of PP-PEC, J774.1 cells and MM46 cells were 91, 92 and 54%, respectively. The lysates were subjected to affinity chromatography on Sepharose 6MB coupled with WGA and the elution pattern of the PP-PEC lysate is shown in Fig. 4. When the column was washed with 10 mM Tris-HCl buffer (pH 7.0) containing 0.2% Triton X-100, approximately 90% of the applied radioactivity was recovered in the flow-through fraction, with slight continuous release of the remaining radioactivity in late fractions. When the release of radioactivity had decreased to a low level, the washing buffer was replaced by a buffer containing 0.5 M glucose and then buffer containing 0.5 M GlcNAc. Although no radioactivity above background was observed with buffer containing 0.5 M glucose, a marked peak of radioactivity was eluted with buffer containing GlcNAc, which is the monosaccharide specifically recognized by WGA, indicating that this peak contained cell-surface glycoproteins specifically recognized by WGA. The elution patterns of J774.1 and MM46 cell lysates were similar to that in Fig. 4, except for the proportions of radioactivity in the GlcNAc-eluate; these were 1.3, 2.0 and 0.8% of the total radioactivity in lysates of PP-PEC, J774.1 and MM46 cells, respectively, which were applied to the affinity column.

The peak fractions eluted with GlcNAc were pooled and dialyzed against ethanol and the distilled water and this dialysis procedure was repeated to remove remaining Triton X-100. After the last ethanol-dialysis, the fractions were concentrated by evaporation, solubilized with SDS and subjected to SDS-PAGE. The autoradiograms are shown in Fig. 5. The major peaks of PP-PEC migrated to positions corresponding to 153 kilo daltons (kDa), 140 and 90 kDa, the 153 kDa protein being predominant. On the other hand, J774.1 cells gave 6 major peaks of 150, 115, 85, 63, 54 and 45 kDa, which were similar to those of MM46 cells of 150, 125, 85, 67, 58 and 44 kDa with little differences in their

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**FIG. 5.** Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoregrams of Radioiodinated Surface Glycoproteins Eluted from WGA-Sepharose

Experimental conditions are described in Materials and Methods.
intensities (Fig. 5).

These results indicated that the composition of WGA-binding surface glycoproteins of PP-PEC, which contained 80 to 90% macrophages differed from those of the macrophage cell line J774.1 cells and MM46 tumor cells which were somewhat similar.

DISCUSSION

There are many reports concerning the role and importance of monocyte-macrophage lineage cells in host defense against infections and malignant diseases. We previously demonstrated that macrophages participate in mediating *in vitro* lysis of tumor cells in the presence of appropriate lectins such as WGA.\(^1\)\(^2\) Essential processes in this cytolysis are the binding of effector and target cells and triggering of the cytolytic mechanism of effector cells by WGA. The triggering process may be the key characteristic of LDMC-inducing lectins.\(^3\) To elucidate the mechanism of triggering the cytolytic action of macrophages by lectins, we investigated the binding of WGA to macrophages and macrophage cell-line cells or tumor cells.

With regard to direct tumoricidal activity of macrophages, Adams and his colleagues defined 4 discrete stages in the development of macrophages by functional, biochemical and immunological characterization of macrophage preparations obtained from animals after appropriate stimulations. These stages were resident, responsive or inflammatory, primed and fully activated macrophages.\(^4\)\(^5\) In this work we demonstrated that peritoneal exudate macrophages in variously stimulated states *in vivo*, (*i.e.*, glycogen-, proteose peptone- and thiglycolate-elicited PEC as responsive or inflammatory macrophages and BCG-elicited PEC as fully activated macrophages) including resident cells, had similar tumor cytotoxicities in the presence of WGA. The requirement for prior activation and the strict dependence on specific lectins such as WGA distinguish lectin-dependent macrophage cytotoxicity from both direct tumoricidal activity by macrophages and lectin-dependent cytotoxicity by lymphocytes.\(^6\)

On the other hand, the macrophage cell-line J774.1 showed a biphasic phenomenon (Fig. 2): at a low E/T ratio of 0.63, J774.1 cells showed optimal cytolysis of MM46 tumor cells comparable to that by glycogen-PEC, while at an E/T ratio of more than 40, at which cytolysis by glycogen-PEC was maximal, J774.1 showed little cytolysis. Similar results were obtained with WEHI-3 cells but cytolysis was less. One possible explanation for this phenomenon is a metabolic side-effect at a high cell density, *e.g.*, mitotic cells (J774.1 and WEHI-3 cells have doubling times of 23 and 13 h, respectively) with metabolically high activity may exhaust the medium of nutrients or release some harmful products to themselves or may rapidly endocytose \(^5\)Cr-labeled materials released from MM46 cells.

Another explanation may be that relatively high affinity of macrophage cell-line cells to WGA (Table 1) prevents their effective WGA-mediated binding to target cells in high macrophage density culture.

There are many reports on the binding characteristics of lectins, including WGA to mammalian cells, erythrocytes, lymphocytes, fibroblasts and malignant cells\(^4\)\(^5\)\(^6\) but little is known about the binding of WGA to monocyte-macrophage line cells. In this report we estimated the number of binding sites and affinity constant of WGA to various elicited PECs, macrophage-line cells and MM46 cells in relation to the event of WGA-dependent macrophage-mediated cytolysis (Fig. 3 and Table 1). Since the addition of GlcNAc in the reaction mixture completely abolished the WGA-dependent cytolysis and the cytotoxicity of macrophages to tumor cells,\(^1\) we examined the GlcNAc-specific binding of WGA. As shown in Table 1, these kinetic analyses of WGA-bindings to cells classified the various cell types into four classes. Four preparations of elicited PEC, glycogen-, TG-, PP- and BCG-PEC, which had similar capacities to lyse MM46 cells in the presence of WGA (Fig. 1), had nearly equal WGA-binding properties which were distinct, within the limits of experimental error, from those of MM46 cells, macrophage-line cells and casein-PEC. In these PEC preparations, 80 to 90% of the cells were macrophages, the rest being polymorphonuclear cells, as judged by Giemsa staining. Since about 80% of the cells in casein-PEC were polymorphonuclear cells, the participation of polymorphonuclear cells in WGA-binding in the four preparations may be small and the kinetic data may reflect the binding characteristics of the macrophages.
This classification also correlated well with the capacity of elicited PEC and of macrophage-cell lines to cytolyze MM46 cells in the presence of WGA, indicating that the difference in binding properties of WGA to cells may directly reflect the distinctive expression of the WGA-receptor molecule(s) (glycoproteins and glycolipids) for triggering cytolysis on effector cells in the cell preparations.

The SDS-PAGE profiles of surface glycoproteins specifically bound to the WGA-column also demonstrated a marked difference between WGA-binding surface-molecules of PP-PEC and J774.1 or MM46 (Fig. 5). The existence of different WGA-binding surface glycoproteins and different amounts of these glycoprotein seems to explain the distinct difference in affinities of WGA binding and in the numbers of binding sites. The similarity in the WGA-binding surface glycoproteins on J774.1 and MM46 cells, which were originally derived from different tissues and strains, suggests that some of the six WGA-binding surface glycoproteins on J774.1 and MM46 cells might control or at least be related to growth of these continuously proliferating cells. Actually, the insulin-receptor is reported to be a glycoprotein that binds to a WGA-column. The differences of profiles of WGA-binding surface glycoproteins between PEC and J774.1 may explain the difference of their LDMC phenotype, although some differences may be due to genomic background (PEC from C3H/He mouse and J774.1 from BALB/c mouse). We have considered that the three major WGA-binding glycoproteins (153, 140 and 90 kDa) on PEC macrophages at least may participate in binding of macrophages and target MM46 cells in the WGA-LDMC reaction.

Furukawa et al. recently reported that O-linked sialyloligosaccharides rather than N-linked efficiently bound WGA-Sepharose gels containing less than 0.5 mg of lectin/ml. The present WGA-Sepharose gels which contain about 5 mg/ml, may bind both O-linked and N-linked glycoproteins. It remains to be determined how many of these molecules are important in the LDMC reaction, especially in the triggering by WGA of cytolysis of target cells by macrophages. In addition, the role of glycolipids in the process of LDMC reaction is still to be determined. It is also important to qualitatively and quantitatively compare the WGA-binding molecules with other LDMC-active lectin binding molecules on the surfaces of macrophages.14)

REFERENCES