Cell. Mol. Life Sci. 65 (2008) 2586 – 2593
1420-682X/08/162586-8
DOI 10.1007/s00018-008-8143-x

Cellular and Molecular Life Sciences

Research Article

Co-localization of galectin-1 with GM1 ganglioside in the course of its clathrin- and raft-dependent endocytosis

R. Fajka-Boja[a,*], A. Blaskó[a], F. Kovács-Sólyom[a], G. J. Szebeni[a], G. K. Tóth[b] and É. Monostori[a]

[a] Lymphocyte Signal Transduction Laboratory, Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Temesvári krt. 62, 6726 Szeged (Hungary), Fax: +36 62 433 503, e-mail: roberta@brc.hu
[b] Department of Medical Chemistry, University of Szeged, Dóm tér 8, 6720 Szeged (Hungary)

Received 20 March 2008; received after revision 04 June 2008; accepted 05 June 2008
Online First 26 June 2008

Abstract. Mammalian galectin-1 (Gal-1), a β-galactoside-binding lectin has a prominent role in regulating cell adhesion, cell growth and immune responses. Downregulation of these biological functions may occur via internalization of Gal-1. In the present study we have investigated the mechanism and possible mediator(s) of Gal-1 endocytosis. We show that internalization occurs at a temperature higher than 22 °C in an energy dependent fashion. After one hour incubation Gal-1 localizes in the Golgi system within the cells, and then disappears without accumulation in degradation compartments, such as lysosomes. Based on their strong intracellular co-localization, two glycoconjugates, GM1 ganglioside and CD7 are implicated in the sorting of internalized Gal-1 into Golgi. Other known Gal-1 binding glycoproteins on T cells (CD2, CD3, CD43 and CD45) do not co-internalize with the lectin. Internalization of Gal-1 depends on its lectin activity and follows dual pathways involving clathrin-coated vesicles and raft-dependent endocytosis.

Keywords. Galectin-1, endocytosis, clathrin-dependent, raft-dependent, GM1 ganglioside.

Introduction

Endocytosis of receptor bound ligands is a common way of signal modulation, receptor recycling and degradation. Different mechanisms exist for entry of ligands into cells such as classical clathrin-mediated endocytosis, caveolin/raft-mediated endocytosis, as well as other clathrin- and caveolin-independent pathways [1]. The regular turn-over of the plasma membrane can also deliver cargo to the intracellular vesicular system [2].

Galectin-1 (Gal-1) is a β-galactoside binding animal lectin with particular properties. Although it is synthesized on free ribosomes as a cytoplasmic protein, it is also secreted from the cells in a non-classical way [3]. As it exits from the cells, it immediately binds to glycoproteins of the secreting and neighboring cells or extracellular matrix proteins [4]. Secreted Gal-1 induces apoptosis of immature and activated T cells and lymphoid cell lines [5–8]. Generally, Gal-1 is considered a negative regulator of inflammatory immune responses: it shifts the balance of Th1/Th2 cytokine secretion [9], influences the mobilization and extravasation of leukocytes [10], and attenuates acute and chronic inflammatory dis-

* Corresponding author.

eases [9]. Binding to the target cells, redistribution of membrane microdomains and initiation of signaling pathways leading to apoptosis or unresponsiveness of T cells are well documented [11, 12, 7, 8], however, the dynamics of Gal-1 binding and shedding or internalization has not been studied previously. Recently, the study of another galectin family member, galectin-8 revealed that its endocytosis occurred by non-clathrin and non-cholesterol dependent pathways, and its intracellular targeting was influenced by fine specificity of the two carbohydrate-recognition domains [13]. We have previously found that Gal-1 was downregulated from the cell surface and internalized by Jurkat T cells at physiological temperature (37 °C) [6]. In the present study we have examined the mechanism and the possible mediator of Gal-1 internalization. Here we show that not only the binding, but also the internalization is mediated by the carbohydrate-binding activity of Gal-1, suggesting a receptor mediated pathway. The internalized Gal-1 co-localizes with GM1 ganglioside and CD7, but not with CD45, CD3 or CD43. The endocytosis of Gal-1 in Jurkat cells follows dual routes, both the clathrin- and raft-dependent pathways.

Materials and methods

Cells and reagents. Jurkat leukemic T cell line was cultured in RPMI 1640 medium (Gibco, Invitrogen) supplemented with 5% heat inactivated fetal calf serum (FCS) (Gibco, Invitrogen). C6 rat glioma cells (GM1 negative [14]), Chinese hamster ovary cell (CHO) and its mutant derivative Lec8 (deficient in translocation of UDP-galactose [15]) were maintained in alpha-MEM (Cambrex) with 5% FCS. Human recombinant galectin-1 was purified and labeled with FITC (Sigma-*Aldrich*) or Alexa Fluor 555 (Zenon Labeling Kits, Invitrogen) as previously described [6]. The monoclonal Gal-1 and CD43 monoclonal antibodies [16] were produced in our laboratory. MitoTracker Red, LysoTracker Red, Brefeldin A-Bodipy 558/568, Cholera toxin B-Alexa Fluor 647 and -Alexa Fluor 488 were purchased from Molecular Probes, Invitrogen. Anti-mouse IgG-R-phycoerythrin and streptavidin-FITC were obtained from DAKO, anti-mouse IgG-Northern-Lights-557 from R&D Systems, anti-human CD45-FITC from ImmunoTools and anti-human CD7-PE/Cy5 from BD Biosciences Pharmingen. Other reagents were purchased from Sigma-*Aldrich*.

Analysis of Gal-1 binding and internalization with flow cytometry. For binding tests the cells were suspended at a concentration of 5×10^6 cells/ml in cell culture medium and incubated with 3.6 μM FITC labeled Gal-1 for 1 h at 4 °C in the presence or absence of 100 mM lactose. To determine the concentration dependence of Gal-1 internalization the cells were treated with different concentrations of Gal-1-FITC for 20 min at 37 °C. The cells were then incubated in cell culture medium containing 100 mM lactose for 30 min at 4 °C to remove the resident cell surface bound Gal-1 and to ascertain the visualization of the internalized Gal-1 fraction only. Finally, the cells were washed with FACS-buffer (PBS supplemented with 1% FCS and 0.1% sodium-azide) and analyzed by a FACSCalibur cytofluorimeter (Becton, Dickinson & Co.).

Analysis of Gal-1 internalization by laser scanning confocal microscope. Jurkat cells were suspended at a concentration of 5×10^6 cells/ml in cell culture medium and incubated with 3.6 μM Gal-1-FITC at 37 °C. Samples were taken at different time points as indicated in the figure legends, and the pattern of the internalized Gal-1-FITC was analyzed. The temperature dependence of Gal-1-FITC internalization was determined after 20 min incubation at different temperature as indicated in the figure legends. Adherent cells, such as C6, CHO and Lec8 were grown overnight on glass cover slips, the cell culture medium was then aspirated and Gal-1-FITC was added for 1 h at 37 °C. For ATP depletion the cells were pre-incubated with 40 mM sodium-azide for 30 min at 37 °C before allowing Gal-1-FITC internalization for 1 h at 37 °C. For the last 15 min of Gal-1 internalization, one of the following organelle specific dyes was added: 100 ng/ml Hoechst 33342 (a DNA minor-groove binding dye staining nuclei), 100 nM MitoTracker Red (accumulating in active mitochondria), 50 nM LysoTracker Red (selectively incorporating into acidic organelles) or 100 nM Brefeldin A-Bodipy 558/568 (localizing into Golgi apparatus and endoplasmic reticulum). The cells were analyzed with an Olympus FV1000 laser scanning microscope using 60x objective magnification. The attached fluorochrome did not influence the intracellular localization of Gal-1, since unlabeled Gal-1, detected with monoclonal antibody after cell fixation and permeabilization, showed a similar intracellular distribution (data not shown). On average, 30–50 cells were analyzed in each laser scanning microscope image and representative cells are shown in the figures. All experiments were repeated three times.

Detection of colocalization of Gal-1 and glycoproteins/glycolipids by laser scanning confocal microscope. CD45 and CD7 were cross-linked with FITC labeled anti-CD45 and PE/Cy5 conjugated anti-CD7

mAbs at 4 °C, respectively. CD3 or CD43 were cross-linked with unlabeled anti-CD3 or anti-CD43 followed by NorthernLights-557 conjugated anti-mouse IgG at 4°C. Next, the cells were warmed from 4 °C to 37 °C, and then Gal-1- FITC or Gal-1-Alexa Fluor 555 was added to the samples and co-internalization was carried out for 1 h. Co-localization of glycosphingolipid GM1 ganglioside and Gal-1 was analyzed using Cholera toxin B subunit (CTX)-Alexa Fluor 647 and Gal-1-Alexa Fluor 555 added simultaneously to the cells for 1 h at 37 °C. After washing, the cells were analyzed with confocal microscope. The specific fluorophores were excited with the following lasers: Argon-ion Laser at 488 nm for FITC, He/Ne Green Laser at 543 nm for Alexa Fluor 555 and NorthernLights-557, and He/Ne Red Laser at 633 nm for PE/Cy5 and Alexa Fluor 647.

Inhibition of clathrin- and raft-dependent endocytosis. For inhibition of clathrin-dependent endocytosis K^+-depletion was used according to the protocol described by Larkin *et al.* [17], with small modifications. Briefly, the cells were subjected to hypotonic shock by adding diluted cell culture medium (RPMI/H_2O 1:1) for 5 min at 37 °C. Then the cells were incubated in isotonic K^+-free buffer A (50 mM Hepes, pH7.4, 100 mM NaCl) for 10 min. Finally, buffer B (50 mM Hepes, pH7.4, 100 mM NaCl, 1 mg/ml BSA) or buffer C (50 mM Hepes, pH7.4, 100 mM NaCl, 1 mg/ml BSA, 10 mM KCl) was added to the cells to analyze the K^+-depleted or K^+-restored (control) samples, respectively. Raft-dependent endocytosis was inhibited with preincubation of the cells in cell culture medium containing 10 mM β-cyclodextrin (BCD) for 30 min at 37 °C. For simultaneous inhibition of clathrin- and raft-dependent pathways, BCD was added to the K^+-depleted samples. Internalization of Gal-1-Alexa Fluor 555 and CTX-Alexa Fluor 488 or transferrin-biotin (5 μg/ml) plus streptavidin-FITC was allowed for 15 min at 37 °C. Short internalization time was chosen to allow exploration of the early endocytotic steps and to avoid loss of the transferrin signal due to its recycling [18]. The resident cell surface-bound Gal-1 was removed with incubation of the cells in cell culture medium containing 100 mM lactose for 30 min at 4 °C to ascertain the visualization of the internalized Gal-1 fraction only. The cells were analyzed with a confocal microscope and the average fluorescence intensity of the internalized proteins was determined using FV10-ASW 1.7 Viewer software. Statistical differences were determined by independent Student's t-test and $P < 0.001$.

Results

Time and temperature dependent internalization of Gal-1 requires energy supply and cell surface presentation of glycosylated ligands. We have previously shown that Gal-1 internalizes to Jurkat cells at 37 °C [6]. Here we used FITC conjugated Gal-1 to follow its internalization and cellular localization with confocal laser scanning microscopy. The internalized Gal-1 was detected in small patches under the plasma membrane as early as 1 min after adding the lectin to Jurkat cells (Fig. 1A), and its quantity increased with time. The smaller particles fused into a bigger compartment at about 20 min of incubation. Counting 30–50 cells per sample, 83 % ± 11.5 % of the cells at any time point showed the typical internalization pattern. Elevation of the mean fluorescence intensity (Mf) with time in the samples demonstrated an increasing amount of bound and internalized Gal-1 (Fig. 1A). The intensity of internalization was also enhanced by increasing the Gal-1 concentration up to 10 μM (Fig. 1B). We used 3.6 μM of Gal-1 in our further experiments to achieve a strong fluorescence signal but avoided aggregation of the cells caused by high concentration of the lectin. Since Gal-1 has been shown to follow a non-classical secretion route [3], it was conceivable that internalization also occurred on a non-classical way. Nevertheless, the patchy intracellular distribution of Gal-1 suggested that it was connected to the vesicular/endosomal fraction. The temperature- and energy-dependence (Fig. 1C and Fig. 2A) also supported the idea that internalization of Gal-1 was mediated by endocytosis rather than free membrane penetration. Gal-1 internalization did not occur below 22 °C (Fig. 1C) and the optimal rate of intracellular accumulation of Gal-1 appeared at the physiological temperature (37 °C). Depletion of ATP-stores with sodium-azide treatment inhibited Gal-1 endocytosis, demonstrating an energy-dependent mechanism (Fig. 2A).

The presence of lactose, the minimal ligand for Gal-1, blocked the binding (Fig. 2B left panel) and internalization (Fig. 2B right panel) of Gal-1, confirming that its internalization was mediated by binding of Gal-1 to cell surface glycosylated structures. These findings were supported by the results obtained using the wild type CHO cell line and its glycosylation deficient mutant counterpart, Lec8 [15, 19]. In contrast to the wild type CHO, Lec8 cells bound Gal-1 at a very low level detected with flow cytometry (Fig. 2C left panel). While internalization of Gal-1 in CHO was similar to that of Jurkat cells, the FITC-labeled protein was below the detectable limit in Lec8, analyzed with a laser scanning confocal microscope (Fig. 2C right panel).

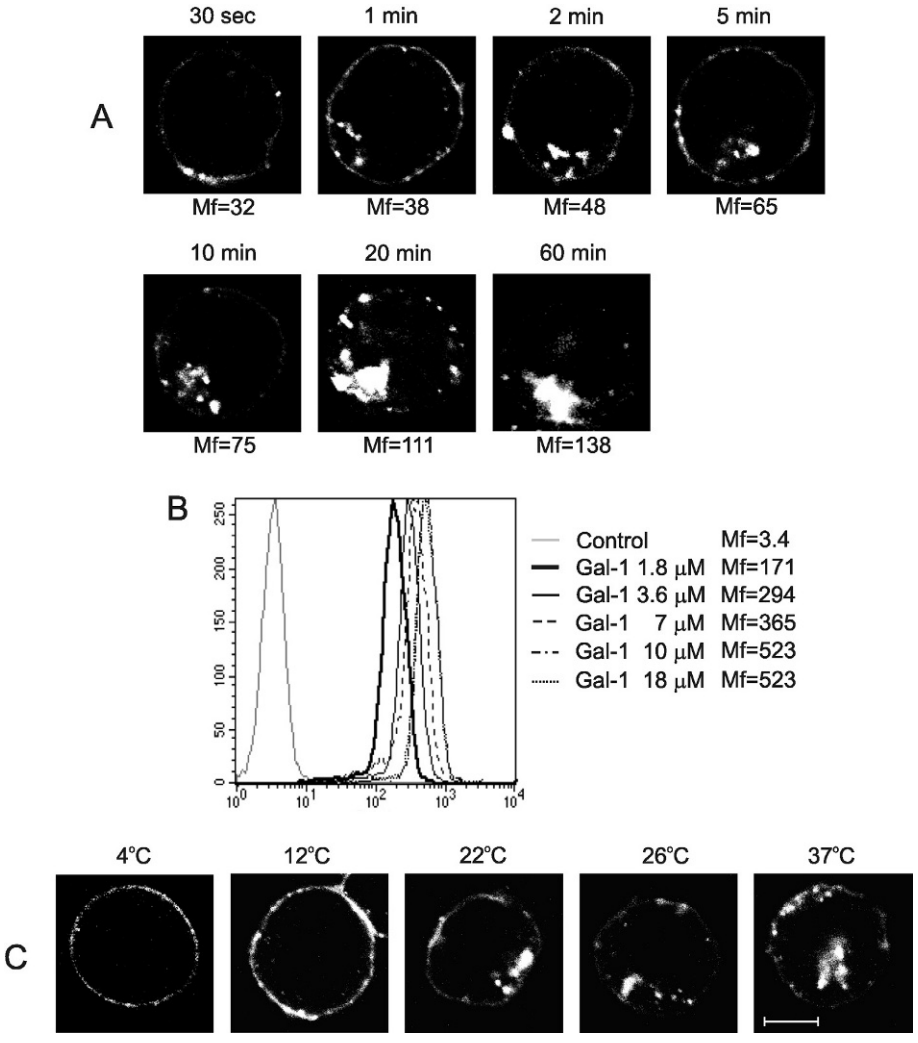

Figure 1. Time- and temperature-dependence of Gal-1 internalization. Jurkat cells were incubated with FITC-labeled Gal-1 for the indicated time at 37 °C (*A*), with different concentration of Gal-1 for 20 min at 37 °C (*B*), or at various temperatures for 20 min (*C*). The samples were analyzed with a confocal microscope and flow cytometry (*A*), with flow cytometry (*B*) or with a confocal microscope (*C*). Mean fluorescence intensity (Mf) obtained from flow cytometry data is presented (*A* and *B*). Scale bar, 5 μm.

Internalized Gal-1 accumulates in Golgi apparatus. The process of Gal-1 internalization was initiated at one particular region of Jurkat cells and then the endocytosed protein assembled in a discrete compartment (Fig. 1A). The intracellular localization of Gal-1 was analyzed using organelle-specific dyes. Gal-1 was not concentrated in the nucleus (stained with Hoechst 33342, Fig. 3A), the lysosomes (LysoTracker Red, Fig. 3C) or mitochondria (MitoTracker Red, Fig. 3B). In contrast, Gal-1 and Golgi system (stained with Brefeldin A) showed significant overlay (Fig. 3D) indicating that the internalized Gal-1 was collected in Golgi. After several hours, the fluorescent intensity was dramatically reduced, suggesting a step into the degradation phase rather than recycling to the cell surface (data not shown).

Gal-1 cointernalizes with CD7 and GM1 ganglioside. On T cells, specific Gal-1-binding glycoproteins, such as CD2, CD3, CD7, CD43 and CD45 have been identified recently [20, 11, 6]. Co-localization of these proteins with internalized Gal-1 was assayed to clarify whether these cell surface receptors were involved in Gal-1 entry into the cells. We had previously shown that the level of CD45 did not decrease on the cell surface during Gal-1 internalization, indicating that Gal-1 endocytosis was not accompanied by the down-regulation of the examined glycoprotein [6]. Accordingly, a strong co-localization of Gal-1 and CD45 was found on the cell surface; however, CD45 was not internalized (Fig. 4A). Two other Gal-1 binding glycoproteins, CD3 and CD43 also failed to co-internalize with Gal-1, although they were transported into the cytoplasm upon cross-linking with the corresponding specific monoclonal antibodies (Fig. 4B and C). CD7 has been implicated previously as the receptor responsible for transmitting Gal-1 induced apoptotic signal on T cells [21]. Supporting these data, Gal-1 and CD7 co-localized both on the cell surface and after internalization (Fig. 4D), indicating that CD7 might be one of the mediators of Gal-1 endocytosis. However, Gal-1 internalization also occurred in a CD7 deficient T cell

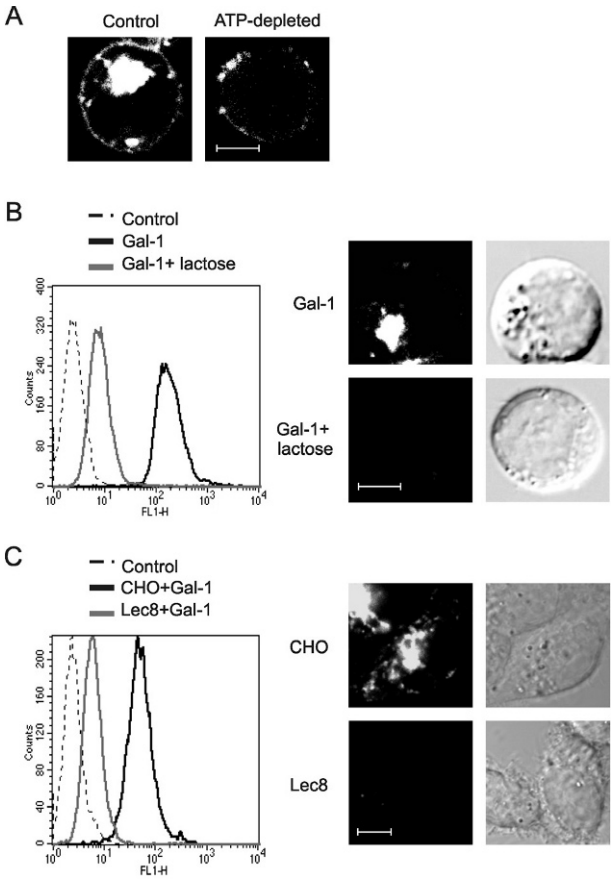

Figure 2. Internalization of Gal-1 is an energy dependent process mediated by cell surface glycoconjugates. (*A*) Jurkat cells were preincubated with (ATP-depleted) or without (control) sodium-azide, then Gal-1-FITC was added. The cells were analyzed with a confocal microscope. (*B*) Jurkat cells were incubated with Gal-1-FITC in the presence (grey line) or absence (black line) of lactose at 4 °C (left) or at 37 °C (right), and then analyzed by flow cytometry (left) or confocal microscopy (right). (*C*) CHO (black line) and Lec8 cells (grey line) were incubated with Gal-1-FITC at 4 °C (left) or 37 °C (right) and the bound and internalized Gal-1 was detected with flow cytometry (left) or confocal microscopy (right). Scale bars, 5 μm.

line, H9, with a similar pattern to that of Jurkat cells (data not shown).

The localization of the sphingolipid GM1 ganglioside, a non-protein Gal-1 binding glycoconjugate [22] was followed by its ligation with cholera toxin B subunit. We detected high extra- and intracellular overlay of GM1 and Gal-1 (Fig. 4E). Hence, GM1 ganglioside was determined as a possible transmitter of Gal-1 entry. Beside T cells, Gal-1 internalization was detected in other cell types of hematopoietic origin (i.e. B lymphoblasts (BL-41 and Daudi), histiocytic (U937) and monocytic cells (MonoMac), data not shown) and in non-hematopoietic cells as well (i.e. Chinese hamster ovarian cells (Fig. 2C), cervix carcinoma cell line (HeLa, data not shown) and glioblastoma (U87, data not shown)). Since these cells lack T cell specific proteins, such as

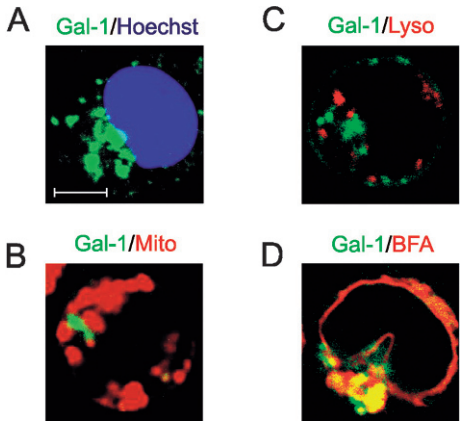

Figure 3. Internalized Gal-1 accumulates in the Golgi apparatus. Confocal microscopic pictures of Jurkat cells were taken after incubation of the cells with Gal-1-FITC for 1 h at 37 °C (green) and stained with organelle-specific dyes: (*A*) Hoechst 33342 – blue, (*B*) MitoTracker Red – red (Mito), (*C*) LysoTracker Red – red (Lyso), (*D*) Brefeldin A-Bodipy 558/568 – red (BFA). Scale bar, 5 μm.

CD7, GM1 might have a general role in mediating Gal-1 endocytosis. These experiments further supported our finding that Gal-1 was transported into the Golgi, since cholera toxin B subunit is a known Golgi-targeted GM1-binding protein [23], showing strong intacellular co-localization with Gal-1 (Fig. 4E).

Gal-1 endocytosis occurs in parallel via clathrin-dependent and -independent pathways. Potassium depletion was used to disrupt coated pits [17] and investigate whether Gal-1 internalization took place via clathrin-dependent or independent endocytosis. Potassium depletion decreased the internalization of Gal-1 significantly but not completely (Fig. 5A and quantitative analysis in Fig. 5B). Endocytosis of CTX was only marginally affected by inhibition of clathrin-coated pits. In contrast, the internalization of transferrin (used as a validation control), occurring via the classic clathrin dependent way [18], was abolished by K^+-depletion of the cells (Fig. 5C). Extra- and intracellular co-localization of Gal-1 with GM1 ganglioside, a component of lipid microdomains (Fig. 4E) raised the possibility of raft-mediated endocytosis. As shown in Fig. 5A and B, β-cyclodextrin (BCD), a raft-disrupting agent, did not affect CTX and Gal-1 endocytosis. Treatment of the cells with the combination of K^+-depletion and BCD during Gal-1 and CTX internalization resulted in a complete inhibition (Fig. 5A and B), suggesting the simultaneous presence of clathrin- and raft-mediated processes during Gal-1 endocytosis. In C6, a GM1 negative glioma cell line [14], K^+-depletion alone dramatically diminished the internalization of Gal-1 (Fig. 5D), indicating that in GM1 deficient cells the clathrin-dependent pathway dominated.

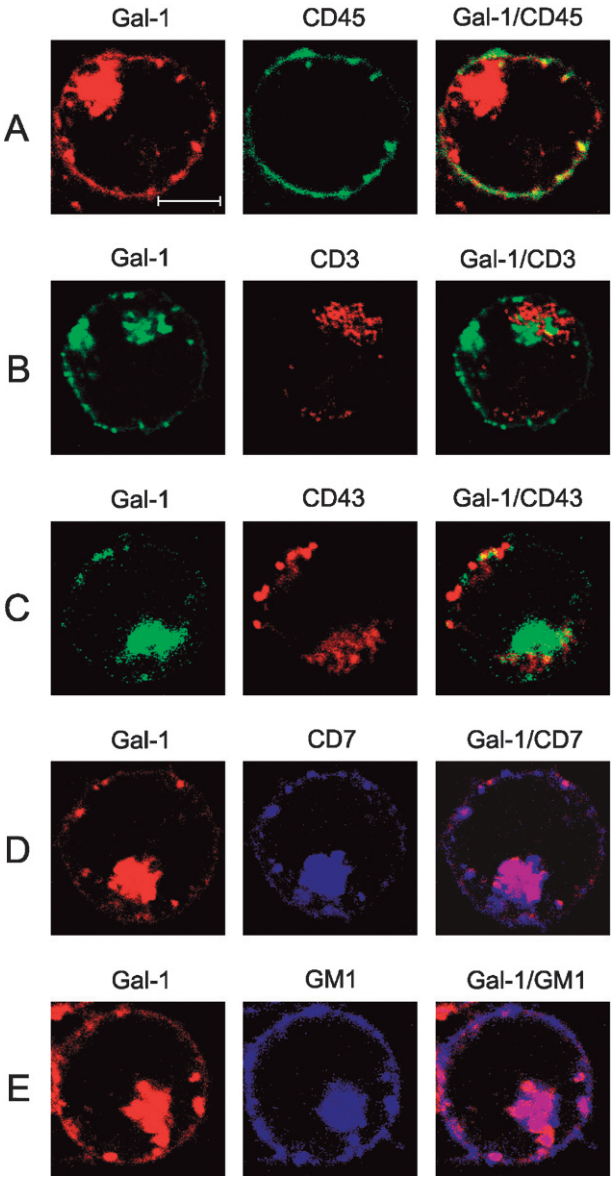

Figure 4. Cointernalization of Gal-1 with glycoproteins and GM1 ganglioside. The examined glycoproteins were cross-linked with fluorochrome labeled specific antibodies (*A*) anti-CD45-FITC, green and (*D*) anti-CD7-PE/Cy5, blue), or unlabeled antibodies and NorthernLights-557 conjugated anti-mouse IgG (*B*) anti-CD3, red and (*C*) anti-CD43, red) at 4 °C. Then the cells were warmed to 37 °C and Gal-1, conjugated with FITC (green, *B* and *C*) or with Alexa Fluor 555 (red, *A* and *D*), was added to them and they were incubated for 1 h. (*E*) Jurkat cells were incubated with Alexa Fluor 555 labeled Gal-1 (red) and Alexa Fluor 647 conjugated cholera toxin B subunit (GM1, blue) for 1 h at 37 °C. The cells were analyzed with a confocal microscope. Scale bar, 5 μm.

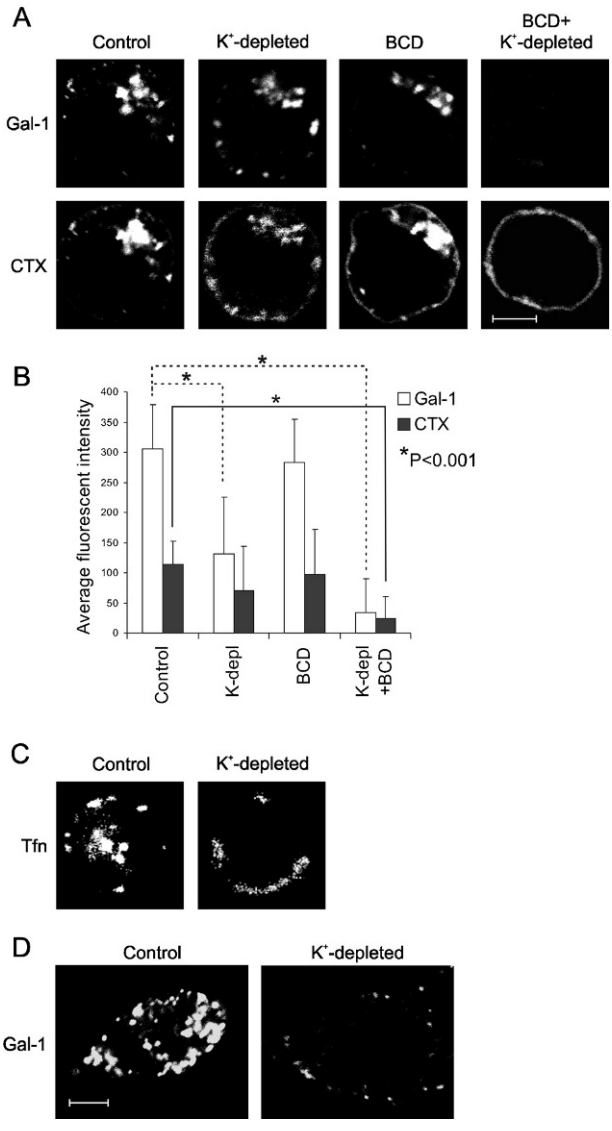

Figure 5. Gal-1 is internalized by clathrin- and raft-dependent pathways. Jurkat (*A*, *B* and *C*) or C6 cells (*D*) were incubated with Gal-1-Alexa Fluor 555 (*A*, *B* and *D*), CTX-Alexa Fluor 488 (*A* and *B*) or transferrin-FITC (Tfn, *C*) for 15 min at 37 °C in different buffers: control without inhibitor, K^+-free (K^+-depleted) buffer (*A*–*D*), in the presence of β-cyclodextrin (BCD) alone (*A* and *B*), or K^+-free buffer combined with BCD (*A* and *B*). After the internalization period the cells were washed with buffer containing lactose to remove the cell surface bound Gal-1. The samples were analyzed with confocal microscope and the average fluorescence intensity of the internalized proteins was determined using FV10-ASW 1.7 Viewer software (*B*). Statistical differences were determined using the Student's t-test and $P < 0.001$. Scale bars, 5 μm.

Discussion

Gal-1, a member of the galectin family, has recently challenged immunologists due to its powerful immunosuppressive function [9]. Soluble Gal-1 induces apoptosis of activated T cells and leukemic T cell lines [5] with a feature of a relatively prolonged time required for induction of T cell death [8]. So far, an explanation for this slow cell response to Gal-1 treatment has not been found. One possible reason could be the continuous and intensive internalization, shown in this paper, which is associated with the presence of Gal-1 in cell culture medium. As we show, the main mediator of Gal-1 endocytosis is GM1

ganglioside, a Gal-1 binding glycosphingolipid, which has not yet been implicated in Gal-1 triggered T cell apoptosis. Receptor mediated transport is suggested by the rapid internalization of Gal-1 (detected as early as 1 min) requiring its carbohydrate-binding activity, a temperature higher than 22 °C and an energy supply. It is known from earlier studies that Gal-1 binding results in raft formation in T cell membrane [8, 12]. The plausible mechanism is that Gal-1 binds to the raft-component, GM1 ganglioside [22], induces rapid reorganization of the lipid microdomains, then is delivered as a cargo to endosomes and the Golgi system following the route of glycosphingolipid trafficking [24]. However, inhibition of the raft-dependent pathway does not reduce Gal-1 (and GM1) internalization, suggesting that this might not be the sole route. Indeed, blocking clathrin-mediated endocytosis along with disruption of lipid rafts leads to the complete arrest of Gal-1 internalization, demonstrating that Gal-1 uses multiple mechanisms for its entry into Jurkat cells. This concept is supported by previous findings, namely that GM1 transports one of its ligands, cholera toxin, into the Golgi system via similar endocytotic machinery [25].

Pace et al. have shown that Gal-1 binding to cells segregates the main Gal-1 binding glycoproteins, CD45 with CD3 and CD43 with CD7, into different microdomains [11], but they have not followed the localization of Gal-1 itself in their study. Our results show a co-localization of Gal-1 with CD45, CD3, CD43 and CD7 on the cell surface, segregated in small patches. However, following internalization, only CD7, the putative receptor for transmitting the apoptotic signal of Gal-1 in T cells [21] displays a general overlay with Gal-1. Nevertheless, Gal-1 is also internalized by a CD7 negative cell line, H9, hence CD7 is not the mediator receptor, rather a bystander, traveling together with GM1-bound Gal-1 in the endocytosed membrane microdomain. Moreover, Gal-1 internalization takes place in cell lines of non-lymphoid origin as well, implying that Gal-1 internalization is not mediated by a glycoprotein specific for T cells, but rather by GM1 ganglioside, which is universally present on various cell types.

Polypeptides synthesized without signal peptide are frequently transported (secreted or internalized) through cellular membranes via an unconventional way. Well known examples are fibroblast growth factor (FGF)-1 and 2, interleukin-1β (IL-1β) and members of the galectin family [26]. It has recently been published that Gal-1 is secreted via inside-out transport involving direct translocation across the plasma membrane of mammalian cells and requiring as yet unidentified integral membrane proteins and cytosolic factors [27]. Galectin-1 transport machinery requires β-galactoside-containing surface molecules for export of intracellular Gal-1 [27] and, as our results show, for entry of Gal-1 as well. It would be intriguing to explore whether GM1 ganglioside also plays a role in the secretion of Gal-1.

The signal of internalized Gal-1 diminishes after several hours, indicating that Gal-1 degrades without accumulation in lysosomes, cytoplasm or nucleus. The role of Gal-1 internalization in biological processes has not yet been defined. Our unpublished results show that monoclonal antibody to Gal-1 is transported as a cargo together with the internalized lectin (R.F.B., unpublished results), suggesting that Gal-1 may function as a transporter for extracellular glycosylated particles. Supporting this theory, Gal-1 can cross-link HIV-1 with target cells and promote a stable adhesion of the virus to the cell surface, thereby augmenting the efficiency of the infection process [28]. The internalization of Gal-1 itself was not shown in the referenced study. Other members of the galectin family have also been suggested as participating in endocytosis and phagocytosis [29–31]. A prominent example is galectin-3, which is a scavenger receptor for advanced glycation end products and modified low density lipoproteins [32].

Further analysis is required to clarify whether Gal-1 internalization simply downregulates Gal-1 biological function, or whether it plays a role, as the above examples indicate, in outside-in transport mechanisms.

Acknowledgements. We acknowledge Andrea Gercsó for excellent technical assistance, Edit Kotogány for handling the flow cytometer, Dr. Ferhan Ayaydin and Zsuzsanna Kószó for help with the confocal microscope. This work was supported by grants from the Hungarian Scientific Research Fund (OTKA TS 049817, OTKA 69047) and the National Office for Research and Technology (Öveges József HEF_06>galektin). R.F.B. is a young research fellow of the Hungarian Academy of Sciences. We are grateful to the Bástyai–Holczer Foundation for supporting the cost of the publication of this paper.

1 Mayor, S. and Pagano, R. E. (2007) Pathways of clathrin-independent endocytosis. Nat. Rev. Mol. Cell. Biol. 8, 603–612.
2 Nichols, B. J., Kenworthy, A. K., Polishchuk, R. S., Lodge, R., Roberts, T. H., Hirschberg, K., Phair, R. D. and Lippincott-Schwartz, J. (2001) Rapid cycling of lipid raft markers between the cell surface and Golgi complex. J. Cell. Biol. 153, 529–541.
3 Cooper, D. N. and Barondes, S. H. (1990) Evidence for export of a muscle lectin from cytosol to extracellular matrix and for a novel secretory mechanism. J. Cell. Biol. 110, 1681–1691.
4 Camby, I., Mercier, M. L., Lefranc, F. and Kiss, R. (2006) Galectin-1: a small protein with major functions. Glycobiology 16, 137R-157R.
5 Perillo, N. L., Pace, K. E., Seilhamer, J. J. and Baum, L. G. (1995) Apoptosis of T cells mediated by galectin-1. Nature 378, 736–739.
6 Fajka-Boja, R., Szemes, M., Ion, G., Legradi, A., Caron, M. and Monostori, E. (2002) Receptor tyrosine phosphatase,

CD45 binds galectin-1 but does not mediate its apoptotic signal in T cell lines. Immunol. Lett. 82, 149–154.
7 Ion, G., Fajka-Boja, R., Tóth, G. K., Caron, M. and Monostori, E. (2005) Role of p56lck and ZAP70-mediated tyrosine phosphorylation in galectin-1-induced cell death. Cell. Death. Differ. 12, 1145–1147.
8 Ion, G., Fajka-Boja, R., Kovacs, F., Szebeni, G., Gombos, I., Czibula, A., Matko, J. and Monostori, E. (2006) Acid sphingomyelinase mediated release of ceramide is essential to trigger the mitochondrial pathway of apoptosis by galectin-1. Cell Signal. 18, 1887–1896.
9 Salatino, M., Croci, D. O., Bianco, G. A., Ilarregui, J. M., Toscano, M. A. and Rabinovich, G. A. (2008) Galectin-1 as a potential therapeutic target in autoimmune disorders and cancer. Expert. Opin. Biol. Ther. 8, 45–57.
10 Kiss, J. Kunstar, A., Fajka-Boja, R., Dudics, V., Tovari, J., Legradi, A., Monostori, E. and Uher, F. (2007) A Novel Anti-Inflammatory Function of Human Galectin-1: Inhibition of Hematopoietic Progenitor Cell Mobilization. Exp. Hematol. 35, 305–313.
11 Pace, K. E., Lee, C., Stewart, P. L. and Baum, L. G. (1999) Restricted receptor segregation into membrane microdomains occurs on human T cells during apoptosis induced by galectin-1. J. Immunol. 163, 3801–3811.
12 Chung, C. D., Patel, V. P., Moran, M., Lewis, L. A. and Miceli, M. C. (2000) Galectin-1 induces partial TCR zeta-chain phosphorylation and antagonizes processive TCR signal transduction. J. Immunol. 165, 3722–3729.
13 Carlsson, S., Carlsson, M. C. and Leffler, H. (2007) Intracellular sorting of galectin-8 based on carbohydrate fine specificity. Glycobiology 17, 906–912.
14 Fishman, P. H., Bradley, R. M., Hom, B. E. and Moss, J. (1983) Uptake and metabolism of exogenous gangliosides by cultured cells: effect of choleragen on the turnover of GMl. J. Lipid Res. 24, 1002–1011.
15 Stanley, P., Caillibot, V. and Siminovitch, L. (1975) Selection and characterization of eight phenotypically distinct lines of lectin-resistant Chinese hamster ovary cell. Cell 6, 121–128.
16 Ando, I., Ramadan, H., Bogdan, G., Vilmos, P. and Monostori, E. (1995) Induction of cell aggregation by the Workshop Adhesion Structure Subpanel 9 (CD43) mAb. In: Leukocute Typing V. White Cell Differentiation Antigens. pp. 1711–1712. Schlossman, S. F. (ed.) Oxford University Press, Oxford.
17 Larkin, J. M., Brown, M. S., Goldstein, J. L. and Anderson, R. G. (1983) Depletion of intracellular potassium arrests coated pit formation and receptor-mediated endocytosis in fibroblasts. Cell 33, 273–285.
18 Willingham, M. C., Hanover, J. A., Dickson, R.B. and Pastan, I. (1984) Morphologic characterization of the pathway of transferrin endocytosis and recycling in human KB cells. Proc. Nat. Acad. Sci. USA. 81, 175–179.
19 Deutscher, S. L. and Hirschberg, C. B. (1986) Mechanism of galactosylation in the Golgi apparatus. A Chinese hamster ovary cell mutant deficient in translocation of UDP-galactose across Golgi vesicle membranes. J. Biol. Chem. 261, 96–100.
20 Walzel, H., Blach, M., Hirabayashi, J., Kasai, K. and Brock, K. (2000) Involvement of CD2 and CD3 in galectin-1 induced signaling in human Jurkat T-cells. Glycobiology 10, 131–140.
21 Pace, K. E., Hahn, H. P., Pang, M., Nguyen, J. T. and Baum, L. G. (2000) CD7 delivers a pro-apoptotic signal during galectin-1-induced T cell death. J. Immunol. 165, 2331–2334.
22 Kopitz, J., von Reitzenstein, C., Burchert, M., Cantz, M. and Gabius, H.-J. (1998) Galectin-1 is a major receptor for ganglioside GM1, a product of the growth-controlling activity of a cell surface ganglioside sialidase, on human neuroblastoma cells in culture. J. Biol. Chem. 273, 11205–11211.
23 Orlandi, P. A., Curran, P. K. and Fishman, P. H. (1993) Brefeldin A blocks the response of cultured cells to cholera toxin. Implications for intracellular trafficking in toxin action. J. Biol. Chem. 268, 12010–12016.
24 Sharma, D. K., Choudhury, A., Singh, R. D., Wheatley, C. L., Marks, D. L. and Pagano, R.E. (2003) Glycosphingolipids internalized via caveolar-related endocytosis rapidly merge with the clathrin pathway in early endosomes and form microdomains for recycling. J. Biol. Chem. 278, 7564–7572.
25 Lencer, W. I. and Saslowsky, D. (2005) Raft trafficking of AB5 subunit bacterial toxins. Biochim. Biophys. Acta 1746, 314–321.
26 Nickel, W. (2003) The mystery of nonclassical protein secretion. A current view on cargo proteins and potential export routes. Eur. J. Biochem. 270, 2109–2119
27 Seelenmeyer, C., Wegehingel, S., Tews, I., Künzler, M., Aebi, M. and Nickel, W. (2005) Cell surface counter receptors are essential components of the unconventional export machinery of galectin-1. J. Cell. Biol. 171, 373–381.
28 Ouellet, M., Mercier, S., Pelletier, I., Bounou, S., Roy, J., Hirabayashi, J., Sato, S. and J. Tremblay, M. J. (2005) Galectin-1 acts as a soluble host factor that promotes hiv-1 infectivity through stabilization of virus attachment to host cells. J. Immunol. 174, 4120–4126.
29 Furtak, V., Hatcher, F. and Ochieng, J. (2001) Galectin-3 mediates the endocytosis of b-1 integrins by breast carcinoma cells. Biochem. Biophys. Res. Commun. 289, 845–850.
30 van den Berg, T. K., Honing, H., Franke, N., van Remoortere, A., Schiphorst, W. E. C. M., Liu, F. T., Deelder, A. M., Cummings, R. D., H. Hokke, C. H. and van Die, I. (2004) LacdiNAc-glycans constitute a parasite pattern for galectin-3-mediated immune recognition. J. Immunol. 173, 1902–1907.
31 Nagaoka, M. R., Strital, E., Kouyoumdjian, M. and Borges, D. R. (2003) Participation of a galectin-dependent mechanism in the hepatic clearance of tissue-type plasminogen activator and plasma kallikrein. Thromb. Res. 108, 257–262.
32 Zhu, W., Sano, H., Nagai, R., Fukuhara, K., Miyazaki, M. and Horiuchi, S. (2001) The role of galectin-3 in endocytosis of advanced glycation end products and modified low density lipoproteins. Biochem. Biophys. Res. Commun. 280, 1183–1188.