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Macrophage surface glycoproteins binding to galectin-3 (Mac-2-antigen)

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Galectin-3 (formerly called Mac-2 antigen) is a ~30 kDa carbohydrate-binding protein expressed on the surface of inflammatory macrophages and several macrophage cell lines. We have purified from lysates of the murine macrophage cell line WEHI-3 glycoproteins that bind to a galectin-3 affinity column. Several of these receptors are labelled after biotinylation of intact cells showing their location at the cell surface. N-terminal aminoacid sequencing of intact galectin-3-binding glycoproteins isolated from preparative SDS-gels or of chemically derived fragments showed several homologies with known proteins and identification was confirmed by immunoprecipitation with specific antibodies. The glycoproteins were shown to be: the α-subunit(CD11b) of the CD11b/CD18 integrin(Mac-1 antigen); the lysosomal membrane glycoproteins LAMPs 1 and 2 which are known in part to be expressed at cell surfaces; the Mac-3 antigen, a mouse macrophage differentiation antigen defined by the M3/84 monoclonal antibody and related immunochemically to LAMP-2; the heavy chain of CD98, a 125 kDa heterodimeric glycoprotein identified by the 4F2/RL388 monoclonal antibodies respectively on human and mouse monocytes/macrophages and on activated T cells. Further studies showed that CD11b/CD18, CD98 and Mac-3 are major surface receptors for galectin-3 on murine peritoneal macrophages elicited by thioglycollate.

***Keywords*: galectin-3, macrophages, receptors**

***Abbreviations*: PBS, phosphate buffered saline; CNBR, cyanogen bromide; PMSF, phenyl methyl sulphonyl fluoride**

Introduction

The galectins are a family of mammalian proteins that bind to galactose-containing glycoconjugates in a Ca^{2+}-independent manner [1]. Galectin-3 is a ~30 kDa multi-domain protein consisting of an N-terminal repetitive sequence enriched in proline, glycine and tyrosine residues and a C-terminal carbohydrate-binding domain homolous to similar domains in other galectins [2]. Although galectin-3, like other family members, is synthesized without a signal sequence and lacks a transmembrane sequence, it is secreted from the cytoplasm by a novel pathway independent of the endoplasmic reticulum-Golgi apparatus and is found expressed on cell-surfaces, presumably by capture of secreted lectin through binding to appropriate glycoconjugate receptors [3]. Indeed galectin-3 was originally described as the Mac-2 antigen expressed on the surface of thioglycollate-elicited peritoneal macrophages and is a widely used surface marker of mouse macrophage development [4–10].

Little is known about the functions of galectin-3 present in the extracellular space or at cell surfaces. Galectin-3 binds to matrix glycoproteins such as tumour-derived laminin and foetally-derived fibronectin and blocks attachment and spreading of cells on substrata coated with these glycoproteins, possibly by steric hindrance of cell-binding domains and modulation of integrin binding [11]. In other cells, ligation of surface receptors by galectin-3 appears to result in cell signalling and activation. Human monocytes treated with galectin-3 up-regulate production of IL-1 [12] and similar treatment of human neutrophils stimulates superoxide production [13]. Galectin-3 also binds the rat mast cell high affinity IgE receptor as well as IgE and causes degranulation and serotonin release from rat basophillic leukemia cells [14, 15]. Other results implicate this lectin in IgE-mediated activation of neutrophils [16].

The finding that galectin-3 has a wide spectrum of effects on different cell-types indicates a variety of specific receptors and is consistent with the broad carbohydrate-binding specificity of the lectin. Galectin-3 binds to Types I and II Galβ1 → 3(4) GlcNAc-R chains, to polylactosamines and with highest affinity to blood group ABH determinants (see for example [11]). In addition to laminin and fibronectin isoforms, galectin-3 binds the matrix glycoproteins tenascin-C and tenascin-R and neural adhesion molecules L1, N-CAM and myelin associated glycoprotein MAG [17]. A neutrophil receptor appears to be NCA-160, a human carcinoembryonic antigen-related glycoprotein [13]. Human tumour cell lines, including colon and breast

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carcinomas and melanomas, secrete glycoprotein ligands containing an N-terminal region highly homologous to the macrophage scavenger receptor cysteine-rich domain [18–21]. Recently, advanced glycation end (AGE) products, the reactive derivatives of nonenzymic glucose-protein condensation reactions, have been shown to be high affinity ligands for galectin-3 [22]. However, in this instance the binding site is distinct from the carbohydrate recognition domain of the lectin.

In order to understand more fully the role(s) of galectin-3 in macrophage activity, we have studied the galectin-3-binding glycoproteins of murine macrophages, some of which are candidate molecules for surface ligation of the lectin *in vivo*.

Materials and methods

Cell culture

WEHI-3 cells were grown in suspension culture at 37 °C in RPMI-1640 medium supplemented with 10% heat inactivated foetal calf serum. Peritoneal macrophages from female BALB/c mice, elicited by intraperitoneal injection of brewers thioglycollate medium as described [10], were harvested after 3 days into RPMI-1640 plus 10% inactivated foetal calf serum. The exudate cells were then cultured in Nunc plates at 37 °C; non-adherent cells were removed after 2 h of culture by replacement of the serum supplemented-RPMI-1640 medium. For metabolic labelling, cells were washed twice with methionine-free medium containing 10% dialysed foetal calf serum and subsequently cultured for various periods at 37 °C in the same medium supplemented with 200 μCi ml^{-1} Trans ^{35}S label. Labelled WEHI-3 cells were harvested by centrifugation at 3000 × g for 15 min as were peritoneal macrophages after scraping.

Antibodies

Monoclonal antibodies used were as follows: rat M3/38 antibody against mouse galectin-3 (Mac-2 antigen) from Boehringer Mannheim, East Sussex, UK; rat M3/84 antibody against mouse Mac-3 antigen [23] from Pharmingen Cambridge Bioscience, UK; rat antibodies ID4B against mouse LAMP-1, ABL-93 against mouse LAMP-2 and M1/70.15.11.5.2 against mouse CD11b from Developmental Studies Hybridoma Bank, The Johns Hopkins University School of Medicine USA; rat RL388 against mouse CD98 from Dr H. R. MacDonald, Ludwig Institute, Lausanne, CH. Rabbit polyclonal antibodies against galectin-3 and its N-terminal domain were as described [24].

Cell surface biotinylation

Adherent macrophage cultures were washed with cold PBS and treated with 1 mM NHS-S-S-biotin reagent (Pierce Warriner, Chester, UK) in PBS at 2 °C for 30 min as described [10].

Affinity chromatography

Recombinant hamster galectin-3 [24] was coupled to CNBr activated Sepharose 4B using recommended procedures (Pharmacia LKB) in lactose-containing buffer. The product containing 0.5–1 mg of lectin ml^{-1} of Sepharose was packed into 10–15 ml columns and washed with 8 mM CHAPS: 50 mM Tris-HCl buffer (pH 7.2): 0.15 M NaCl: 100 μM leupeptin: 1 μM pepstatin: 1% aprotinin: 1 mM EDTA: 2 mM PMSF (Elution buffer). Cells (up to 10 ml packed volume) labelled with Trans ^{35}S Label for 24 h were suspended and lysed in 40 mM CHAPS: 50 mM Tris-HCl (pH 7.5): 0.15 M NaCl: 100 μM leupeptin: 1 μM pepstatin: 1% aprotinin: 1 mM EDTA: 2 mM PMSF (Lysis buffer) at 2 °C. The supernatant after centrifugation at 100 000 × g for 60 min at 2 °C was applied to the column which was washed at 2 °C with Elution buffer followed by 0.15 M lactose in Elution buffer to elute bound glycoproteins. Fractions (2 ml) were analysed for radioactivity. The lactose-eluted fraction was dialysed against elution buffer and concentrated. In other experiments, intact metabolically labelled cells were subjected to vectorial biotinylation before lysis and application to the galectin-3 affinity column. The lactose-eluted fraction was passed through a 5 ml column of Streptavidin-agarose (Sigma) equilibrated with elution buffer. After thorough washing to elute unbound material, biotinylated galectin-3 binding glycoproteins were eluted with 0.5 M dithiothreitol in Elution buffer.

Electrophoresis and microsequencing

SDS-PAGE was carried out under reducing or non-reducing conditions in 7.5% polyacrylamide gels. To isolate individual proteins, gels were stained for 5 min with 0.2% Coomassie Blue in 50% (v/v) methanol, briefly destained and the gel pieces containing single protein bands were destained completely with washing in 50% methanol-50 mM Tris-HCl (pH 8.8) followed by water. Proteins were removed from the gel by electroelution and freeze dried. Freeze dried samples were dissolved in 75 μl of freshly prepared BNPS-Skatole (2 mg ml^{-1} in 75% acetic acid) and incubated for 16 h at 37 °C. The cleavage reaction was stopped by adding 0.25 ml of H_2O, precipitated BNPS-Skatole was removed by centrifugation and peptides were then separated by Tricine gel electrophoresis [25]. For amino acid sequencing, unstained gels were electroblotted for 60 min at 90 V onto Immobilon-P membranes. Bands cut from the membranes after brief Coomassie Blue staining for 5 min were used for microsequencing on Applied Systems 470 A gas-phase and 477A pulsed liquid-phase sequencers with applied Biosystems 120A on-line phenyl thiohydantoin analysers. For Western blotting, unstained gels were transferred to nitrocellulose filters. The filters were blocked with 10% bovine serum

albumin in PBS at 2 °C overnight, incubated overnight with galectin-3 solution (1 μg ml^{-1}) in 20 mM Tris-HCl (pH 7.2): 150 mM NaCl: 0.1% Nonidet P40 and bound lectin was located by sequential addition of rabbit antibody against galectin-3 and anti-rabbit Ig: alkaline phosphatase conjugate.

Immunoprecipitation

Samples of [^{35}S] methionine-labelled cell lysates or column fractions diluted in 1% Nonidet P40: 0.5% deoxycholate: 0.1% SDS: 50 mM Tris HCl (pH 8.0): 150 mM NaCl: 1 mM PMSF: 2 units aprotinin, were pre-cleared by addition of normal rabbit serum followed by Protein A-Sepharose. Cleared supernatants were precipitated, usually sequentially, with various monoclonal antibodies. After addition of one primary antibody (1:100 dilution) and incubation overnight at 2 °C, polyclonal rabbit anti rat Ig (1:100 dilution) was added and immune complexes were collected on Protein A-Sepharose after 2 h at 2 °C. Immune complexes were dissolved in Laemmli reducing buffer for SDS-PAGE, followed by autoradiography.

Flow cytometry

Freshly harvested peritoneal exudate cells or WEHI-3 cells from suspension cultures were washed twice with PBS: 0.1% BSA: 0.1% NaN_3(PAB) and aliquots (approximately 5×10^5 cells) were incubated with monoclonal antibody at saturating concentration (usually 10 μg ml^{-1}) in PAB for 30 min at 2 °C. The treated cells were washed twice with PAB and incubated for 30 min at 2 °C with FITC: conjugated goat anti: rat Ig diluted 1:100 in PAB. Other aliquots were incubated with galectin-3 (20 μg ml^{-1}) in PAB for 30 min at 2 °C, washed and treated sequentially with M3/38 monoclonal antibody followed by FITC: conjugate anti rat Ig antibody. Controls included omission of the first antibody step. Cells were fixed with 1% paraformaldehyde in PBS for 10 min, washed twice in PAB and analysed in a FACStar–plus (Becton Dickinson, Mountain View, CA) instrument using appropriate gating for pure macrophage populations.

Results and discussion

Purification of WEHI-3 receptors for galectin-3

Flow cytometric analysis (Figure 1b) showed that the mouse macrophage cell line WEHI-3 expresses little galectin-3 on the cell surface, unlike more mature macrophage cell lines in agreement with previous studies [9]. It was also shown that WEHI-3 cells do carry abundant cell surface receptors for galectin-3 when added exogeneously (Figure 1b). We decided to use WEHI-3 cells for isolation of galectin-3 receptors to overcome the possible shielding of high affinity receptors by large amounts of endogenous galectin-3.

WEHI-3 cells were labelled with [^{35}S]-methionine over 24 h and the washed cells were then biotinylated with

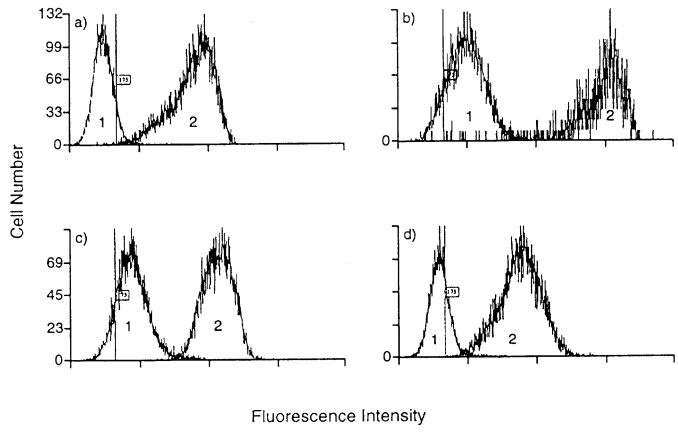

Figure 1. Surface expression of galectin-3-binding glycoproteins on mouse macrophage cell line WEHI-3. Cells were treated with monoclonal antibodies against murine antigens followed by FITC-conjugated second antibody and analysed by fluorescence flow cytometry. (a) Control with no primary antibody (line 1); anti Mac-1 α-subunit, CD11b (line 2). (b) Anti Galectin-3 before (line 1) or after (line 2) incubation of cells with exogenous galectin-3. (c) Anti LAMP-1 (line 1); anti CD98 heavy chain, RL388 antigen (line 2). (d) Anti LAMP-2 (line 1); anti Mac-3 (line 2).

a cleavable reagent NHS-S-S- biotin. Cells were lysed in CHAPS-containing buffer and the soluble fraction was fractionated by affinity chromatography on a galectin-3 column. Glycoproteins bound to the column were eluted with lactose (Figure 2a). SDS-PAGE followed by autoradiography of the bound fraction showed seven major bands a–g with apparent molecular weights ranging from 88 to 203 kDa (Figure 2b, Table 1) and several smaller, minor bands. In order to identify cell surface components, the lactose eluate from the galectin-3 column was passed through Streptavidin-Agarose and the bound fraction containing biotin were released by disulphide-bond cleavage. SDS-PAGE and autoradiography showed that the major cell surface components binding to galectin-3 in affinity chromatography were bands b, d, e and g of apparent molecular weights 172, 125, 100 and 88 kDa respectively (Figure 2b, Table 1). In order to obtain individual bands, preparative SDS-PAGE was performed. Each purified component was identified by Commassie Blue staining and a blot with galectin-3 showed that each reacted with the lectin (Figure 3). Binding was blocked in each case by lactose (result not shown). Thus, the components isolated from affinity chromatography are recognized directly by galectin-3 and their isolation in the bound fraction from the galectin-3 column was not due to non-specific interactions with the matrix or with other bound components. Notably, the bands on SDS-PAGE were broad in appearance indicating the presence in each of several glycoproteins or of glycoprotein isoforms varying in extent of glycosylation.

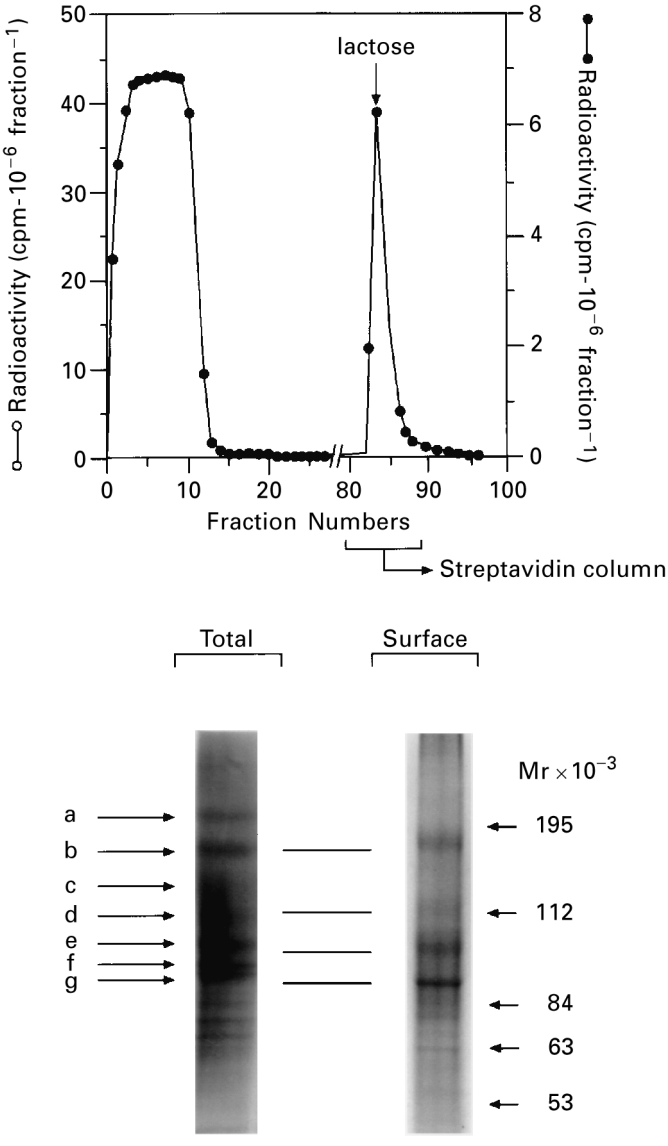

Figure 2. Affinity chromatography of WEHI-3 cell lysates. (a) Cells labelled with [^{35}S]-methionine were surface biotinylated and cell lysates were subjected to chromatographed on galectin-3 Sepharose. The bound fraction was eluted with lactose and biotinylated components were isolated by passage through a Streptavidin-Agarose Column. The bound components were released by reductive cleavage. (b) SDS-PAGE of affinity purified receptors of WEHI-3 cells. Radioactive binding fractions from galectin-3-Sepharose (Total receptors) and from Streptavidin-Agarose (Surface receptors) as described in panel (a) were dialysed, concentrated and subjected to electrophoresis under reducing conditions followed by radioautography. Major bands a–g are indicated. The migration of protein standards of known molecular weights is indicated.

Partial aminoacid sequencing of glycoproteins

Glycoproteins purified by preparative SDS-PAGE (Figure 3) were transferred to Immobilon-P membranes and subjected to gas phase N-terminal aminoacid sequencing. To obtain internal sequences, purified bands were cleaved chemically

Table 1. Galectin-3 binding glycoproteins of murine macrophage cell line WEHI-3 isolated by affinity chromatography.

Band[a,b]	~ $M_r \times 10^{-3}$
a	203
b	172
c	146
d	125
e	100
f	92
g	88

See Figure 2.
[a] Bands a b d e and g were obtained reproducibly. Bands c and f showed variation in different experiments.
[b] The Streptavidin binding fraction (Figure 2) contained bands b, gp 172; d, gp 125; e, gp 100; g, gp 88.

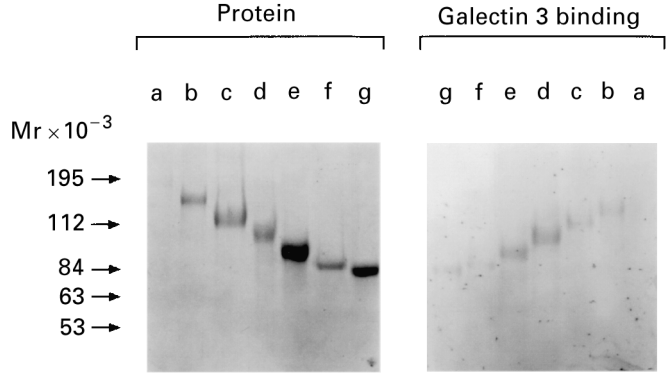

Figure 3. Galectin-3 binding glycoproteins of WEHI-3 cells purified by preparative SDS-PAGE. Glycoprotein bands from Figure 2 were electroeluted from several polyacrylamide gels and re-examined by SDS-PAGE under reducing conditions. (A) Commassie Blue staining; (B) Western blotting: the glycoproteins shown in panel (A) were transferred to a nitrocellulose filter after SDS-PAGE and probed with galectin-3 followed by polyclonal antibodies against galectin-3. The migration of protein standards is indicated.

Table 2. Peptide sequences of galectin-3 receptors.

Band[a,b]	*Sequence analysed*	*Sequence*
b gp172	N-terminal	GHLDTEHPM
	Skatole	FHLDTEHPR
Mouse CD11b	N-terminal	FNLDTEHPM
d, gp 125	N-terminal	LPEVKNDGTTXIMA
Mouse LAMP-1	N-terminal	LFEVKNDGTTCIMA
e gp 100	Skatole	DQNERYLVVLNE
Mouse CD98	Internal	DQNERYLVVLNE

[a] Band c (Table 1) gave an identical N-terminal sequence as band d.
[b] No useful sequence data were obtained for bands a and g.

by Skatole treatment and fragments, separated by SDS-PAGE and blotted, were analysed similarly. Sufficient sequence data were collected for Bands b, d and e to establish probable identity of glycoprotein constituents of these bands with known proteins (Table 2). No useful sequence data were obtained for bands a and g.

Band b of apparent molecular weight 172 kDa contained the α-subunit, CD11b, of the major macrophage integrin CD11b/CD18, originally described as the Mac-1 surface antigen [26]. The CD11b/CD18 integrin is a receptor for iC3b opsonized particles and fibrinogen and plays a major role in adhesion-dependent functions of myeloid cells, through binding to the third N-terminal Ig-like domain of ICAM-1 on partner cells [27]. This binding appears to be influenced by the glycosylation of the ICAM-1 molecule [28]. It is present in intracellular granules as well as on the cell surface and stimuli such as Ca^{2+} ionophores elicit increased translocation to the surface from intracellular storage sites. The CD11b/CD18 molecule is itself capable of transducing a Ca^{2+} signal that is critically dependent on the state of cell activation or differentiation [29]. The CD11b/CD18 integrin present on the myeloid cell surface requires one or more activation step in order to adhere to its cellular or soluble ligands. The CD11b subunit is reported to have 170 kDa molecular weight and contains 18 potential N-glycosylation sites, about half of which are occupied in the mature molecule [27]. The data available on the glycan structures present in the β_2 integrins, including CD11b/CD18, indicates branched complex-type glycans, some of which have polylactosamine extensions [30], having expected affinity for galectin 3 [11].

Band d contained an N-terminal aminoacid sequence showing identity with lysosomal membrane glycoprotein LAMP-1 [31]. The molecular weight of LAMP-1 in murine P388 macrophage cell line is reported variously to be 115–160 kDa, consistent with the size of band d. Interestingly, band c gave an N-terminal sequence identical to band d indicating that it contains LAMP-1 presumably originating in internal pools of the cells. LAMP-1, which contains polylactosamine glycans, is known to be expressed at low levels at cell surfaces, in addition to its primary localization in lysosomes [31].

A constituent of band e contained an internal sequence showing identity with the heavy chain of the leukocyte CD98 antigen (Table 2). The CD98 antigen is a 120–130 kDa disulphide-bonded heterodimer composed of a glycosylated heavy chain and an unglycosylated light chain of molecular weight 40 kDa [32–35]. The heavy chain varies in size according to cell source from approximately 85–93 kDa due to differences in N-glycosylation, details of which are unfortunately not available. However, the N-glycans of the mature glycoprotein are endo H-resistant showing that complex type chains having affinity for galectin-3 are likely to be present. The CD98 antigen is present on peripheral blood monocytes and macrophages but absent from peripheral blood T- and B-cells. It appears on lectin or alloantigen activation of T-cells and is present on cytotoxic T-cells and most permanent leukocyte cell lines. The function of CD98, recognized on murine cells by monoclonal antibody RL388 and on human cells by 4F2 monoclonal antibody, is unknown. Indirect evidence has suggested that it may function in Ca^{2+} channel activities in some tissues [36, 37].

Immunochemical identification of galectin-3 receptors

Further identification of the putative galectin-3 receptors was carried out with a series of sequential immunoprecipitations using specific monoclonal antibodies of the [^{35}S]-methionine labelled glycoproteins isolated from WEHI-3 cell lysates by galectin-3 affinity chromatography. Equal proportions of total labelled WEHI-3 cell lysate, unbound and bound fractions from affinity chromatography (see Figure 2a) were examined.

The results showed (Figure 4) that LAMP-1, migrating on SDS-PAGE as a broad 115–160 kDa band, was removed quantitatively from the cell lysate by galectin-3. Similarly, galectin-3 bound quantitatively the total CD11b/CD18 content of WEHI-3 cells; under reducing conditions in SDS-PAGE, the $\alpha\beta$ subunits ran separately as 190 and 105 kDa polypeptides respectively. Immunoprecipitation of the galectin-3 bound fraction with monoclonal antibody RL388, directed against heavy chain CD98 epitopes, co-precipitated in addition to the $\sim$90–93 kDa heavy chain the $\sim$35–40 kDa molecular weight light chain. In non-reducing SDS-PAGE, the immunoprecipitated component gave a single $\sim$120 kDa molecular weight band.

The macrophage Mac-3 antigen binds galectin-3

The Mac-3 glycoprotein was described originally as a macrophage surface antigen [23], but it is present in many tissues and may be closely related if not identical to lysosomal associated membrane protein, LAMP-2 [31]. The average molecular weight of Mac-3 varies according to source due to glycosylation differences in the 79 kDa molecular weight polypeptide.

When labelled WEHI-3 cell extracts were immunoprecipitated with monoclonal antibody M3/84 against murine Mac-3, SDS-PAGE of the immune complex gave a broad band migrating in the 130–170 kDa molecular weight range (Figure 5). The size of Mac-3 in WEHI-3 cells is reported to be 170 kDa [23]. All of the Mac-3 content of whole WEHI-3 cell extracts was bound to the galectin-3-affinity column and was eluted with lactose (Figure 5A), a result confirmed by Western blotting with M3/84 antibodies (Figure 5B). Very similar results were obtained using a LAMP-2-specific monoclonal antibody and sequential immunodepletion experiments showed that LAMP-2 and Mac-3 glycoproteins are immunochemically related (result not shown), in agreement with previous findings

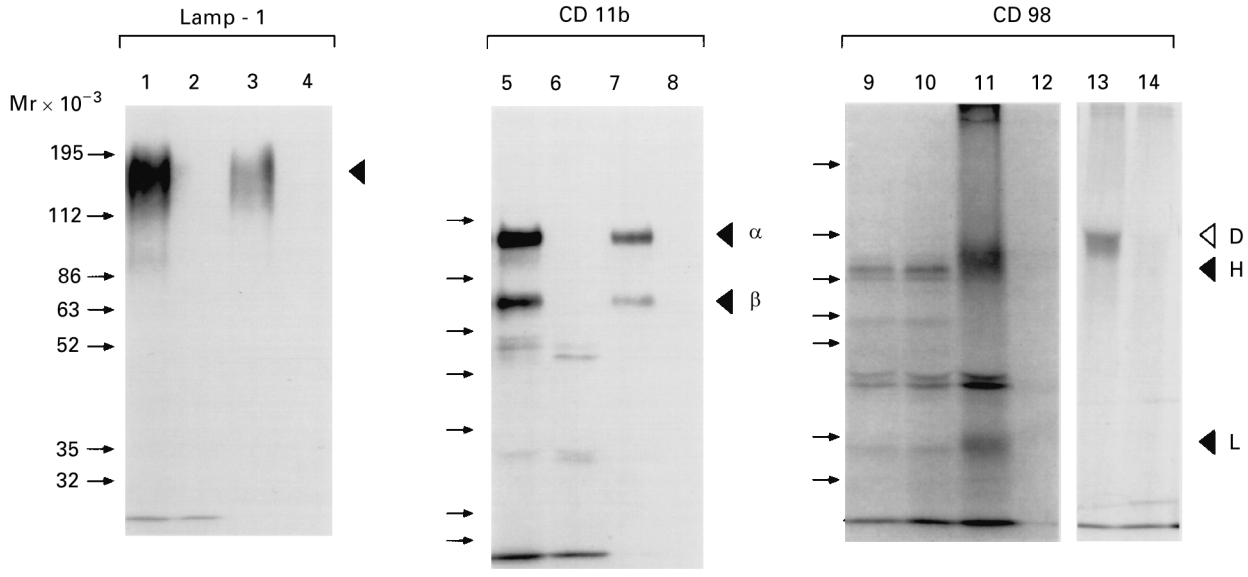

Figure 4. Immunoprecipitation of [^{35}S]-methionine labelled galectin-3-binding glycoproteins of WEHI-3 cells. Equal portions of total cell lysates or fractions obtained after affinity chromatography on galectin-3 Sepharose were immunoprecipitated with monoclonal antibodies against murine LAMP-1, CD11b or CD98. Immune complexes were analysed by reducing [1–12] or non-reducing [13, 14] SDS-PAGE and autoradiography. 1 and 5, whole cell lysate plus specific antibodies; 2, 6 and 9, unbound fraction from galectin-3-Sepharose plus specific antibodies or 10, control antibody; 3, 7, 11 and 13, bound fraction from galectin-3-Sepharose plus specific antibodies; 4, 8, 12 and 14, bound fraction plus control antibody. Arrow heads indicate subunits of LAMP-1, CD11b(*α*)/CD18(*β*) integrin, CD98 heavy (H) and light (L) chains and dimer (D).

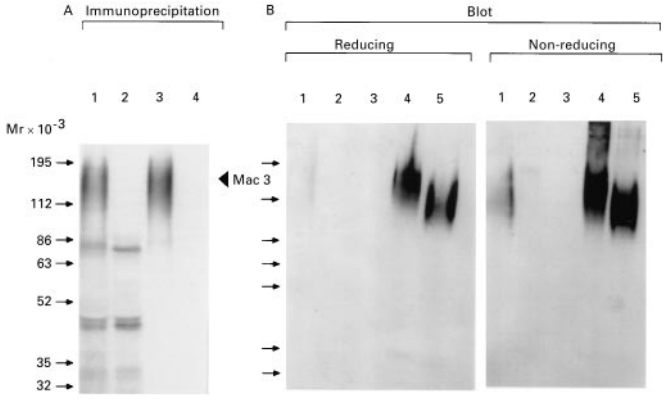

Figure 5. Immunochemical identification of Mac-3 antigen as a galectin-3-binding component in WEHI-3 cells. (A) [^{35}S]-methionine labelled cell extracts were immunoprecipitated with monoclonal antibody M3/84 against murine Mac-3. The immune precipitates were analysed by SDS-PAGE and autoradiography; (1) Total extract; (2) Unbound fraction from galectin-3-Sepharose; (3) and (4) Bound fraction from galectin-3-Sepharose. Tracks 1–3 contained M3/84 antibody; track 4 contained control primary antibody; (B) Equal amounts of WEHI-3 cell extracts were separated by SDS-PAGE, transferred to nitrocellulose and blotted with M3/84 antibody. Tracks 1, total extract; 2, unbound fraction from galectin-3 sepharose; 3, final wash before lactose elution; 4. and 5. bound fractions from galectin-3-Sepharose in two independent experiments. Migration of Mac-3 is indicated by an arrow head and protein standards by arrows.

[31]. The apparent size of Mac-3/LAMP-2 in separate experiments using WEHI-3 cells was variable, as shown for two independently obtained cell lysates (Figure 5B, tracks 4 and 5).

Surface expression of galectin-3 binding glycoproteins on WEHI-3 cells

In order to estimate the relative contributions of the galectin-3-binding glycoproteins, identified after affinity chromatography, to lectin binding at the cell surface flow cytometric analysis was carried out after treatment of intact cells with specific antibodies. On WEHI-3 cells, the major galectin-3 receptors appeared to be (Figure 1a, c) integrin CD11b/CD18 (Mac-1 antigen) and CD98(4F2/RL388 antigen) (Figure 1c). Neither LAMP-1 nor LAMP-2 were expressed at high levels on WEHI-3 cells, suggesting that these glycoproteins are minor contributors to galectin-3 binding by these cells. Lamps 1 and 2 are also intracellular ligands for galectin 3 in human melanoma cells [19]. Interestingly, the Mac-3 antigen as defined by the M3/84 monoclonal antibody was detected at moderately high levels (Figure 1d), although other studies [34] have indicated close similarity with LAMP-2. Our present results argue against an exact identity or alternatively the monoclonal antibody ABL 93 used to detect the LAMP-2 molecule reacts with a cryptic epitope in the membrane-bound glycoprotein.

Galectin-3 receptors on murine peritoneal macrophages

Thioglycollate-elicited macrophages express surface galectin-3 at high levels [5, 7]. To identify surface receptors, peritoneal exudate macrophages from thioglycollate-treated mice were labelled in culture with [^{35}S] methionine and cell

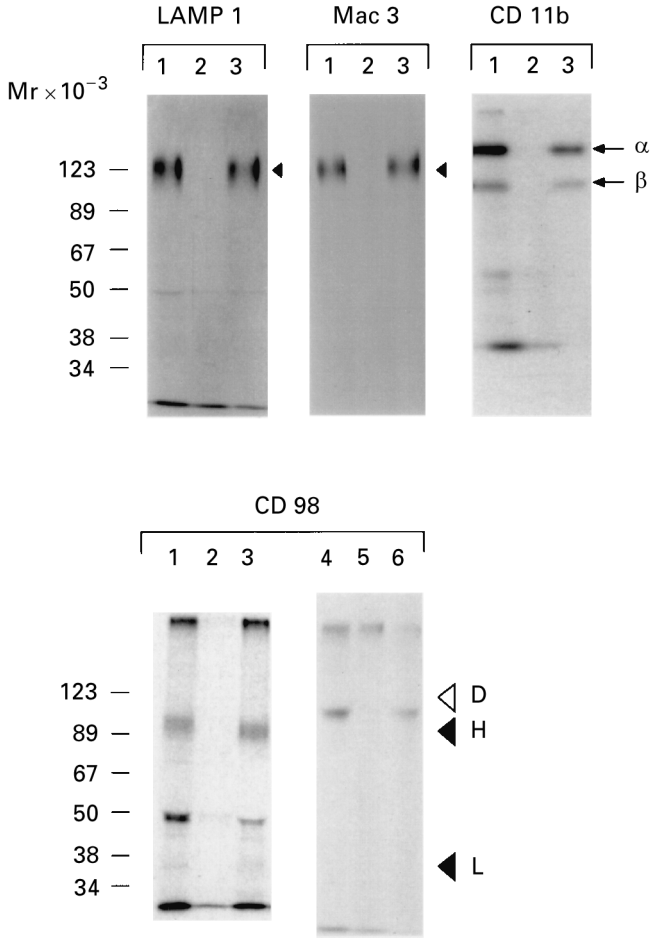

Figure 6. Galectin-3-binding components of murine peritoneal exudate macrophages. Immune complexes prepared from [^{35}S] methionine-labelled cell lysates (tracks 1 and 4), unbound (tracks 2 and 5) or bound (tracks 3 and 6) fractions from galectin-3-Sepharose affinity chromatography with monoclonal antibodies were analysed by SDS-PAGE under reducing (tracks 1–3) or non-reducing (tracks 4–6) conditions. See Figure 4 for other details.

lysates fractionated by galectin-3 affinity chromatography. Immunoprecipitation experiments showed (Figure 6) that integrin CD11b/CD18, CD98 and Mac-3 all bound quantitatively to the galectin-3 column. Other data show that these glycoproteins are abundantly expressed on thioglycollate-elicited murine peritoneal macrophages [6, 27, 32]. Our immunoprecipitation data also showed binding of LAMP-1 to galectin-3 (Figure 6). However, FACStar analyses indicate very low surface expression of LAMPs on thioglycollate-elicited macrophages (results not shown).

Concluding remarks

Our results show that major macrophage surface receptors for galectin-3 (Mac-2 antigen) include the Mac-1 and Mac-3 antigens, described [4] in the same screening of monoclonal antibodies reactive with the macrophage surface that produced the Mac-2 monoclonal antibody. In the original screen [4], plasma membrane glycoproteins from C57 BL/6 mouse thioglycollate-elicited peritoneal cells were isolated by affinity chromatography on *Lens culinaris* lectin and used for immunization of rats and subsequent monoclonal antibody production. Galectin-3 (Mac-2 antigen) is not glycosylated, and presumbly was represented in the glycoprotein fraction from pea lectin affinity chromatography by virtue of its binding to macrophage plasma membrane glycoproteins, including Mac-1 and -3 antigens. A similar possibility may explain the co-precipitation of ~ 32 kDa and ~ 18 kDa molecular weight polypeptides with CD98 from extracts of mouse EL-4 thyoma cells with CD98-specific monoclonal antibodies [34], and similarly sized polypeptides co-precipitated with CD11b/CD18 integrin from mouse macrophage extracts with specific α and β integrin subunit antibodies [38]. This polypeptide may be galectin-3, its carbohydrate binding domain produced by removal of the N-terminal protease-sensitive domain [24], or possibly other galectins with affinity for CD98 and CD11b/CD18 glycans.

Endogenous galectin-3 expressed at the surface of thioglycollate-elicited mouse peritoneal macrophages was shown to be in association with three major glycoproteins of 92, 125, and 180 kDa by chemical cross-linking [10]. Although these molecular weights are similar to several components described in the present work, immunoprecipitation experiments [10] with material isolated from the cross-linked conjugates failed to establish identity with β_2 integrins, LAMP-1 or LAMP-2, indicating either that additional galectin-3 receptors exist on these cells or that the antigenic epitopes recognized by the monoclonal antibodies used for immunoprecipitation did not survive chemical treatment. Recently it was shown that a major receptor for galectin-3 in murine macrophages is an integral membrane protein of the scavenger receptor cysteine-rich domain SRCR superfamily [20, 21]. This glycoprotein is homologous to the surface receptor for cytophilin C, a small polypeptide that lacks a signal sequence and is secreted from cells by a novel pathway, as is galectin-3. The SRCR-family galectin-3 receptor is also homologous to the ~ 98 kDa galectin-3 binding glycoprotein secreted by carcinoma cell lines [18, 19]. However, this glycoprotein was not detected in macrophage cell lines [18] and its expression required prolonged culture of peritoneal adherent macrophages or treatments with inducers of macrophage activation such as TNF and γ-interferon [21].

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