ISSN 0006-2979, Biochemistry (Moscow), 2011, Vol. 76, No. 8, pp. 958-967. © Pleiades Publishing, Ltd., 2011.
Published in Russian in Biokhimiya, 2011, Vol. 76, No. 8, pp. 1173-1184.
Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM11-041, April 24, 2011.

Galectins Promote the Interaction of Influenza Virus with Its Target Cell

E. S. Chernyy[1], E. M. Rapoport[1], S. Andre[2], H. Kaltner[2], H.-J. Gabius[2], and N. V. Bovin[1]*

[1]*Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, 117997 Moscow, Russia; fax: (495) 330-5592; E-mail: bovin@carb.ibch.ru*
[2]*Institute of Physiological Chemistry, Faculty of Veterinary Medicine, Ludwig Maximilians University, Veterinar str. 13, 80539 Munich, Germany*

Received January 31, 2011
Revision received March 21, 2011

Abstract—Influenza virus is known to bind sialoglycans located on the surface of the host cell. In addition, recent data suggest the involvement of other molecular targets in viral reception. Of note, a high density of terminal galactose residues is created on the surface of virions because of the influenza virus' own neuraminidase activity. Thus, we suggested the possibility for an interaction of the influenza virus with galactose-binding proteins – galectins. In the present work we studied the influence of several galectins on the adhesion and further internalization of virus into the cell; six virus strains and three cell lines were studied. Chicken galectins CG-1A and -2 as well as human galectins HGal-1 and -8 promote virus binding in dose dependent manner, but they do not influence the internalization stage. Also, galectins are able to restore the ability of influenza virus to infect desialylated cells up to the level of native cells. When CG-1A in physiological concentrations was loaded onto viruses, the adhesion level was higher than in the case of on-cell loading. The effect of adhesion increase depends on the glycan structure of target-cell as well as of virus. The aggregated data suggest a promotional effect of galectins during the stage of influenza virus binding with the surface of target-cell.

DOI: 10.1134/S0006297911080128

Key words: influenza virus, galectins, glycans

For a long time sialoglycans have been considered as exclusive cell receptors for influenza virus. In particular, carbohydrate chains of glycoproteins with terminal Neu5Acα2-6Gal residues were required for human viruses, and Neu5Acα2-3Gal residues were required for avian viruses [1-7]. Later, other molecules, which could promote the infection in some way, were identified. Particularly, influenza virus is able to interact with 6-O-sulfated glycans [8], known as structural motifs of mucins and glycolipids [9, 10]. In general, sialidase treatment of cells reduces the rate of viral infection, but the removal of sialic acids not always abolishes the infection [11, 12]. Knock-out of one of the two sialyltransferase genes, responsible for synthesis of Neu5Acα2-6Gal terminated glycans in mice, did not exclude the infection of airway epithelium with "human" phenotype influenza viruses [13]. It is necessary to mention that during the budding process from the surface of an infected cell, receptor proteins of the host-cell, which are not actually encoded by the viral genome, can get into virus particles [14] and possibly promote the reception.

Hemagglutinin and neuraminidase are major surface glycoproteins of influenza virus [15], and both of them carry glycans of complex- and oligomannose-type. As a result, the virus surface is densely covered with glycans [16, 17]. It has been proved for human immunodeficiency virus that its own glycans can promote the infection process by interacting with human cell lectins [18, 19]; in the case of influenza virus, there are also some experimentally shown prerequisites for additional interactions involving mannose- and galactose-terminated glycans of virus [8]. Besides mannose and galactose, sialic acids are at terminal positions of HA and NA carbohydrate chains,

Abbreviations: BSA, bovine serum albumin; C-virus, virus passaged in chicken embryos; CG, chicken galectins; FITC, fluorescein isothiocyanate; HA, hemagglutinin; HGal, human galectins; M-virus, virus passaged in MDCK (Madin–Darby Canine Kidney) cells; NA, neuraminidase; Neu5Ac, N-acetylneuraminic acid; PBA, phosphate buffered saline containing 0.2% BSA; PBS, phosphate buffered saline.

* To whom correspondence should be addressed.

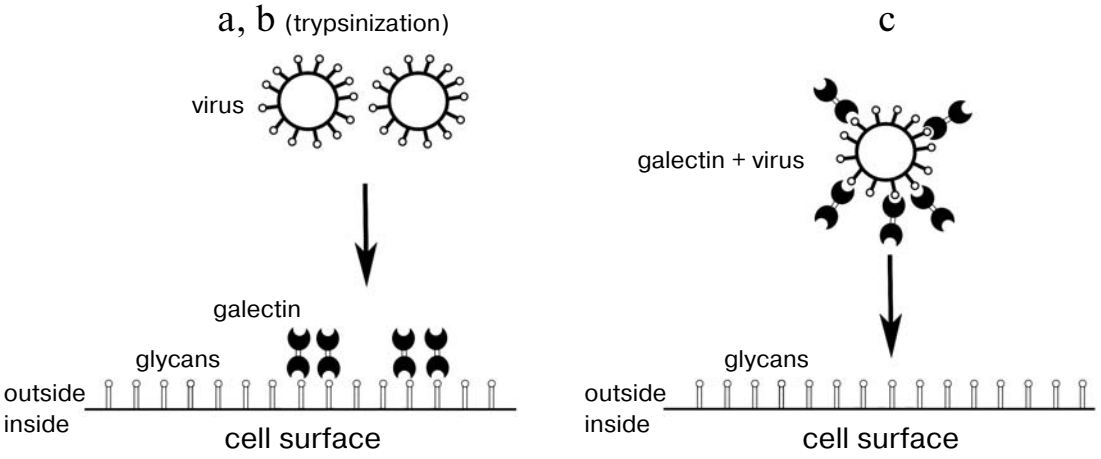

Fig. 1. Scheme illustrating the methods of infection of cells in the presence of galectins: a) *method 1*; b) *method 2*, which is similar to *method 1* (a) but has an additional stage of trypsinization; c) *method 3*.

but virus neuraminidase removes them and, as a result, β-galactose becomes the dominating terminal residue [16, 17, 20-22]. Thus, owing to high density of HA and NA, bearing several N-glycosylation sites, and total desialylation, a uniquely high density of galactose residues is generated on the surface of virus, suggestive for galectin anchoring.

Galectins are a family of small size β-galactose binding lectins [23]. Galectins -1, -3, -8, and -9 are found in the human respiratory tract [24, 25], whose epithelium is a primary target for influenza virus [4, 13]. Galectins are synthesized and localized in cytoplasm but under specific conditions (usually during the inflammation process) are secreted into extracellular space and can be found on the cell surface [26]. Previously, we have found that on the surface of the cell even proto- and chimera-type galectins are able to bind exogenous glycans [27]; therefore, we suggested their interaction with surface glycoproteins of influenza virus. Galectin-mediated anchoring can also proceed by an alternative mechanism (Fig. 1): after virus exit from the cell, or during the assembling process, virions adsorb galectins, which (with the help of NA, generating herein new galactose termini) promote virus binding with nearby uninfected cells. Here we present results demonstrating the ability of galectins to facilitate the adhesion of influenza virus on the cell surface.

MATERIALS AND METHODS

Reagents. The following chemicals and materials were used: BSA from Merck (Germany); sialidase from *Vibrio cholerae* which cleaves the α2-3 and α2-6 bound sialic acids, fluorescein isothiocyanate, streptavidin conjugated with FITC, and anti-rabbit IgG with FITC from Sigma (USA); fetal calf serum (FCS), DMEM/F12, gentamicin, trypsin, and L-glutamine from Gibco (UK). Digoxigenin-labeled lectin from *Maackia amurensis* (MAA) binding Neu5Acα2-3Gal-containing glycans and FITC-labeled anti-digoxigenin antibodies were from Roche (Germany). Biotinylated lectin from *Sambucus nigra* (SNA) binding Neu5Acα2-6Gal-containing glycans was from Vector Laboratories (USA); polyclonal antibodies to influenza A virus (H1N1) and anti-goat antibodies conjugated with FITC were from Abcam (UK).

Cell cultures. MDCK cell were obtained from the Institute of Cytology (Russian Academy of Sciences); MDCK cells transfected with the α2,6-sialyltransferase gene (MDCK6SIAT) were obtained from Dr. M. N. Matrosovich (Marburg, Germany). Swine testis cells (ST) were obtained from Dr. T. C. Harder (Greifswald-Insel Riems, Germany). Cells were cultured in DMEM-F12 containing 10% FCS and 2 mM glutamine.

Viruses. Viruses A/Mallard/NPV/41/04 (H7N1), A/Guinea fowl/Germany/DZ3/85 (H2N2), A/Lesser black-backed gull/Finland/R2265/09 (H13N2), and A/Rheinland Pfalz/14/07 (H1N1) were obtained from Dr. T. C. Harder. Viruses A/FPV/Rostock/34 (H7N1) (FPV-H7N1) and A/NIB/23/89-MA (H1N1) (NIB-H1N1) were given by Dr. A. S. Gambaryan (Moscow, Russia). All viruses were grown in chicken embryo. A/NIB/23/89-MA virus was passed in chicken embryo (C-variant) and then in the MDCK cell culture (M-variant). Viruses were purified as described elsewhere [28].

The viruses were labeled with FITC according to a previously developed method [29]. Briefly, to the virus-containing solution (10 mg/ml in PBS) was added an equal volume of FITC solution (0.1 mg/ml in 0.5 M $NaHCO_3$, pH 9.5). The mixture was incubated for 1 h at room temperature. FITC-labeled virus was purified on a column of Sephadex G-50 and stored in PBS containing 0.01% NaN_3 at 4°C.

Galectins and anti-galectin antibodies. Chicken galectins CG-1A (CG-16), CG-1B (CG-14), CG-2, CG-3, CG-8I, human galectins HGal-1, HGal-2, HGal-3, HGal-4, HGal-7, HGal-8, and rat galectin-2 and anti-galectin antibodies were obtained, purified, and tested as described elsewhere [30-40].

Study of the interaction of influenza virus with cells. *Method 1.* Adhesive cells were removed from plastic with Versene solution and washed twice with PBA using centrifugation at 800 rpm. Aliquots of the cell suspension ($1 \cdot 10^5$ cells in 50 μl) were incubated with 50 μl of galectin-containing solution (20-400 μg/ml in PBA) for 30 min at 10°C under intensive agitation on a shaker (to avoid aggregation and precipitation of cells) [27]. The cells were washed using centrifugation and incubated with FITC-labeled virus for 1 h at 37°C. Depending on the strain, the virus was used in concentration 2-40 μg/ml. The cells were then washed three times with PBA using centrifugation under the same conditions. After that cells were intently mixed with 2 ml of PBS and transferred into cytometry tubes. Fluorescence of viruses on cells was analyzed on a FACScan laser flow cytometer (Becton-Dickinson, USA) at 488 nm at room temperature. The data was analyzed using Dako Summit v.4.3 software. At least 3000 cells were analyzed in each sample.

A part of the experiments was carried out with unlabeled virus. Antibodies to the virus were used for the detection: after the incubation with virus the cells were washed and then incubated with anti-virus antibodies (5 μg/ml) and with the FITC-labeled anti-goat IgG (dilution 1 : 400 in PBA), both for 20 min at 4°C.

Method 2. Cells were grown on 24-well plates (Grenier, Switzerland). After formation of monolayer, the cells were washed three times with PBA and the galectin solution in PBA (150 μg/ml) was added. Cells with galectin were incubated for 30 min at 37°C. Then galectin solution was removed and the FITC-labeled virus was added and incubated for 1 h at 37°C. The monolayer was treated with trypsin for 10 min at 37°C, washed three times with PBA using centrifugation, and then analyzed by flow cytometry as described above.

Method 3. Unlabeled virus was mixed with an equal volume of galectin solution in PBA (200 μg/ml) and incubated for 30 min at 37°C. In parallel, adhesive cells were removed from plastic with Versene solution and washed twice with PBA using centrifugation. The galectin-loaded virus was added to the cells and incubated for 1 h at 37°C followed by washing three times with PBA using centrifugation, and the virus was detected using antibodies. The samples were analyzed on the flow cytometer as in *method 1.*

All the infection experiments were repeated at least three times to check their internal validity.

Desialylation of cells. Cells were removed from plastic with Versene solution and washed three times with DMEM/F12 medium. The cells were then treated with neuraminidase (2 U/ml) for 3 h at 37°C in DMEM/F12 medium. After that the cells were washed three times with PBA using centrifugation at 800 rpm. The desialylation was monitored by binding with SNA and MAA. For that purpose, 50 μl of lectin solution labeled with digoxigenin (MAA, 1 μg/ml) or biotin (SNA, 20 μg/ml) was added into the wells of a U-shaped plate containing $2 \cdot 10^5$ cells in 100 μl of PBA and incubated for 30 min at 4°C. The cells were then washed with PBA and incubated with FITC-labeled antibodies to digoxigenin (dilution 1 : 10 in PBA), or FITC-labeled streptavidin (dilution 1 : 50 in PBA) for 30 min under the same conditions. After the reaction was completed, the cells were washed with PBA under the same conditions and analyzed by flow cytometry as described above.

RESULTS

Interaction of influenza viruses with galectin-loaded cells. Here we use the following terminology. *Adhesion* – the first stage of interaction, before virus enters the cell. *Internalization* – the stage of virus penetration through the plasma membrane of the cell. *Infection* – the result of realization of both mentioned stages; the further infection process occurring inside the cell was not studied in the present work. The virus was incubated with cells for one hour at 37°C, thus there was adhesion of virions to the cell surface, and their internalization also started. *Method 1* (Fig. 1a) allows estimation of the total number of internalized viruses and viruses still found on the surface of the cell. *Method 2* (Fig. 1b) has the additional trypsinization stage and allows determination of only (or predominantly) the number of internalized virions. *Percent of infection* was registered as ratio of cells with fluorescent signal (i.e. cells with virus) to the total number of cells.

Concentration of the virus was optimized individually for every strain so that no more than 50% of cells were infected (without galectin) allowing detection of reliable decrease or increase of the percent of infection.

The galectins were loaded onto cells (400 μg/ml generally) before the infection using the previously developed method [27]; after that the results of infection with or without galectin were compared. When the loading concentration of galectins was reduced to 20 μg/ml, the influence of galectins on the infection could still be detected; using lower concentrations showed no effect.

To exclude the possible contribution of fluorescent label (attached to HA or NA) of the virus on the examined effect, *method 1* in its standard variant (labeled virus) was compared with the method wherein the cells were infected with non-labeled virus and quantitation was done by antiviral antibodies. Galectins exhibited an analogous effect in both variants of this method to allow the more practical variant with labeled virus to be used in the following study.

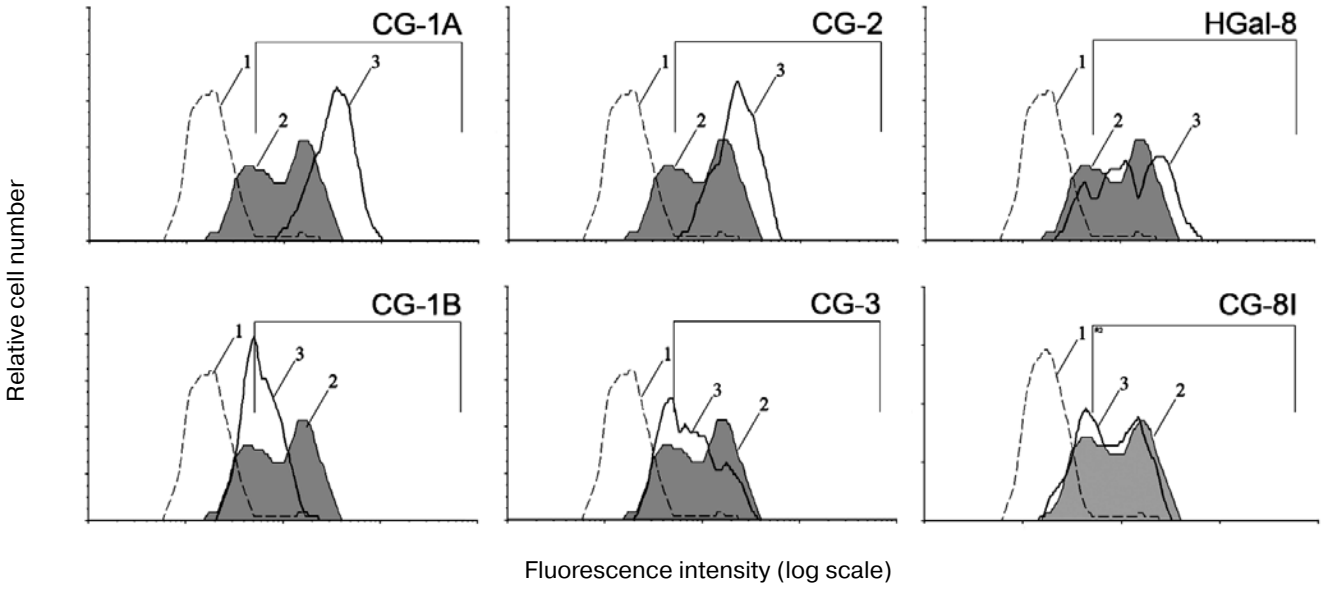

Fig. 2. Activity of galectins in the process of interaction of influenza virus A/Rheinland Pfalz/14/07 (H1N1) with ST cells (*method 1*). *1*) Control cells without virus; *2*) cells with virus without galectin; *3*) galectin-loaded cells with virus. Cytofluorimetry data: horizontal axis, fluorescence intensity of the selected cell population (in arbitrary units); vertical axis, relative frequency of occurrence.

The influence of chicken galectins CG-1A, CG-1B, CG-2, CG-3, and CG-8I and three human galectins HGal-1, HGal-3, and HGal-8 on the infection of ST-cells with human influenza virus H1N1 was studied. Galectins CG-1A and CG-2 and, to a smaller degree, HGal-8 promoted the adhesion and further internalization of viruses (Fig. 2), while CG-1B, CG-3, CG-8I, and the other studied human galectins (HGal-1 and HGal-3, results not shown) did not show any effect.

Figure 3 reports on the influence of CG-1A on the infection of cells with avian viruses H7N1, H2N2, and H13N2 and also with H1N1 virus. Chicken galectins CG-1A and CG-2 also increased level of infection of cells with H13N2 and H1N1, and to a smaller degree with H2N2 and H7N1 viruses, while human galectins including HGal-8 (but not for H1N1; Fig. 2) did not exert any influence (results not shown).

Notably, similar results were obtained on MDCK cells (results not shown). Additionally, it was shown that human HGal-2, HGal-4, and HGal-7 and also rat galectin-2 did not effect the infection of MDCK cells with viruses used in this work serving as inherent specificity controls (results not shown).

Comparison of the action of galectins CG-1A and CG-2 on MDCK and MDCK6SIAT cells, when infected with H1N1, H2N2, H7N1, FPV H7N1, and H13N2 viruses, revealed that the influence of galectins was relatively weak on transfected cells (results not shown).

Stage of internalization (*method 2*). The difference from *method 1* was that *method 2* included an additional step of trypsinization (Fig. 1b). Hereby, virions, which had adsorbed on the surface but had not entered the cell, will likely be removed by trypsin and thus not detected. The corresponding method was previously used for the detection of HIV virus inside the cell [19]. As illustrated in Fig. 4, when cells were infected with virus H1N1 and treated with trypsin, the number of virus-positive cells was about twofold lower than in *method 1*, i.e. only a part of bound viruses was able to enter the cell under these experimental conditions (data shown for MDCK6SIAT cells; for MDCK cells and other virus strains the results were similar).

When cells were loaded with CG-1A or CG-2 and infected under the conditions of *method 2*, it turned out that the level of infection increase was less than 5% (results not shown), i.e. these galectins did not show any influence on the stage of internalization. In the following experiments, to study the effect of galectin loading, only *method 1* was used.

Influence of galectins on virus binding with desialylated cells. MDCK cells were sequentially treated with neuraminidase, loaded with galectin, and infected with virus NIB H1N1 (C-virus). The effectiveness of the enzymatic activity was monitored by the decrease in binding of SNA and MAA, which recognize terminal 6- and 3-sialosides, respectively (results not shown). As shown in Fig. 5, desialylation of cells leads to significant reduction in viral adhesion. At the same time, the desialylated cells loaded with galectins CG-1A and CG-2 (but not CG-3 used as control) restored the infection ability almost up to the level of cells not treated with sialidase.

Role of virus glycans in binding with galectins. The influence of the viral carbohydrate chains on galectin-mediated cell infection was studied using C- and M-vari-

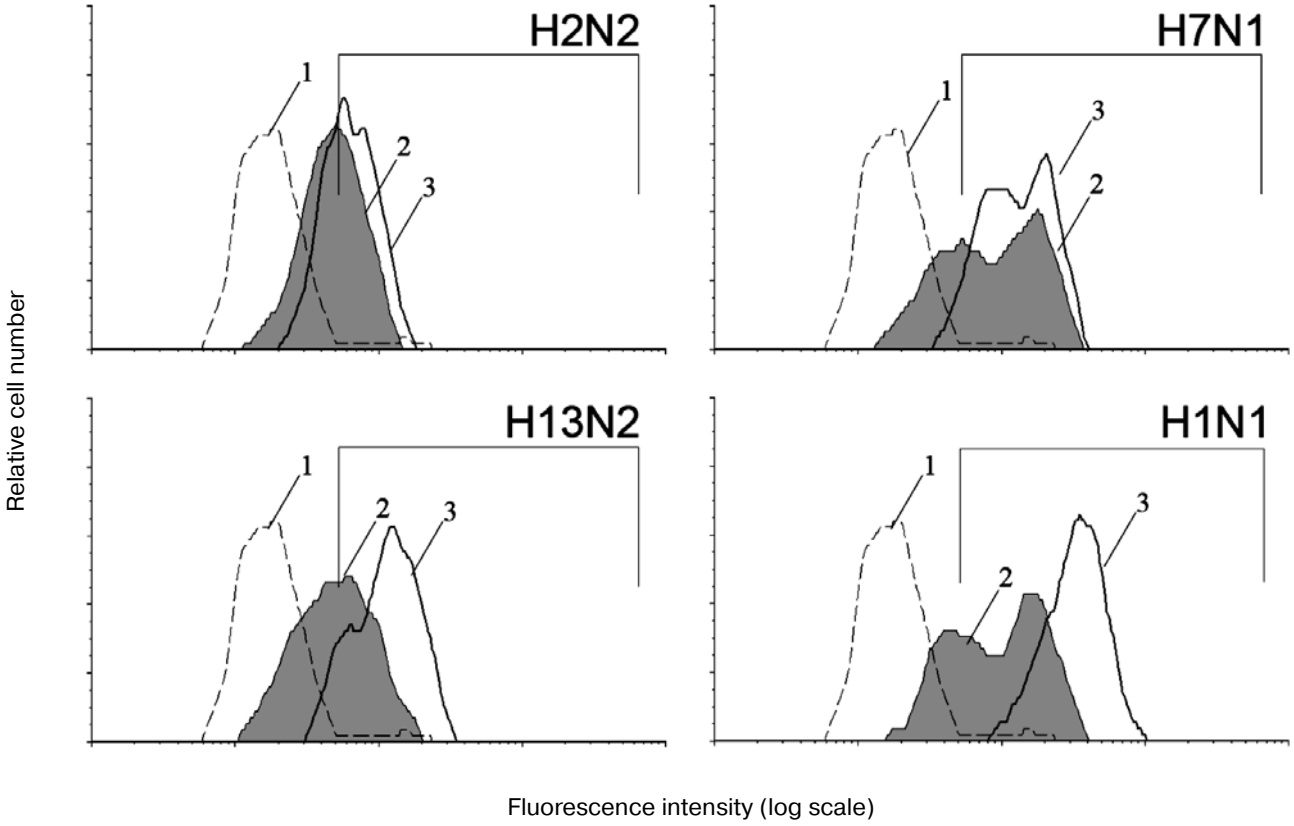

Fig. 3. Activity of galectins in the process of interaction of influenza viruses A/Guinea fowl/Germany/DZ3/85 (H2N2), A/Mallard/NPV/41/04 (H7N1), A/Lesser black-backed gull/Finland/R2265/09 (H13N2), and A/Rheinland Pfalz/14/07 (H1N1) with ST cells (*method 1*). *1*) Control cells without virus; *2*) cells with virus without galectin; *3*) galectin-loaded (CG-1A) cells with virus.

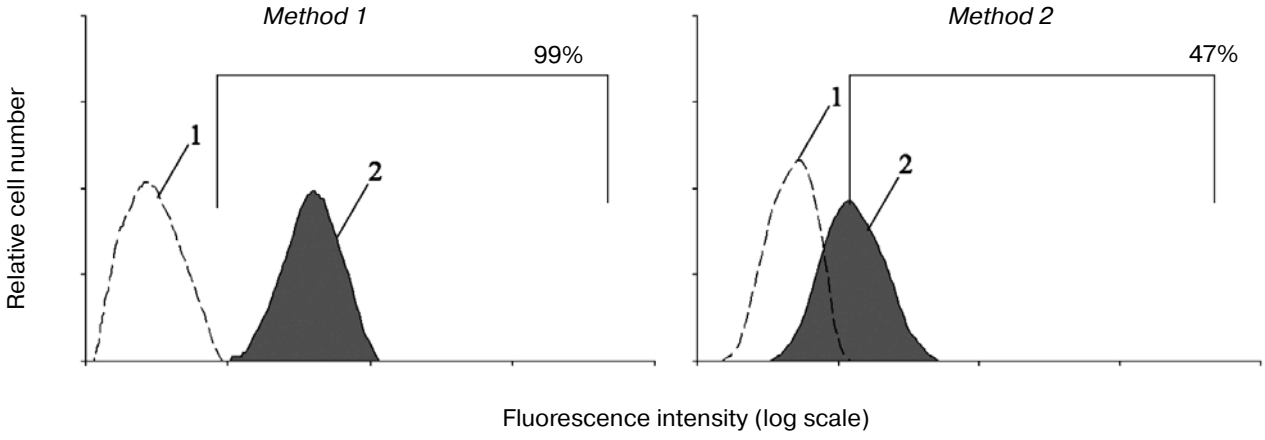

Fig. 4. Interaction of influenza virus A/Rheinland Pfalz/14/07 (H1N1) (10 μg/ml) with MDCK6′SIAT cells (*methods 1* and *2*): *1*) control cells without virus; *2*) cells with virus.

ants of the same virus. The results obtained for the virus passaged in chicken embryos (C-virus) were compared with results obtained with virus grown in MDCK cells (M-virus). In this study we used CG-1A, CG-2, HGal-1, and HGal-8 galectins (150 μg/ml). Collectively, these galectins promoted the infection of cells with M- to a greater degree than with the C-variant of virus (Fig. 6).

Infectivity of influenza virus loaded with galectins (*method 3*; Fig. 1c). In this series of experiments, galectins were loaded on the surface of virions rather than

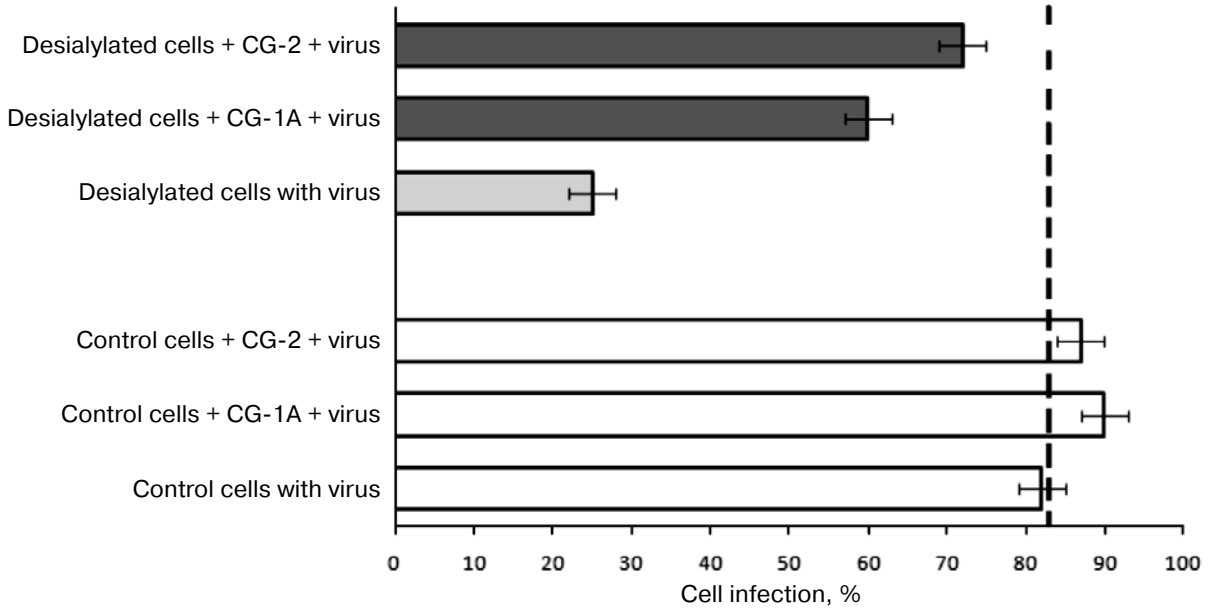

Fig. 5. Interaction of influenza virus A/NIB/23/89-MA (H1N1) (C-virus) with MDCK cells in the presence of galectins (*method 1*).

on cells: influenza virus NIB H1N1 (C-virus) was incubated with galectin and after that galectin-loaded virus was used to infect the MDCK cells. Comparison with *method 1* showed that loading of CG-1A on the virion surface more effectively increased the infection than in the case of loading the same galectin on cells (Fig. 7). The opposite result was obtained for CG-2, which better promoted the infection when presented on the surface of the cell than on virus, revealing disparities between the two homodimeric proto-type CGs.

DISCUSSION

It is reasonable to assume that the high density of terminal galactose residues on the influenza virus surface can favor binding with galectins; but the result of this interaction has so far not been studied in detail. On one hand, galectins can in principle bind the viral carbohydrate chains located near the receptor binding site of hemagglutinin [41], and therefore spatially impair virus attachment to the cellular sialosides. Additionally, it is known that carbohydrate chains are located near the cleavage site of HA [42] important for the fusion of viral and cell membranes. Galectin located near this site can prevent the release of viral RNA. On the other hand, the binding of galectins can promote the infection, as it was indicated for HIV [19] and HTLV [43] viruses. The bi- to penta-valency enables galectins to bridge cells or cells and pathogens [44-46]. In this context, the galectin can serve as bridge between cell and virus, which may then favor internalization. Also, a masking by galectins in the process of interaction with the host-organism antibodies with the virus can be anticipated. As a result, it allows the virus to escape recognition by the innate and adaptive immune system of the host. Summing up, galectin binding favored by high-density galactose presentation can have different effects, a question attracting our interest.

Building on previous work, a cell model has been developed for studying carbohydrate specificity of galectins [27]. Galectins were loaded on the cell surface. The principle of this approach was applied in this work to study the influence of galectins on the process of influenza virus to cell interaction. Human and avian viruses were used in the infection experiments, but all viruses were passaged on chicken embryos, a reason why the study of the action of chicken galectins was our primary goal. Physiological concentrations of galectins in the human body may vary considerably in different tissues, e.g. 0.5 μg/ml in serum [47] or 300 μg/ml in lymphoid tissue of tonsils [18]. After infection by pathogenic microorganisms galectin concentration can locally increase to 600 μg/ml [48]. Based on these numbers, we used a concentration of 400 μg/ml and lower.

According to our results galectins generally promote adhesion and thus indirectly facilitate the penetration of virus inside the cell. We did not observe any examples of retroaction. Notably, the proto-type chicken galectins CG-1A and CG-2 increased cell binding for all of the investigated virus strains. At the same time, galectin CG-1B, which is closely related to CG-1A, did not. It was recently showed that cell staining on Capan-1 pancreatic cancer cells was lower for CG-1B than CG-1A/2 [36].

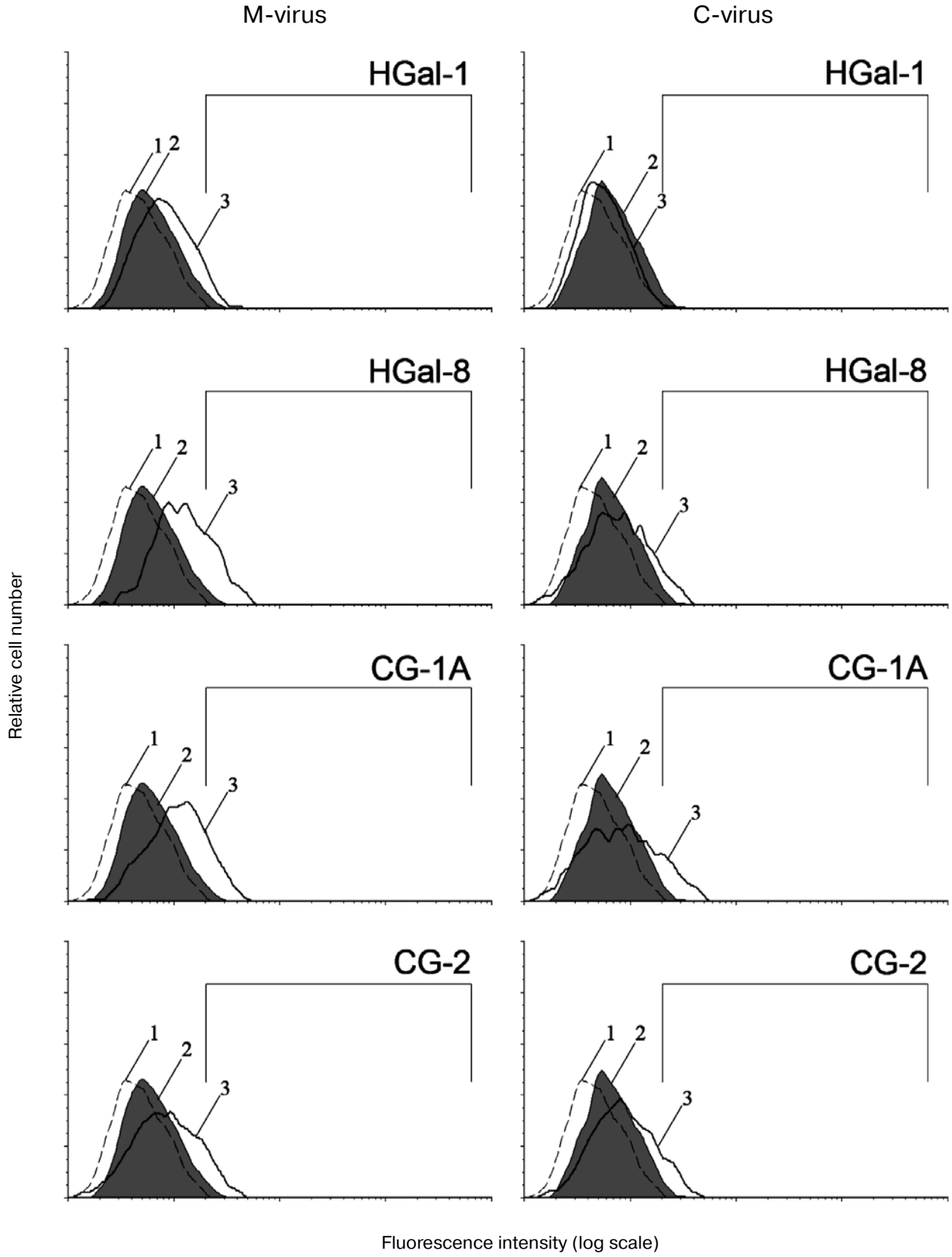

Fig. 6. Interaction of cultural (M-virus) and allantois (C-virus) variants of influenza virus A/NIB/23/89-MA (H1N1) with MDCK cells (*method 1*). *1*) Control cells without virus; *2*) cells with virus without galectin; *3*) galectin-loaded cells with virus.

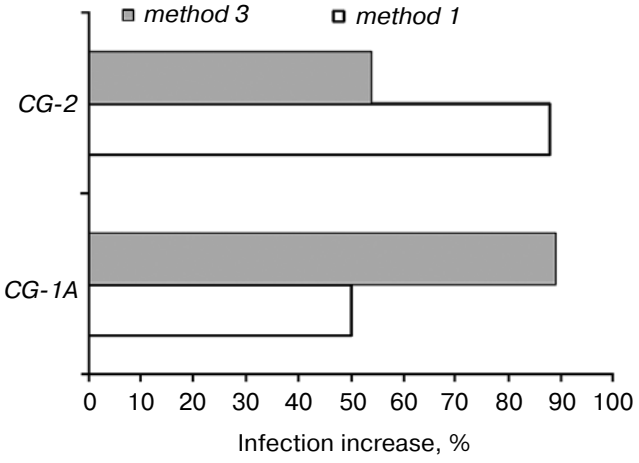

Fig. 7. Interaction of influenza virus A/NIB/23/89-MA (H1N1) (C-virus) with MDCK cells in the presence of galectins. Different results are obtained when galectin was loaded on the virus (grey bars) or on the cell surface (white bars). Horizontal axis, percentage of infection increase compared to control (no galectin).

Together with previous reports [49-52] data on binding neoglycoconjugates indicate affinity differences to N-acetyllactosamine repeats, spatially privileged sites on cells. These results could explain the inability of CG-1B to act as a bridge between the cell and virus and thus promote the infection.

Looking at HGal variants, several proteins such as HGal-1 and HGal-8 selectively affected the infection of cells with H1N1 and NIB H1N1 viruses. Thus HA and NA of these viruses appear to bear carbohydrate chains of suited structure that serve as distinctive ligands for certain galectins.

As mentioned above, the density of galectin ligands on the surface of any influenza virus is unusually high, so a similar effect can be expected, independent of virus type and strain. Indeed, as it can be seen from our data, both human and avian viruses bind target cells better in the presence of galectin, that the virus phenotype apparently has no significant impact on galectin effect under these conditions.

For human galectins it has recently been shown that 6- (but not 3-) sialylation prevented the binding of most galectins with lactosamines [53, 54]. We assume that this is also true for chicken galectins because part of the amino acid sequence of carbohydrate binding domain of the galectins is conservative [55]. The small effect, if any, of galectins on viral adhesion on MDCK6SIAT cells, which have a higher level of terminal Neu5Acα2-6Gal residues [56], is likely to the impairment of ligand reactivity by α2,6-sialylation.

The initial process of infection involves two stages – specific adhesion and subsequent internalization of the virus, leading to the question at what stage galectins play the enhancing role. After changing the experimental conditions we concluded that galectins mainly affect the primary stage of virus-to-cell binding.

According to results of fluorescence microscopy, after loading CG-2 on MDCK cells and infection with A/NIB/23/89-MA (H1N1) or A/Puerto Rico/8/1934 (H1N1) viruses, co-localization of virus and galectin on the cell surface is observed (data not shown), corroborating our data on galectins promoting the infection.

Additional information was provided from the infection studies of desialylated cells. On one hand, the number of potential ligands for influenza virus on the cell surface is decreased after the desialylation, on the other hand, the number of terminal galactose residues is increased. Accordingly, the galectin-binding capacity is elevated. As a result of desialylation of MDCK cells we observed the expected reduction of binding of virus to the cell surface. However, the loading of galectins increased virus adhesion and, in some cases, restored the binding, up to the level provided by the canonical receptor system HA/sialoside. We should note that HA and NA are antagonist proteins, since HA binds sialic acid, while NA cleaves it. Imbalance of HA/NA activity may reduce virus replication efficiency [57]. In this situation, galectin functionality can compensate the lack of sialoreceptors and thus correct a failure in activity of HA and NA.

It is known that HA of influenza virus facilitates fusion besides receptor binding. Because of that the studied galectin-mediated pathway may not necessarily accomplish complete infection. Galectins can thus obviously play an active role in the reception process during the interaction of virus with hyposialylated surface. An activity ensuing the contact formation could explain the mechanism of virus infection of cells that do not have the HA ligands on their surface, i.e. cells treated with sialidase [11].

Since carbohydrate chains are key ligands for galectins, the attachment of these proteins to the virus depends on the glycosylation of its surface glycoproteins – hemagglutinin and neuraminidase. In addition, specific host cell glycolipids can be inserted into the membrane of virus particles. As a result, the carbohydrate pattern of the virion surface is largely dependent on the type of cells these virus particles are produced in. We compared the virus passaged on MDCK cells with the same virus originating from chicken embryos. It is known that viruses grown in cell cultures (in this case, the M-virus) tend to have longer and more branched carbohydrate chains than those derived from the allantoic fluid of chicken embryos (C-virus) [58]. Moreover, viruses can lose sites for glycosylation on HA when passed on chicken embryos. The results of this study showed that difference in glycosylation reflected (though not dramatically) the interaction of galectins with the virus. Though CG-1A and CG-2 promoted the infection with both C- and M-variants, in the case of M-virus the increase of virus adhesion was enhanced. The maximum difference between the viruses

was detected for HGal-8 and HGal-1, which interacted better with M-virus and showed almost no effect on the infection with C-virus.

As galectins are secreted from cells during the spread of infection, they may remain anchored in the glycocalyx or bind to virions. The topography of galectins on the virus or on the cell may differ significantly. On the cell surface galectins can be involved in *cis*-interaction with nearby glycans, and such a galectin will not have a free glycan-binding site able to bind external ligands [27]. Moreover, according to our recent unpublished data galectins can be deeply immersed in the glycocalyx and thus be unable to bind external high-affinity ligands. In contrast, galectin accessibility on the virus surface may be different. Considering these particular features, we separately examined the effect of galectins on cells and on viruses. As expected, the loading of CG-1A on virions more efficiently promoted the infection of cells than loading of the same galectin on the cell membrane. At the same time, the opposite result was obtained for CG-2, drawing attention to disparate characteristics of even closely related family members.

According to our data, galectins in physiological concentrations are able to bind with glycans of the target cells and of influenza virus. As a result, this affords enhanced adhesion ability of the virus and increases the probability for cell infection. Of course, our model experiments cannot unambiguously answer the question whether galectin-mediated binding of virus to target cell takes place in infection *in vivo*. Considering the dynamics of infection spread, one can envision that galectins may be able to be actively engaged at a later stage of infection and play an important role in the further reception of influenza virus. It is obvious that at the focus of infection the concentration of viral NA is significantly increased. The resulting loss of sialic acids can lead to a situation when on the surface of potential target cells (not yet infected) the density of the classical influenza virus receptors is significantly reduced. If the infection process involves a secretion of galectin, as known for galectin-3 (Mac-2 antigen) [52], their local concentration can be significantly increased. In such a situation the contact of galectins with virions compensates for deficiency in classical receptors and helps the virus to overcome the obstacles encountered during the infection spread.

We thank Dr. A. S. Gambaryan (M. P. Chumakov Institute of Poliomyelitis and Viral Encephalitides, Moscow, Russia), Dr. M. N. Matrosovich (Institute of Virology, Philipps University, Marburg, Germany), and Dr. T. C. Harder (Friedrich-Loeffler-Institute, Greifswald-Insel Riems, Germany) for the viruses and cell cultures and methodological support of this work.

This work was supported by the Russian Foundation for Basic Research (project Nos. 10-04-00959 and 10-04-01693), the Fundamental Research Program "Molecular and Cell Biology" of the Russian Academy of Sciences Presidium, and the EC GlycoHIT consortium.

REFERENCES

1. Gottschalk, A. (1959) *The Viruses* (Burnet, F. M., and Stanley, W. M., eds.) Academic Press, N. Y., pp. 51-61.
2. Matrosovich, M. N., Gambaryan, A. S., Teneberg, S., Piskarev, V. E., Yamnikova, S. S., Lvov, D. K., Robertson, J. S., and Karlsson, K. A. (1997) *Virology*, **233**, 224-234.
3. Matrosovich, M. N., Tuzikov, A., Bovin, N., Gambaryan, A., Klimov, A., Castrucci, M. R., Donatelli, I., and Kawaoka, Y. (2000) *J. Virol.*, **74**, 8502-8512.
4. Matrosovich, M. N., Matrosovich, T. Y., Gray, T., Roberts, N. A., and Klenk, H.-D. (2004) *Proc. Natl. Acad. Sci. USA*, **101**, 4620-4624.
5. Pinto, M., Robine-Leon, S., Appay, M. D., Kedinger, M., Triadou, N., Dussaulx, E., Lacroix, B., Simon-Assman, P., Haffen, K., Fogh, J., and Zweibaum, A. (1983) *Biol. Cell*, **47**, 323-330.
6. Rogers, G. N., Paulson, J. C., Daniels, R. S., Skehel, J. J., Wilson, I. A., and Wiley, D. C. (1983) *Nature*, **304**, 76-78.
7. Suzuki, Y., Ito, T., Suzuki, T., Holland, R. E., Chambers, T. M., Kiso, M., Ishida, H., and Kawaoka, Y. (2000) *J. Virol.*, **74**, 11825-11831.
8. Rapoport, E. M., Mochalova, L. V., Gabius, H.-J., Romanova, J., and Bovin, N. V. (2006) *Glycoconj. J.*, **23**, 115-125.
9. Takahashi, T., Suzuki, T., Nishinaka, D., Murakami, M., Fujii, S., Jwa Hidari, K. I.-P., Miyamoto, D., Li, Y.-T., and Suzuki, Y. (2003) *FEBS Lett.*, **553**, 355-359.
10. Suzuki, T., Sometani, A., Yamazaki, Y., Horiike, G., Mizutani, Y., Masuda, H., Yamada, M., Tahara, H., Xu, G., Miyamoto, D., Oku, N., Okada, S., Kiso, M., Hasegawa, A., Ito, T., Kawaoka, Y., and Suzuki, Y. (1996) *Biochem. J.*, **318**, 389-393.
11. Stray, S. J., Cummings, R. D., and Air, G. M. (2000) *Glycobiology*, **10**, 649-658.
12. Thompson, C. I., Barclay, W. S., Zambon, M. C., and Pickles, R. J. (2006) *J. Virol.*, **80**, 8060-8068.
13. Glaser, L., Conenello, G., Paulson, J., and Palese, P. (2007) *Virus Res.*, **126**, 9-18.
14. Shaw, M. L., Stone, K. L., Colangelo, C. M., Gulcicek, E. E., and Palese, P. (2008) *PloS Pathog.*, **4**, 1-6.
15. Gamblin, S. J., and Skehel, J. J. (2010) *J. Biol. Chem.*, **285**, 28403-28409.
16. Keil, W., Geyer, R., Dabrowski, J., Dabrowski, U., Niemann, H., Stirm, S., and Klenk, H.-D. (1985) *EMBO J.*, **4**, 2711-2720.
17. Mir-Shekari, S. Y., Ashford, D. A., Harvey, D. J., Dwek, R. A., and Schulze, I. T. (1997) *J. Biol. Chem.*, **272**, 4027-4036.
18. Ouellet, M., Mercier, S., Pelletier, I., Bounou, S., Roy, J., Hirabayashi, J., Sato, S., and Tremblay, M. J. (2005) *J. Immunol.*, **174**, 4120-4126.
19. Mercier, S., St.-Pierre, C., Pelletier, I., Ouellet, M., Tremblay, M. J., and Sato, S. (2008) *Virology*, **371**, 121-129.
20. Nakamura, N., and Compans, R. W. (1979) *Virology*, **93**, 31-47.

21. Deom, C. M., and Schulze, I. T. (1985) *J. Biol. Chem.*, **260**, 14771-14774.
22. Arbatsky, N. P., Derevitskaya, V. A., Zheltova, A. O., Kochetkov, N. K., Likhosherstov, L. M., Senchenkova, S. N., and Yurtov, D. V. (1988) *Carbohydr. Res.*, **178**, 165-181.
23. Leffler, H. (ed.) (2002) Special Issue on Galectins, *Glycoconj. J.*, **19**, 433-630.
24. Yang, R. Y., Rabinovich, G. A., and Liu, F. T. (2008) *Expert. Rev. Mol. Med.*, **10**, e17.
25. Rapoport, E. M., Kurmyshkina, O. V., and Bovin, N. V. (2008) *Biochemistry (Moscow)*, **73**, 393-405.
26. Hughes, R. C. (1999) *Biochim. Biophys. Acta*, **1473**, 172-185.
27. Rapoport, E. M., Andre, S., Kurmyshkina, O. V., Pochechueva, T. V., Severov, V. V., Pazynina, G. V., Gabius, H.-J., and Bovin, N. V. (2008) *Glycobiology*, **18**, 315-324.
28. Mochalova, L., Gambaryan, A., Romanova, J., Tuzikov, A., Chinarev, A., Katinger, D., Katinger, H., Egorov, A., and Bovin, N. V. (2003) *Virology*, **313**, 473-480.
29. Yoshimura, A., and Ohnishi, S. (1984) *J. Virol.*, **51**, 497-504.
30. Schneller, M., Andre, S., Cihak, J., Kaltner, H., Merkle, H., Rademaker, G. J., Haverkamp, J., Thomas-Oates, J. E., Losch, U., and Gabius, H.-J. (1995) *Cell. Immunol.*, **166**, 35-43.
31. Gabius, H.-J. (1990) *Anal. Biochem.*, **189**, 91-94.
32. Solis, D., Romero, A., Kaltner, H., Gabius, H.-J., and Diaz-Maurino, T. (1996) *J. Biol. Chem.*, **271**, 12744-12748.
33. Kaltner, H., Seyrek, K., Heck, A., Sinowatz, F., and Gabius, H.-J. (2002) *Cell Tissue Res.*, **307**, 35-46.
34. Andre, S., Kaltner, H., Furuike, T., Nishimura, S.-I., and Gabius, H.-J. (2004) *Bioconjug. Chem.*, **15**, 87-98.
35. Andre, S., Pei, Z., Siebert, H.-C., Ramstrom, O., and Gabius, H.-J. (2006) *Bioorg. Med. Chem.*, **14**, 6314-6326.
36. Kaltner, H., Solis, D., Kopitz, J., Lensch, M., Lohr, M., Manning, J. C., Murnseer, M., Schnolzer, M., Andre, S., Saiz, J. L., and Gabius, H.-J. (2008) *Biochem. J.*, **409**, 591-599.
37. Kaltner, H., Solis, D., Andre, S., Lensch, M., Manning, J. C., Murnseer, M., Saiz, J. L., and Gabius, H.-J. (2009) *Biochemistry*, **48**, 4403-4416.
38. Lopez-Lucendo, M. F., Solis, D., Saiz, J. L., Kaltner, H., Russwurm, R., Andre, S., Gabius, H.-J., and Romero, A. (2009) *J. Mol. Biol.*, **386**, 366-378.
39. Saussez, S., de Leval, L., Decaestecker, C., Sirtaine, N., Cludts, S., Duray, A., Chevalier, D., Andre, S., Gabius, H.-J., Remmelink, M., and Leroy, X. (2010) *Histol. Histopathol.*, **25**, 541-550.
40. Kaltner, H., Kubler, D., Lopez-Merino, L., Lohr, M., Manning, J. C., Lensch, M., Seidler, J., Lehmann, W. D., Andre, S., Solis, D., and Gabius, H.-J. (2011) *Anat. Rec.*, **294**, 427-444.
41. Wiley, D. C., Wilson, I. A., and Skehel, J. J. (1981) *Nature*, **289**, 373-378.
42. Lazarowitz, S., and Choppin, P. W. (1974) *Virology*, **68**, 440-454.
43. Gauthier, S., Pelletier, I., Ouellet, M., Vargas, A., Tremblay, M. J., Sato, S., and Barbeau, B. (2008) *Retrovirology*, **5**, 1-15.
44. Ahmad, N., Gabius, H.-J., Andre, S., Kaltner, H., Sabesan, S., Liu, B., Roy, R., and Brewer, C. F. (2004) *J. Biol. Chem.*, **279**, 10841-10847.
45. Rabinovich, G. A., Toscano, M. A., and Jackson, S. S. (2007) *Curr. Opin. Struct. Biol.*, **17**, 513-520.
46. Nieminen, J., Kuno, A., Hirabayashi, J., and Sato, S. (2007) *J. Biol. Chem.*, **282**, 1374-1383.
47. Sakaki, M., Oka, N., Nakanishi, R., Yamaguchi, K., Fukumori, T., and Kanayama, H. O. (2008) *J. Med. Invest.*, **55**, 127-132.
48. Kohatsu, L., Hsu, D. K., Jegalian, A. G., Liu, F.-T., and Baum, L. G. (2006) *J. Immunol.*, **177**, 4718-4726.
49. Wu, A. M., Wu, J. H., Tsai, M. S., Kaltner, H., and Gabius, H.-J. (2001) *Biochem. J.*, **358**, 529-538.
50. Hirabayashi, J., Hashidate, T., Arata, Y., Nishi, N., Nakamura, T., Hirashima, M., Urashima, T., Oka, T., Futai, M., Muller, W. E. G., Yagi, F., and Kasai, K. (2002) *Biochim. Biophys. Acta*, **1572**, 232-254.
51. Wu, A. M., Singh, T., Liu, J.-H., Krzeminski, M., Russwurm, R., Siebert, H.-C., Bonvin, A. M. J. J., Andre, S., and Gabius, H.-J. (2007) *Glycobiology*, **17**, 165-184.
52. Krzeminski, M., Singh, T., Andre, S., Lensch, M., Wu, A. M., Bonvin, A. M. J. J., and Gabius, H.-J. (2011) *Biochim. Biophys. Acta*, **1810**, 150-161.
53. Stowell, S. R., Arthur, C. M., Mehta, P., Slanina, K. A., Blixt, O., Leffler, H., Smith, D. F., and Cummings, R. D. (2008) *J. Biol. Chem.*, **283**, 10109-10123.
54. Ahmad, N., Gabius, H.-J., Kaltner, H., Andre, S., Kuwabara, I., Liu, F.-T., Oscarson, S., Norberg, T., and Brewer, C. F. (2002) *Can. J. Chem.*, **80**, 1096-1104.
55. Barondes, S. H., Castronovo, V., Cooper, D. N. W., Cummings, R. D., Drickamer, K., Felzi, T., Gitt, M. A., Hirabayashi, J., Hughes, C., Kasai, K., Leffler, H., Liu, F., Lotan, R., Mercurio, A. M., Monsigny, M., Pillai, S., Poirer, F., Raz, A., Rigby, P. W. J., Rini, J. M., and Wang, J. L. (1994) *Cell*, **76**, 597-598.
56. Matrosovich, M., Matrosovich, T., Carr, J., Roberts, N. A., and Klenk, H.-D. (2003) *J. Virol.*, **77**, 8418-8425.
57. Wagner, R., Matrosovich, M. N., and Klenk, H.-D. (2002) *Rev. Med. Virol.*, **12**, 159-166.
58. Schulze, I. T. (1997) *J. Infect. Dis.*, **176**, Suppl. 1, S24-28.