Glycoconjugate Journal 15, 615–622 (1998)

Inhibition of phagocytic activity by the *N*-acetyl-D-galactosamine-specific lectin from *Amaranthus leucocarpus*

Guadalupe Maldonado[1], Patricia Gorocica[2], Concepción Agundis[1], Armando Pérez[3], Juan Molina[4] and Edgar Zenteno[1]*

[1] *Laboratorio de Inmunología, Departamento de Bioquímica, Facultad de Medicina UNAM; P.O. BOX 70159, 04510 México*
[2] *Departmento de Bioquímica, Instituto Nacional de Enfermedades Respiratorias, Tlalpan, 14080 México,*
[3] *Departmentos de Biología Celular y Tisular and* [4] *Farmacología, Facultad de Medicina UNAM, 04510 México*

Amaranthus leucocarpus **lectin (ALL), specific for *N*-acetyl-D-galactosamine, induces inhibition of the erythrophagocytic activity of resident murine peritoneal macrophages and of the macrophage-like cell line J-774. This effect was observed only in macrophages that were Mac-2 (CD11c/CD18 or CR4) negative, indicating that macrophage activation induces important modification to the glycosylation (mainly O-glycosylation) of the membrane. Receptors for IgM and C3b remain unaltered after lectin treatment. Ultrastructural analysis revealed (a) that ALL induced the formation of pinocytic vacuoles, and (b) a regular distribution over the macrophage membrane as well as endosomal vesicles of the gold labeled ALL. Our results suggest that macrophage membrane glycoproteins with constitutive *N*-acetyl-D-galactosamine residues participate in the regulation of pinocytic-phagocytic vacuole formation.**

Keywords: ***Amaranthus*** **lectin, lectins, glycoproteins, macrophage, phagocytic activity**

Introduction

Macrophages specialize in ingesting and sequestering a wide variety of foreign bodies and cell debris, but the exact mechanism by which they engulf such particles is not completely understood [1, 2]. The initial steps of phagocytosis and pinocytosis involve ligand binding to specific receptors on the cell surface. Macrophage phagocytosis can be mediated by receptors for the Fc portion of IgG or IgM, receptors recognizing complement fragments C3b and C3bi, fibronectin receptors, receptors for mannose/fucose residues of glycoconjugates, and non-specific receptors that mediate the uptake of several kinds of particle. In foreign cell phagocytosis, facilitated interaction with adjacent macrophage receptors through ligand patching seems to be important for the initial binding [3, 4]. Weir [5] suggested that macrophage recognition is established by an interaction between surface glycoproteins of the phagocytic cell and the carbohydrates located on the surface of the foreign particle.

Lectins have the capacity of specifically recognize carbohydrate moieties and allow isolation of glycosylated compounds; they have been extensively used to show the glycoside nature of distinct structures [6] and to investigate cell differentiation processes [7]. Some studies have demonstrated that lectins are capable of modifying the phagocytic activity of the macrophage. When germ agglutinin (*N*-acetyl-D-glucosamine specific) enhances the phagocytic and bactericidal activity of macrophages [8]; also, an important increase in vacuole formation in macrophages has been demonstrated after treatment with Concanavalin A (Con A, specific for glucose and mannose) [9, 10]; Ofek and Sharon [11] reviewed data showing an increase in the breakdown rate of ingested particles when they were coated with lectins. Inhibition of the macrophage phagocytic activity has been demonstrated with lectins that recognize complex galactose- and *N*-acetyl-D-galactosamine-containing structures, such as *Machaerocereus eruca* lectin [12, 13] and peanut agglutinin (PNA) [14], suggesting a downregulation role of galactose/*N*-acetyl-D-glucosamine-containing receptors. Based on the above, we decided to use the lectin from *Amaranthus leucocarpus*, which is specific for the core of O-glycosidically linked glycans [15], and induces immunosuppression in animals treated with the lectin against particulated antigens [12], in order to identify the participation of O-glycosidically linked glycans from membrane in the regulation of the phagocytic activity of macrophages.

* To whom correspondence should be addressed. Fax: 52 (5) 616-2419; E-mail: ezenteno@servidor.unam.mx.

Materials and methods

Lectins

Amaranthus leucocarpus lectin (ALL) was purified from seeds from Tulyehualco, Mexico, by affinity chromatography on stroma from human erythrocytes type O, as described in [15]. Concavalin A and all the sugars used in this study were obtained from Sigma Chemical Co. (St. Louis, Mo., USA).

Macrophages

Peritoneal macrophages from CD-I mice were purified by adherence on glass coverslips and suspended in DMEM culture medium supplemented with 10% fetal bovine serum, 10 U penicillin/ml, and 10 μg streptomycin/ml [16]. Cell cultures were incubated at 37 °C in a humidified chamber with a 5% CO_2 atmosphere. Cell viability (>90%) was determined by trypan blue dye exclusion. Purified adherent cells (1×10^5 per ml) were seeded on glass coverslips and cultured until a uniform monolayer was formed; the cells on the coverslips were then washed with non-supplemented DMEM culture medium, counted, and used. The macrophages-like cell line J-774, generously provided by the Immunology Department, National School of Biological Sciences (Mexico), was seeded with an identical cell density.

Phagocytic activity

Phagocyte activity of murine macrophages as well as of the J-774 cell line was studied by incubating the coverslip-adherent cells with sheep red blood cells (SRBC) at a ratio of 1:10 for 1 h at 37 °C in a humidified chamber with a 5% CO_2 atmosphere. Exhaustive washing of the coverslips with PBS (0.14 mM sodium phosphate, 0.15 M NaCl, pH 7.4) eliminated non-ingested erythrocytes and those cells attached to the side were fixed with methanol and stained with Giemsa dye. Quantification of the phagocytic process was expressed as a phagocytic index (P.I.), which represented the number of erythrocytes attached to, or phagocytized by, 100 macrophages [14]. Sheep red blood cells, obtained from the Faculty of Veterinary Medicine, UNAM, Mexico, were centrifuged and washed three times by centrifugation at 200 × g, 5 min each, with phosphate buffered saline (PBS).

Effect of ALL on Phagocytic Activity

Cells were incubated for 30 min with different concentrations of ALL (from 0.2 to 200 μg ml^{-1}), prior to performing the phagocytic test. At the end of the incubation period, cells were washed and their phagocytic activity determined. The specificity of the lectin-macrophage interaction for carbohydrates was determined by incubating the macrophages for 30 min at room temperature with 100 mM of different sugars before adding the lectin, or by incubating the lectin with 100 mM of carbohydrates in DMEM for 10 min, before adding the lectin to the monolayer. Con A (20 μg ml^{-1}) was used as a positive stimulator of phagocytic activity [14, 17].

Rosette-Formation Assay

The binding assay of Fc and C3b receptors on murine peritoneal macrophages was performed as described [16]. Fc receptors were tested using SRBC incubated for 1 h at 37 °C, with rabbit hemolysin (IgM anti SRBC, kindly donated by Dr Ruben Dario Martínez, Faculty of Medicine, UNAM, Mexico) diluted 1:200. The cells were then washed thrice with PBS and diluted to a final concentration of 1% in PBS. C3b receptors were analyzed with SRBC incubated with human serum diluted 1:200, at 37 °C, for 30 to 60 min, centrifuged at 200 × g, and suspended to a final concentration of 1% with PBS. Hemolysin or human serum-treated erythrocytes were added to macrophages previously incubated with different concentrations of ALL and/or 100 mM GalNAc in a proportion of 1 macrophage for each 10 erthrocytes. Rosette formation was tested after 30 and 60 min incubation; a group of macrophages without lectin treatment was used as a control. Positive binding was considered when three or more treated erythrocytes, on average, were bound by a macrophage. Each determination was made in triplicate and about 100 cells were examined for each sample.

Cell ELISA

Macrophages were seeded in sterile flat bottom microwell plates (Nunc Delta) (1.5×10^5 macrophages per well). The macrophages were fixed with 0.05% glutaraldehyde in PBS at 4 °C, for 30 min; then washed with PBS and covered with 1% bovine serum albumin fraction V (BSA) at room temperature for 4 h in PBS. After removing the BSA, the wells were incubated for 60 min at room temperature with 50 μl monoclonal antibody against the mouse macrophage-2 (Mac 2) antigen (clone M3/38, Boehringer Manheim, Germany) diluted 1:400 in PBS; after five washes with PBS, the wells were incubated with polyclonal sheep anti-rat Ig-biotin, F (ab) 2 fragment (Boehringer Mannheim, Germany) diluted 1:200 for 60 min at room temperature [18]. To evaluate the binding sites for ALL, we performed the same procedure as for Mac 2, but using 2 μg of biotin-labeled ALL (as the optimal dose), at room temperature for 1 h. Excess monoclonal antibody or ALL was eliminated by washing five times with PBS, and the wells were incubated for 1 h with peroxidase-labeled extravidine in PBS. Unbound conjugate was removed by washing with PBS containing 0.05% Tween 20 followed by two washes with PBS alone. The presence of ALL on the cells was revealed by incubating the wells with 100 μl o-phenylenediamine hydrochloride and 0.015% hydrogen peroxide in 0.1 M citric acid, 0.2 M phosphate buffer, pH 5.4, for 30 min at room temperature. Absorbency of the reaction was read at 490 nm

on a Biotek EL311 microplate reader. Absorbency was corrected by using labeled sheep anti-rat Ig-biotin as a non-specific binder, or the auto-oxidation or o-phenylenediamine, and by subtracting the absorbency; the result was termed specific absorbency [18]. Each experiment was performed six times, and the specific absorbency expressed as the mean ±S.E.M.

Morphological Analysis of Macrophages

Monolayers of peritoneal macrophages adherent to coverslips and treated with ALL and Con A (see above) were observed with Nomarski differential-interference-contrast microscopy while others were processed for ultrastructural studies. The cells were fixed with 1.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (SCB, pH 7.2) for 2 h at 4 °C and postfixed in 1% osmium tetroxide in 0.15 M SCB for 45 min at 4 °C. After washing with 0.2 M sodium cacodylate buffer, pH 7.2, the specimens were dehydrated and embedded in Araldite 6005 (Polysciences Inc., Warrington, PA, USA) as follows: the coverslips were placed with fixed cells facing down over Beem capsules (Polysciences Inc., Warrington, PA, USA) filled with the resin and left overnight at room temperature to permit adequate infiltration of the Araldite into the monolayer. This was followed by heating the Beem capsules at 55 °C for 48 h to allow proper polymerization. Finally, the Beem capsules with the coverslips were frozen, allowing the coverslip to be snapped off leaving the monolayer embedded in Araldite. Ultrathin sections were obtained with a diamond knife and then contrasted with 2% aqueous uranyl acetate and 1% lead citrate, and observed with a Zeiss EM-10 electron microscope [19].

Pre-embedding staining of *A. leucocarpus* lectin binding sites for transmission electron microscopy (TEM)

A. Leucocarpus lectin binding sites were visualized by an indirect method according to [20, 21]. Briefly, peritoneal macrophages (5×10^5 cells per coverslip) were cultured for 1 h on glass coverslips, washed with an non-supplement DMEM culture medium and then cooled to 4 °C. Cells were incubated wth biotinylated ALL (3–4 $\mu g\,ml^{-1}$) diluted in PBS (pH 7.2), for 15 min at 4 °C. After washing twice with cold PBS-0.1% BSA (pH 7.2), the cells were incubated at 4 °C for 30 min with 10 nm gold particles-coupled streptavidin (Sigma Chemical Co) at a dilution 1:40 in PBS-0.1% BSA (pH 7.2) and then washed twice with cold PBS (pH 7.2). Cells were then either fixed immediately as described above and processed for TEM, or incubated at 37 °C for the following intervals: 5, 10, 15, 30 and 60 min, and 24 h before fixation and then processed for TEM. Control were incubated with the culture medium or PBS in the first step of the procedure instead of biotinylated ALL.

Results

Effect of ALL on mouse peritoneal and line J-774 macrophages

As shown in Figure 1 the optimal phagocytic activity of untreated macrophages was found after 30 to 60 min. Treatment of macrophages with Con A, before the addition of RBCs, increased their phagocytic activity. This effect was more pronounced at 60 min of incubation; however, ALL treatment diminished three- and nine-fold the phagocytic activity at 60 min of incubation with respect to the control

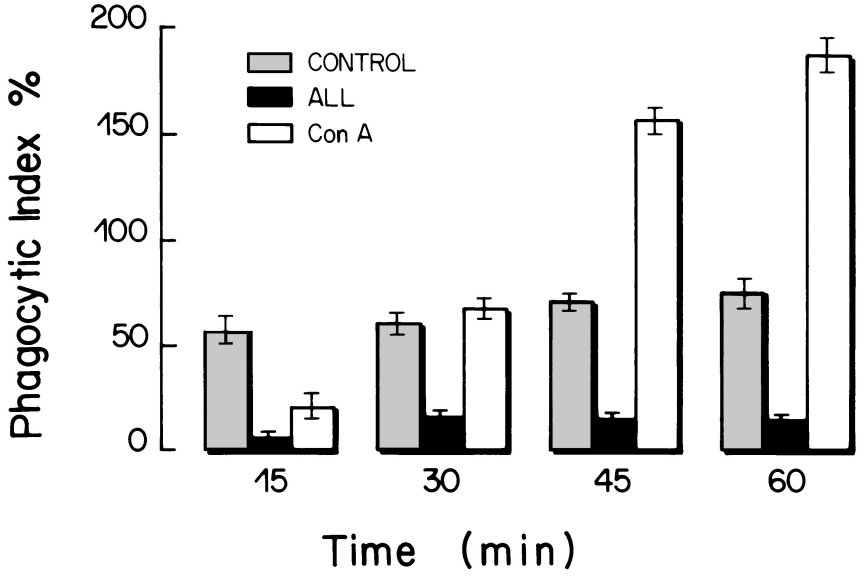

Figure 1. Phagocytic activity of murine peritoneal macrophages (50 μg per 2×10^5 macrophages) in the presence of Con A or ALL (20 μg each). Quantification of the phagocytic process was expressed as a phagocytic index (P.I.), which represents the number of erythrocytes attached to or engulfed by 100 macrophages. Results are shown as the mean and standard deviations of three replications per point.

(untreated) and to Con A-treated macrophages, respectively (Figure 1). The inhibitory effect of ALL was already evident after 15 min incubation, which was also observed in the macrophage-like cell line J774 (data not shown). The optimal inhibitory effect of ALL on erythro-phagocytosis was obtained using 20 $\mu g\,ml^{-1}$ of the lectin (representing 2 μg lectin per 2×10^5 macrophages). No effect on the phagocytic index was observed when the lectin was added to the macrophages at the same time as the SRBCs (not shown). The inhibition induced by ALL was abolished with 100 mM of GalNAc, added 10 min prior to the lectin or when ALL was pre-incubated in the presence of this sugar. Other sugars tested at the same concentration without effect on the inhibitory effect of the lectin were *N*-acetyl-D-galactosamine, *N*-acetyl-neuraminic acid, D-glucose, D-mannose, D-galactose and D- and L-fucose.

Rosette formation

The percentage of positive rosette forming macrophages in the presence of erythrocytes, treated with rabbit hemolysin or human serum, increased from 40% at 30 min to 95% at 1 h in control experiments using untreated macrophages. ALL-treated macrophages showed no significant difference from control macrophages, since the values of rosette forming cells were 43% at 30 min to 91% at 1 h (not shown).

Expression of Mac-2 on murine macrophages

The expression of ALL receptors and Mac-2 in murine peritoneal macrophages was determined by cell ELISA using biotin-labeled ALL lectin and a peroxidase-labeled monoclonal antibody directed against the mouse macrophage Mac-2 antigen, as shown in Figure 2. The appearance of Mac-2 on the monolayered macrophages is observed after 45 min of incubation of 37 °C. In contrast, adherence of macrophages induces an important decrease of receptors for ALL (Figure 2).

Morphological analysis of treated macrophages

Effect of ALL

ALL-treated macrophages were predominantly round, with almost no spreading and a few macrophages had one or two vesicles (Figure 3a). In the phagocytic activity assay, macrophages showed an evident but heterogeneous spreading. In comparison with Con A-treated macrophages, fewer macrophages were phagocytic, with one or two red blood cells internalized. The most conspicuous feature of ALL treated macrophages was the presence of coated pits and vesicles and a complex composed of membrane-bound tubules and vesicles, predominantly located near the nucleus and sometimes in the periphery of the cell. These structures are clearly distinct from rough endoplasmic reticulum and Golgi complex, and probably correspond to the endosomal compartment (Figure 3b).

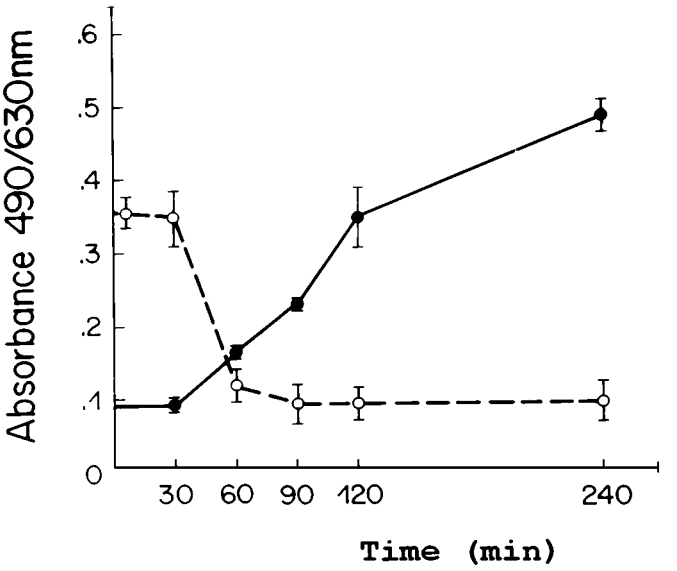

Figure 2. Determination of Mac-2 antigen (●) and ALL (○) receptors by cell ELISA. Macrophage monolayers (1.5×10^5 macrophages per well) fixed with 0.05% glutaraldehyde. The Mac 2 antigen was identified with a monoclonal antibody to the mouse macrophage-2 (Mac-2) antigen and revealed with polyclonal sheep anti-rat Ig-biotin, $F(ab)_2$ fragments and streptavidin peroxidase. ALL receptors were identified using biotin-labeled ALL and revealed with streptavidin peroxidase. Absorbency of each reaction was read at 490/630 nm. Negative control Optical Density (OD) was the same for the 0 and 30 min time. Each experiment was performed six times and the specific absorbency expressed as means ± S.E.M.

Effect of Con A

Peritoneal macrophages treated with Con A showed a rounded shape, broad spreading, well-developed vesicles (Figure 4a) which had a polarized distribution, and peripherally displaced nucleus (Figure 4b). Phagocytosing macrophages showed a similar aspect, but the observed vesicles appeared to be occupied by the internalized erythrocytes. Ultrastructurally, Con A-treated macrophages are characterized by the presence of large vesicles, frequently containing debris (Figure 4b). The mean diameter of these structures was 3 μm (range: 0.8 to 6 μm). In the periphery of the cells, several membrane-bound tubules and vesicles were observed, some in continuity with the plasma membrane and communicating with the extracellular space. Other organelles and a well-defined cytocenter were also observed (Figure 4b).

As presented in Figure 5, localization of ALL receptors on murine peritoneal macrophages in the plasma membrane was based on the presence of gold particles. Lectin labeling appeared to be present homogeneously distributed over the plasma membrane at 4 °C (Figure 5a). Moreover after 5–15 min of incubation at 37 °C we identified the presence of gold particles associated with endosomal compartment, confirming the lectin-membrane interaction as an inductor of pinocytic vesicles (Figure 5b, c). Blocking the lectin-binding site with 100 mM GalNAc inhibited the gold labeling.

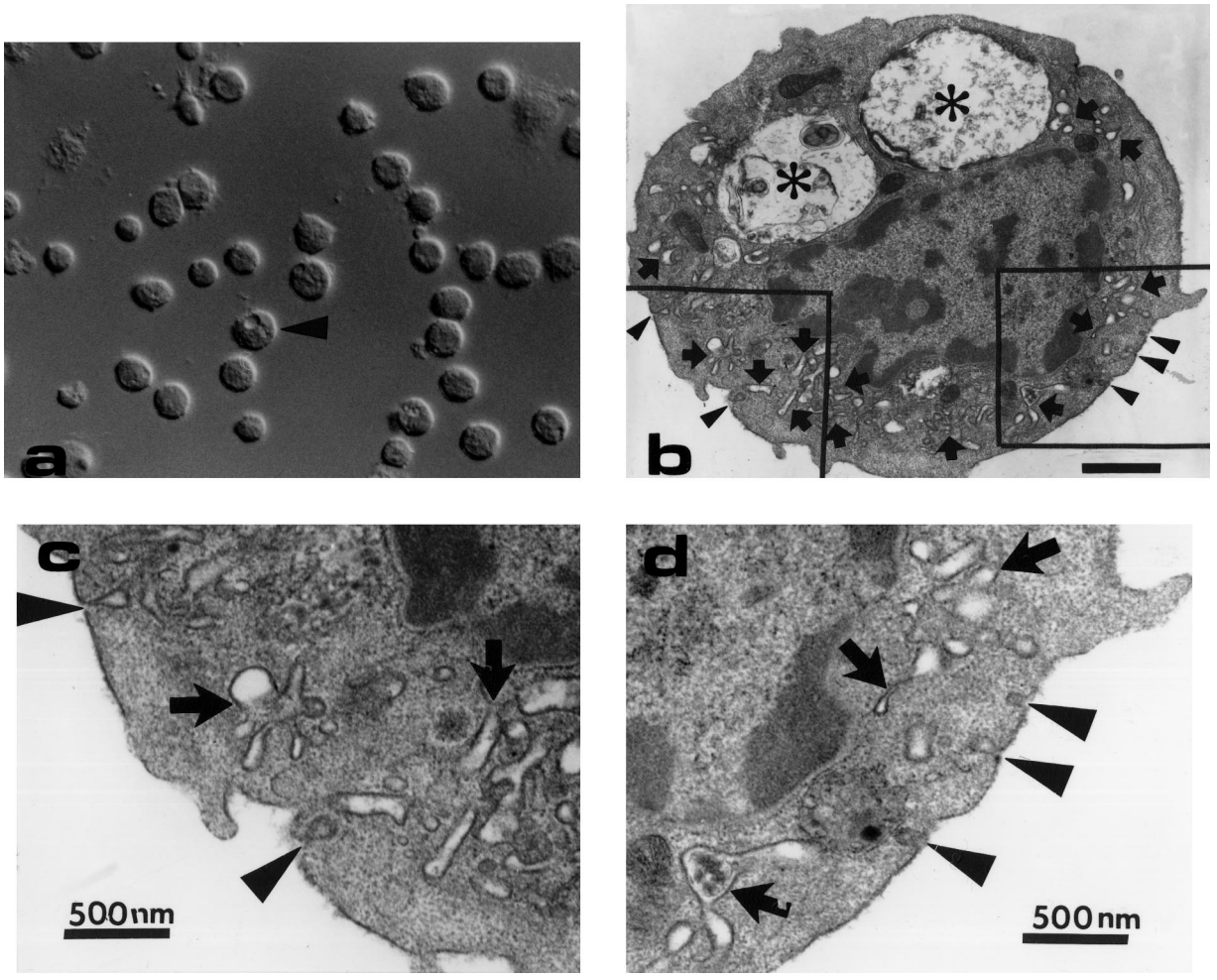

Figure 3. Adherent peritoneal macrophages treated with *A. leucocarpus* lectin. Macrophages observed with Nomarski differential-interference-contrast microscopy had a rounded shape, were poorly spread and few contained vesicles (a, arrowhead) (400 ×). Ultrastructurally (b), *A. leucocarpus* lectin seems to induce formation of coated vesicles (arrowheads) and a prominent endosomal complex that consist of heterogeneous membrane-bound tubes and vesicles localized in the periphery of the cell and the perinuclear region (arrows). Scarce phagocytic vesicles could be observed (asterisk). More details of identified structures are observed in higher magnification (c, d) of the boxed area. (Bar in (b) = 1 μm.)

Discussion

Particle uptake is a specialized activity of mononuclear and polymorphonuclear phagocytes: dead cells, immune complexes, and microorganisms are all avidly internalized. After fusion with hydrolase-rich lysosomes, the ingested particles are degraded. The clearance and digestive functions of phagocytes contribute to wound healing, tissue remodeling and host defense [22]; however, some intracellular pathogens such as *Hystoplasma capsulatum* [23], *Leishmania* [24] or *Cryptococcus neoformans* [25], evade the lytic effects of the macrophages in which some functions, i.e., macrophage priming, oxidative burst, and anti-fungal activities are inhibited. Although the exact mechanism inducing these effects has not been identified, the possibility of down regulation of macrophage activity induced by specific signals, generated by pathogen-macrophage interactions, cannot be ruled out.

Lectin studies have suggested the regulation of macrophage activity through specific interaction with surface glycoproteins. Lectins with specificity of GlcNAc (such as wheat germ agglutinin [8]), α-Man Con A [10]), or for *N*-acetyllactosaminic type glycans (such as *Phaseolus vulgaris* [26]), induce an increase in vacuole formation and in the capacity to form internal vesicles [6] and engulf bacteria [3]; however, PNA and soybean agglutinin, specific for the Gal or GalNAc (in non-reducing position), inhibit phagocytic activity [14, 27]. In this work, we tested the ability of the lectin from *A. leucocarpus* to induce modifications on the activity of murine peritoneal macrophages and of the macrophage cell-line J-774.

ALL sugar specificity is directed to the T-antigen, containing Galβ(1–3) GalNAcα(1–3) Ser/Thr, and the Tn-antigen, which contains GalNAcα(1–0) Ser/Thr [15]. ALL suppressed the response of mice, when the lectin was

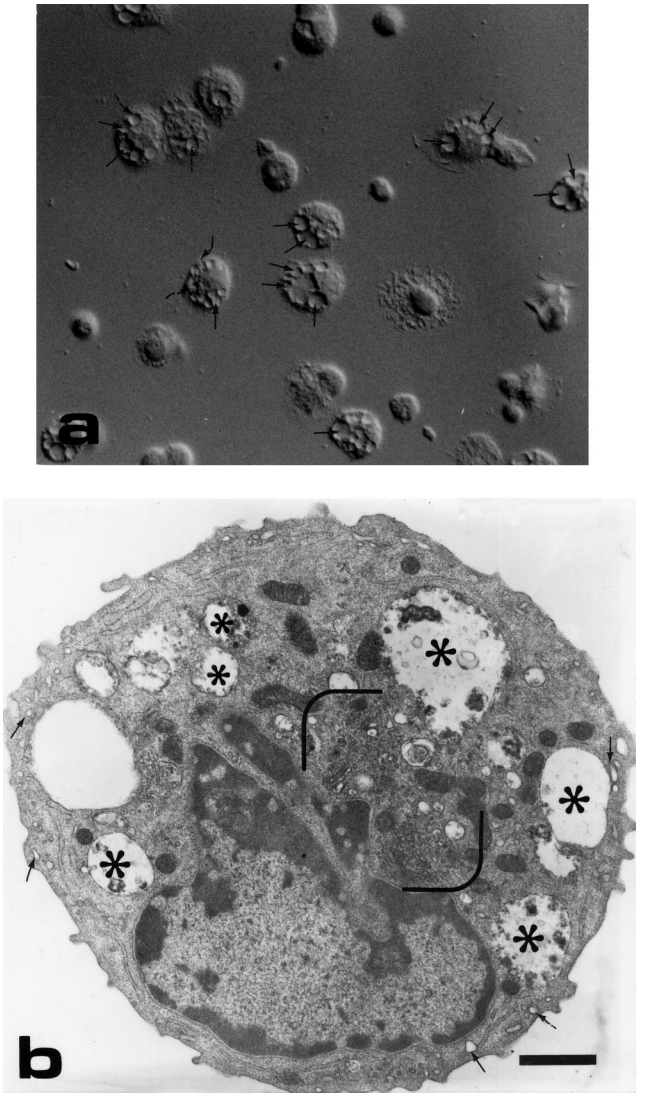

Figure 4. Adherent peritoneal macrophages treated with Con A. The effect of Con A is characterized by a broad spreading of macrophages and the presence of numerous vesicles (a, arrowhead) (400 ×). Ultrastructurally (b), a well-developed endosomal complex was not observed and only a few tubular and vesicular structures were identified in the periphery of the cells (arrows). The numerous phagocytic vesicles frequently contain debris (asterisk). Note the presence of an evident juxtanuclear cytocenter (in brackets). (Bar in (b) = 1 μm.)

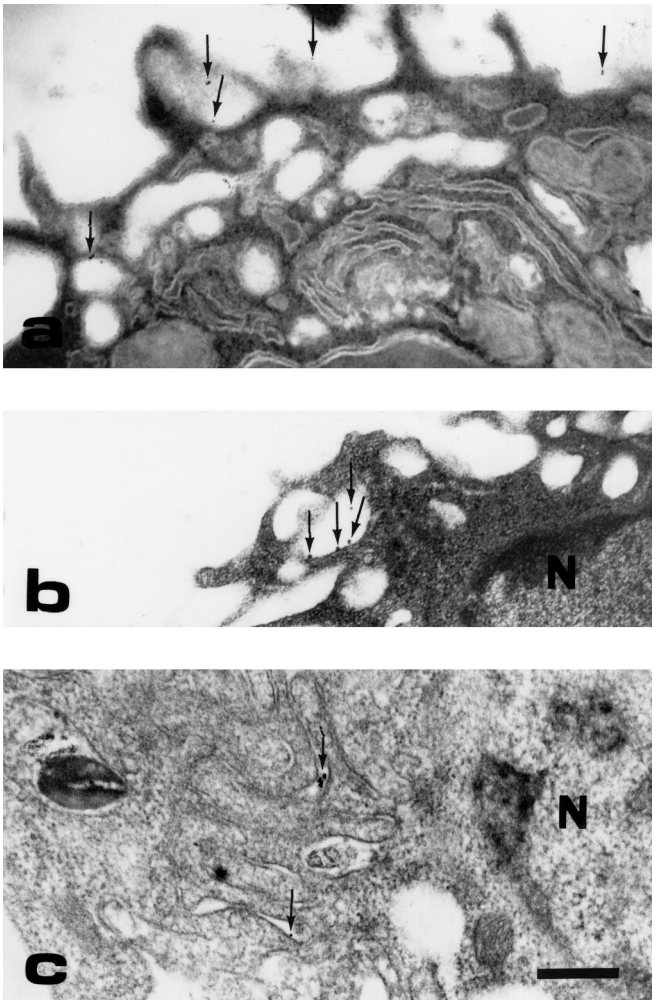

Figure 5. Peritoneal macrophages incubated with biotinylated-*A. leucocarpus* followed to 10 nm gold particle-coupled streptavidin. The label was found in the cell membrane (top, arrows) at 4 °C. After incubation for 30 (b) and 60 min (c) at 37 °C, gold particles were bound to the membrane of vesicles (b, arrows) and in the lumen and membrane of tubular structures (c, arrows). (Bar = 250 nm.)

administered intraperitoneally before the antigen [12]. As shown in the present study, incubation of murine peritoneal resident macrophages with ALL induces inhibition of the phagocytic activity toward sheep erythrocytes; this inhibition was also observed in the macrophage-like cell line J-774. The inhibitory effect of ALL on the phagocytic activity of the macrophages tested was demonstrated by a qualitative method, i.e., phagocytic activity identified by optical microscopy and reported as a phagocytic index. Our results indicate that the inhibitory effect of ALL was dependent on the lectin concentration and, apparently, on the activation state of the macrophage. This consideration has been suggested by the fact that the inhibitory effect of ALL lectin decreased with the incubation time of macrophages, so we also tested the presence of an activation specific marker, such as Mac 2, in order to correlate the activation state of the cell with the presence (or absence) of specific GalNAc-containing glycoproteins on the surface of macrophages. Mac 2 (CD11c/CD18 or CR4) is part of the β2-integrin family of molecules and mediates phagocytosis by binding to immune complexes coated with iC3b and iC4b; Mac 2 are molecules strongly expressed by macrophages and their expression increases proportionally with cellular activation [28]. Our results indicate that there is an inverse correlation between the ALL-receptor and the presence of Mac 2, suggesting that O-glycosylation is altered by the activation of the cell. Similar modifications have been demonstrated with macrophage mannose-receptors, which diminish according to the stimulation state of the cells [29].

ALL induces inhibition of the phagocytic activity of macrophages by a mechanism independent from those controlling Fc and C3b receptors, since the expression of these receptors on macrophages remains unaltered after lectin treatment. The macrophage like-cell line J-774 also shows the effect of this lectin, confirming that mannose receptors are not needed for lectin activity; as demonstrated previously, this cell line lacks this receptor [29].

Ultrastructural evaluation of the macrophages treated with ALL and with Con A (as a positive stimulation control) was performed by TEM. Our results indicate that ALL treated macrophages, in contrast to the Con A treated macrophages, increase the formation of the endosomal compartment, and this effect seems to explain the marked reduction in erythrophagocytic activity. Many investigations aimed at identifying the morphology and functional aspects of macrophages have suggested that contractile cytoskeletal filaments are involved in the phagocytic process [30]. Specific microfilament attachments to the cytoplasmic surfaces of nascent phagosomes are very rare and few cytoskeletal filaments are located in the membrane, suggesting that only an array of membrane receptors could control the formation of phagocytic or pinocytic vacuoles [30]. The main receptor for ALL in erythrocytes is glycophorin [15] and this protein is linked by the band 4.1 protein to the membrane skeleton in erythrocytes [31]. In leukocytes, integral membrane glycoprotein CD43 (leukosialin or sialophorin), contains, like glycophorin, many units of Gal $\beta(1, 3)$ GalNAc $\alpha(1, 0)$ Ser/Thr, and plays an important role in the plasma membrane-actin filament association; moreover, this protein is tightly associated with microvilli, providing membrane attachment sites for actin filaments [32]. The results presented here, suggest that regulation of filament attachment to the plasma membrane structures could be modulated specifically by the ALL membrane receptors. Thus ALL induces formation of endosomal compartment, but does not alter the organization of other organelles and the cytocenter, by binding close to the peptide backbone and crosslinking its receptor. Work is in progress in order to define the nature of the ALL receptor, although the evidence presented herein indicates that it is CD43. In this case, it is the first report suggesting the role of O-glycosidically linked glycoprotein in the regulation of the phagocytic/pinocytic formation of vacuoles in macrophages.

The ALL receptor on macrophages should be considered as a phenotypic marker with constitutive O-glycosidically linked glycans present in non-stimulated macrophage populations. Finally, it would be interesting to evaluate the participation of this receptor in antigen processing and presentation by macrophages to demonstrate whether this mechanism participates in the suppression of the immune response induced by ALL [12].

Acknowledgments

This work was supported by the Consejo Nacional de Ciencia y Tecnología (2151 PM and F643-M9406) and PAPIIT-DGAPA program, UNAM, Mexico.

References

1 Lewis EC, Macgee JO'DM (1992) In *The Biology of the Macrophage*: *The Macrophage*, pp. 1–35. Oxford, Irl Press.
2 Gordon S, Perry VH, Rabinowitz S, Chung L, Rosen H (1988) *J Cell Sci* **9**: 1–14.
3 Aggeler J, Werb Z (1984) *Adv Inflamm Res* **8** 35–54.
4 Chandler DB, Kennedy JI, Fulmer JD (1984) *Am Rev Resp Dis* **134**: 85–9.
5 Weir DM, Ögmundsdo'ttir MH (1977) *Clin Exp Immunol* **30**: 323–9.
6 Kreipe H, Radzun HJ, Schumacher U, Parwaresch MR (1986) *Histochem* **86**: 201–6.
7 Lascurain R, Chávez R, Gorocica P, Perez A, Montaño LF, Zenteno E (1984) *Immunology* **83**: 410–13.
8 Gallily R, Vray B, Stein I, Sharon N (1988) *Immunology* **52**: 679–86.
9 Bodo M, Becchetti E, Baroni T, Mocci M, Merletti L, Giammarioli M, Calvitti M, Sbaraglia G (1995) *Cell Mol Biol* **41**: 297–302.
10 Goldman R (1974) *FEBS letters* **46**: 203–8.
11 Ofek I, Sharon N (1988) *Infect Immun* **56**: 539–47.
12 Zenteno E, Ochoa JL, Parra C, Maldonado G, Montaño LF, Carvajal R (1985) In *Lectins-Biology*, *Biochemistry*, *Clinical Chemistry* (Bög-Hansen TC, Breborowicz J, eds) Vol 4, pp. 537–46. Berlin: Walter de Gruyter.
13 Zenteno E, Vázquez L, Chávez R, Córdoba F, Wieruszeski JM, Montreuil J, Debray H (1985) *Glycoconjugate J* **12**: 699–706.
14 Maldonado G, Porras F, Fernandez L, Vazquez L, Zenteno E (1994) *Immunol Invest* **23**: 429–36.
15 Zenteno E, Lascurain E, Montaño LF, Vazquez L, Debray H, Montreuil J (1992) *Glycoconjugate J* **9**: 204–8.
16 Stuart AE, Habeshau JA, Davison AE, (1989) *Phagocytosis in vitro*. In *Handbook of Experimental Immunology* (Weir DM ed) pp 24.1–24.26. Oxford: Blackwell Scientific Publications.
17 Corradin SB, Mauel J, Gallay P, Heumann D, Ulevitch RJ, Tobias PS (1992) *J Leukocyte Biol* **52**: 363–72.
18 Ho M-K, Timothy AS (1982) *J Immun* **128**: 1221–5.
19 Becker I, Monfort R, Perez-Torres A, Rondan-Zarate A, Monfort I (1996) *Exp Parasitol* **82**: 171–81.
20 Takata K, Hirano H (1984) *Histochemistry* **81**: 435–9.
21 De Panfilis G, Manara GC, Ferrari C, Torresani C (1988) *J Invest Dermatol* **91**: 547–52.
22 Isberg RR, Tran Van Nihieu G (1994) *Annu Rev Genet* **27**: 395–422.
23 Wolf JE, Abegg AL, Sharon JT, Kobayashi GS, Little JR (1989) *Infect Immun* **57**: 513–19.
24 Wilson ME, Pearson RD (1988) *Infect Immun* **56**: 363–9.
25 Cross CE, Bancroft GJ (1995) *Infect Immun* **63**: 2604–11.
26 Vargas F, Maldonado G, Montaño LF, Zenteno E (1988) In *Lectins-Biology*, *Biochemistry*, *Clinical Chemistry* (Bög-Hansen TC, Freed D eds) Vol 6, pp 321–6. Denmark: Textop, Hellerup.

27 Goldman R, Sharon N, Lotan R (1976) *Exp Cell Res* **99**: 408–22.
28 Nagpal ML, Browdown JC (1980) *Int J Biochem* **11**: 27–138.
29 Imber JM, Pisso SV, Johnson WJ, Adams DO (1988) *J Biol Chem* **257**: 5129–33.
30 Male D, Champion B, Cooke A, Owen M (1991) *Advanced Immunology*, 2 ed, p 7.5. London: Gower Medical Publishing.
31 Anderson RA, Lovrien ER (1984) *Nature* **307**: 16–18.
32 Yonemura S, Nagafuchi A, Sato N, Tsukita S (1993) *J Cell Biol* **120**: 437–9.

Received 29 July 1997, revised 14 October 1997, accepted 20 October 1997