CELL SURFACE GLYCANS AS AN ADHESION FACTOR FOR EXOSOMAL VESICLES OF *TRITRICHOMONAS* SPP. TYPE PARASITES

V. D. Bets[1, a], N. V. Shilova[2], I. B. Gribchenko[1], X. E. Evstropova[3], E. A. Litvinova[1]

1. Novosibirsk State Technical University, Faculty of Physics and Technology, 630073, Novosibirsk, Russia

2. Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Miklukho-Maklaya Str, 16/10, Moscow, 117997, Russia

3. Novosibirsk State University, Pirogova 2, 630090, Novosibirsk, Russian Federation

(a) betsvd.bio@gmail.com

Annotation

Protozoal infection is a serious factor in the decline of reproductive health in humans and animals. The example of *T. vaginalis* shows how the parasite-host interaction is mediated. It is known that signaling between cells and parasites is carried out with the help of specific connections. These bonds can be represented by glycans and lectins. In this work, we examined the glycan profile of cells and lectin profile of exosomal vesicles of the intestinal trichomonad *Tritrichomonas* sp. in an intestinal epithelial model. The cytotoxicity of active trophozoites on cells of the CaCo-2 tumor cell line was shown, and the absence of cytotoxic effect of exosomal vesicles on cells was also demonstrated. We investigated the glycan profile of CaCo-2 cells by treatment with biotinylated lectins and found a high percentage of binding to the lectins RCA120, AAL, and UEA-1. These lectins bind galactose and fucose-containing glycans. When we determined the lectin profile of vesicles on glycan chips, we found vesicles bound to alpha-Gal beta-Gal and GalNAc, as well as Fuca1. We hypothesize that exosomal vesicles interact with the host cell through these glycan-lectin chains. By selecting blockers of these interactions, parasite adhesion can be inhibited. These mechanisms may form the basis of new antiprotozoal drugs.

Keywords: protozoa, trichomonads, vesicles, lectins, glycans, glycan profile

Introduction

Trichomonas spp. is a group of protozoa parasitizing epithelial cells in mammals, including humans. They include the well-studied species *Trichomonas vaginalis*, which parasitizes humans and affects the genital system, causing inflammation. *Trichomonas foetus* parasitizes animals, causing abortion and infertility in cattle. At the same time, the same species in cats localizes in the intestine and is the causative agent of enteritis.

To date, factors of parasitism of protozoa of the *Trichomonas spp*. type on host cells have been considered [1,2]. These studies have been conducted for vaginal trichomonads, for *T. vaginalis* there is even a vaginal epithelium model to study *in vitro* parasite-host interactions [3]. However, intestinal trichomonads also have deleterious effects on the body as they participate in the regulation of the microbial community and contribute to inflammation in the gut.

Active trophozoites of *Trichomonas spp.* are known to recruit exosomal vesicles to attach to the cell, which perform cell signaling and enable the trophozoite to approach and adhere [4,5].

In this respect, the signaling pathways between the host cell and parasite vesicles become interesting. Since signaling between cells is via glycan-lectin bonds, it is of interest to study the glycan and lectin profile of cells and vesicles to better understand these relationships [6].

The adhesion mechanism for different species of *Trichomonas spp.* can obviously be similar, but there is a dependence on the environment and specific epitopes on the epithelial cell surface. Therefore, to study the interaction of intestinal trichomonads with enterocytes *in vitro*, it is necessary to mimic the intestinal epithelium.

CaCo-2 cells are a widely used *in vitro* model of human intestinal epithelium [7]. They are derived from a human colon adenocarcinoma cell line, and although they are cancerous, when cultured under the correct conditions they exhibit many characteristics of differentiated intestinal epithelial cells. This makes them a valuable tool for studying intestinal absorption, metabolism and drug transport [8].

This cell line is contentedly like intestinal epithelial cells because when grown on permeable supports (such as Transwell inserts), CaCo-2 cells spontaneously differentiate to form a polarized monolayer. This monolayer mimic the intestinal epithelial barrier, having apical (luminal) and basolateral (serosal) sides. This polarization is important as it allows the study of transepithelial transport [9].

CaCo-2 cells also express many transporters and enzymes, dense contact proteins (such as claudins, occludins and ZO-1) that create a selectively permeable barrier between the apical and basolateral compartments. This barrier regulates the passage of molecules through the epithelium, mimicking the function of the intestinal barrier.

In addition, one of the important features of this cell line is the secretion of mucins. The cells secrete glycoproteins that form a layer of mucus that covers the intestinal epithelium. This mucus layer plays a role in protecting the epithelium and influencing microbial interactions. In this way, CaCo-2 can maximally modulate the behavioral conditions of intestinal epithelial cells.

Naturally, it should be considered that this is only a simplified model of intestinal epithelium, as it is not possible to form a complex three-dimensional structure of the epithelium under *in vitro* conditions. It should also be borne in mind that the cells are cancerous and cannot demonstrate aspects of normal cell physiology.

However, it can be concluded that CaCo-2 cells represent a useful and relatively simple *in vitro* model for studying many aspects of intestinal epithelial function.

Mammalian cells are known to possess an amazing array of complex carbohydrate chains on the surface of their membranes that bind proteins and lipids. These chains are called glycans, and play a key role in all cellular processes, making them critical for proper cellular function [10,11].

Lectins are carbohydrate-binding proteins that specifically recognize and bind to glycans (sugar chains) on the surface of cells and other molecules. They generally do not trigger a signal transduction cascade like receptors; their primary function is binding [12]. Different lectins have

different specificities for sugars, allowing researchers to use them as tools to identify and characterize specific glycans. A cell's lectin profile is essentially a map of its glycosylation [13].

Components of the lectin profile include the specific types of glycans present (e.g., N-linked, O-linked, glycolipids); the detailed structure of each glycan, including the types and arrangement of monosaccharides; the density of glycans on the cell surface and in intracellular spaces; and their distribution of glycans inside and outside the cell (e.g., concentrated in specific membrane regions, associated with specific organelles) [14].

Such tissue specificity is critical for cell-to-cell recognition and communication. The nature of cellular glycosylation changes throughout development, reflecting the dynamic nature of cell differentiation and function [15].

Because the receptor system is based on glycan interactions with signalling proteins and immune patterns, glycans and lectins on immune and epithelial cells as well as on pathogens play important roles in the immune response, including activation, suppression and antigen recognition, which provides modulation of the immune system [16,17]. Due to their specific binding, glycans are a target for viruses and bacteria to attach and enter cells, contributing to the pathogenesis of diseases. The same is true for parasitic protozoa, allowing adhesion on cells through lectin-glycan binding [18].

Several papers have examined the bombardment of host cells by a cloud of exosomal vesicles from various pathogenic protists [5,19–21]. Parasite-produced exosomes are characterized by the presence of RNA and nucleus, conserved exosomal proteins as well as parasite-specific proteins. Thus, Twu et al [5] showed that *T. vaginalis* exosomes integrate into host cells, deliver their contents into them and modulate the immune response of host cells. Moreover, exosomes from highly adherent parasite strains increase the adhesion of weakly adherent parasites to vaginal and prostate epithelial cells. In addition to establishing that parasite exosomes act to modulate host-parasite interactions, these studies reveal for the first time a potential role for exosomes in facilitating parasite-parasite communication and host cell colonization.

Currently, there are urgent problems related to the therapy of protozoal infection, including trichomoniasis. This is since protozoa are resistant to antibiotics [22,23], which reduces the effectiveness of conventional treatment regimens in both humans and animals. The only drug today that can affect protozoa is metronidazole. However, its use has several limitations, including the fact that the drug is known to have hepatotoxic effect. Hence, there is a need to search for new drugs. Manipulation of glycan structures for therapeutic purposes, for example, to create antibodies with improved specificity or to develop vaccines with enhanced efficacy, is an active area of research. In addition, the study of glycan-lectin interactions between parasite and host may allow the identification of new therapeutic agents to combat protozoal diseases.

For example, various oligosaccharides can block the attachment sites of cell glycans to lectins of parasitic vesicles, or vice versa. It is therefore of interest to search for such compounds and to develop drugs.

Thus, in this work, we examined the effect of active trophozoites of *Tritrichomonas* sp and their exosomal vesicles on human colon adenocarcinoma cells and determined the glycan profile of cells and lectin profile of vesicles.

Materials and methods

Cultivation of cell lines. CaCo-2 and $2x10^6$/ml cell lines were cultured on 25 cm^2 filtered mattresses (60 ml, TPP, Switzerland) with DMEM nutrient medium (PanEco, Russia), 1% antibiotics (penicillin-streptomycin in r-re, 10000 U/ml penicillin and 10000 µg/ml streptomycin, PanEco, Russia) and addition of 10% fetal bovine serum (Sigma-Aldrich, USA) at 37 ℃, with 5% CO_2. Cells were transplanted every 3 days, and cells were removed from the matrix using 0.25% trypsin with EDTA. The cells were cultured for up to 28 days. Differentiated cells were kept for 14 days after the 3rd passaging.

Treatment of cells with lectins. For treatment of cells with lectins, cells were removed from the mat, cell suspension was washed in 1X PBS (Phosphate-Salt Buffer, Thermo, USA). Cells after washing were aliquoted into 12 tubes, 700,000 in each, 11 of which were treated with lectins, and one was used as a control. Then 10 µl of lectin (1 µg MAL II, 2 µg SNL, 2 µg SBA, 5 µg Lacalin, 1 µg RCA 120, 5 µg ECL, 2 µg PNA, 2 µg GSL I, 1 µg UEA-I, 2 µg GSL II, 1 µg AAL) at a rate of 5 µl per 100,000 cells, gently resuspended and incubated at room temperature, in the dark for 30'. After incubation, 200 µl of PBS was added to the tube and centrifuged at 3000 rpm, 3'. The supernatant was removed and 100 µl PBS and 4 µl Streptavidin (ThermoFisher, USA) were added at a rate of 2 µl per 100,000 cells, incubated in the dark, room temperature 30'. Streptavidin was not added to the UEA-I sample. Next, 200 µl PBS was added, centrifuged at 3000 rpm, 3', the supernatant was removed and 200 µl PBS was added. An aliquot of the sample was transferred to a cytometric tube for further analysis on a flow cytometer.

Trichomonads culturing. The strain of *Tritrichomonas spp*. [24], previously isolated from the intestines of laboratory mice, was used.

Active trophozoites at a concentration of $3.8x10^7$/ml were incubated in nutrient medium trypticase- yeast extract-maltose (TYM) supplemented with 10% inactivated bovine serum, penicillin (1000 IU/ml) and streptomycin sulfate (1 mg/ml) at 37°C with 5% CO_2. pH of TYM medium was adjusted to 7.2. The trophozoites were crossed every 48 h, they showed motility of more than 80% and normal morphology.

To isolate trichomonads from the nutrient medium, 500 µl of sediment from the medium was transferred into 1.5 ml eppendorf-type tubes (SOVTECH, Russia), 500 µl of sediment from the medium was added with 500 µl of cold PBS and centrifuged at 3000 rpm 5', +4°C. After stranding, the supernatant was removed and washed again. After washing, the samples were placed on ice. A gradient was prepared, for this purpose Percoll (Cytiva, Sweden) 100% was diluted to 80% and 40% according to the instructions, then 500 µl of 80% Percoll was gently layered into a 2 ml eppendorf, 500 µl of 40% Percoll on top and then 500 µl of sample in PBS. Centrifuged 1000 g brakes off, 15', +4°C. In the interphase, the contents (approximately 200 µl) were carefully collected, placed in a clean 1.5 ml eppendorf and 800 µl of cold PBS was added, gently resuspended and centrifuged 3000 rpm 5', +4°C. Washed from the gradient twice.

Vesicle production. Density gradient separated and washed trophozoites at a concentration of $2.4x10^5$/ml were stained with fluorescent dye Cell Tracker deep red. For this purpose, 10 µl of

1 M Cell Tracker deep red (Invitrogen, USA) was added to 1ml of trophozoite filtrate, gently resuspended and incubated for 40 minutes at 37℃. After incubation, the samples were centrifuged at 3000 rpm 5', +4℃ and washed from the dye with cold PBS twice. After staining, trophozoites were placed on serum-free TYM at a ratio of 1 ml of sample per 4 ml of medium. It was incubated at 37℃ for 4 hours. Next, the contents of the tubes were filtered through a 0.22 μm syringe filter. The resulting filtrate contained exosomal vesicles at a concentration of $1x10^9$/ml.

Biotinylating of vesicles. For vesicle biotinylating, a solution of biot-CMG2-DOPE (Syntavr Ltd.) in PBS was prepared with a concentration of 1 mg/ml (461 μM). In a test tube with live trichomonads in culture medium, DOPE-biot was added with stirring to a final concentration of 10 μM. The solution was incubated for 1 h at room temperature, stirring periodically so that all trophozoites had an opportunity to contact the lipid derivative. Then they were washed with medium 2-3 times and the vesicle production protocol were repeated. The collected vesicles were concentrated using a microcon (Millipore, USA) with a pore size of 100 kDa.

Printing of vesicles on lectin chips. Microvesicles were analyzed in a solid-phase assay format on activated slides [25]. Immobilized vesicles were incubated with a 10 μg/ml solution of biotinylated lectins (VectorLabs) in PBS containing 1%BSA and 0.1% Tween20 for 1 h at 25C and stirring. Unbound lectins were then removed with PBS+0/05% Tween20. Slides were manifested with Alexa555-labelled streptavidin (Invitrogen, USA). Results were read using an Innoscan 1100AL microarray scanner.

Vesicle specificity studies. The glycochip (Semiotic LLC, Russia) was blocked with standard blocking buffer (50mM EA (pH~8.5): 1195μl ethanolamine (50mM), 3.8g boric acid (150mM) and 800μl Tween 20 (0.2%) were dissolved in 400ml distilled H2O) for 2 hours under constant stirring at room temperature. Afterwards, it was washed with phosphate isotonic buffer containing 0.05% Tween20 (PBS-0.05% (phosphate buffer saline (PBS) with 0.05% Tween 20)) twice. 1 ml of solution containing biotinylated vesicles and 0.1% Tween20 was applied to the chip and incubated for 16 h at +4°C under high humidity. The glycochip was then washed in PBS-0.05% twice. Next, 1 ml of a solution of Alexa555-labelled streptavidin (Str-Alexa555) at a dilution of 1:2000 in PBS-0.1% was applied to the chip and incubated for 45 minutes at room temperature. After that, the glycochip was washed in PBS-0.05% and finally in bidistilled water, and then the chips were air dried and scanned using a fluorescence reader (resolution - 10 μm). The images were then converted into an Excel spreadsheet using the reader software and GAL.file.

Co-culture of cells with vesicles and trophozoites. CaCo-2 cells were grown in 6 cm Petri dishes, on sterile coverslips until a monolayer was formed. One hour before the interaction assay, cells were washed with sterile 1X-m PBS, the growth medium was replaced with fresh medium and incubated at 37°C for at least 1 hour before interaction. After parasites reached a density of 1x106/ml, culture tubes were cooled to detach parasites and centrifuged at 150×g. The supernatant was removed, and the precipitate was resuspended in interaction medium (DMEM: TYM, 2:1).

Cytotoxicity. The effect of parasites and their vesicles on CaCo-2 cell viability was evaluated after 1, 2 h of incubation of cells (monolayer) with vesicles and trophozoites in 12-well plates (TPP, Trasadingen, Switzerland). Cells were seeded in plates (at least 6×10^4 cells per cm^2) on medium consisting of DMEM (PanEco, Russia) with 10% fetal serum (Sigma-Aldrich, USA) and the combined antibiotics 100 IU/ml penicillin and 100 μg/ml streptomycin (PanEco, Russia). The plates were incubated in a CO_2 incubator at +37 °C in air with 5% CO_2. After reaching

monolayer, cells were treated with vesicles ($1x10^4$/ml) and live trophozoites ($1x10^6$/ml). Cocultivation was performed in three repeats.

In the control group, cells did not interact with vesicles and trophozoites. At the end of the incubation period, cells were washed with Dulbecco's phosphate-buffered saline (DPBS) (Gibco, USA). They were then separated using 0.25% trypsin with EDTA in a 1:1 ratio (PanEco, Russia) and centrifuged for 10 min at 1100 rpm. After removing the supernatant and resuspending the precipitate in 1 ml PBS, the cells were washed twice. After that, 500 µl of DPBS (Gibco, Carlsbad, CA, USA) containing 100 nM Calcein AM Viability Dye (eBioscience Invitrogen, San Diego, CA, USA) fluorescent dye for live cell visualization was added to the cells and incubated for 15 min in the dark at room temperature. Then, 5 µl of fluorescent dye 7-AAD Viability Staining Solution (Bioscience Invitrogen, San Diego, CA, USA) was added to the solution to visualize dead cells and stained for 5 min in the dark at room temperature. To identify and quantify live and dead cells, samples stained with the indicated dyes were analyzed on a FACS Canto II flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) using FACS Diva 6.0 software (Becton Dickinson, Franklin Lakes, NJ, USA).

Results

The trophozoites of *Tritrichomonas* sp. strain, previously isolated from laboratory mice from their intestines [24], were used to isolate exosomes (**Fig. 1a**). According to literature data [5], the size of exosomal vesicles of vaginal trichomonads does not exceed 120 nm; therefore, we performed size determination to find out what vesicles are recruited by the intestinal trichomonad strain we used. As a result, we obtained data that the size of vesicles, is about 100 nm (**Fig.1b**), which is close to the value for the already studied trichomonads. When cells were co-cultured with exosomes and trophozoites, we observed that exosomal vesicles, at a concentration of 1x104, did not show a strong toxic effect, as only 16% of dead cells were found, provided that the control lost 10% (**Fig. 1c**). Importantly, there were no colossal differences at different time points in cells incubated with EVs. For example, after a two-hour incubation, the live cell titer decreased by 1.7% in contrast to the one-hour incubation. This result seems logical, since exosomes as a signaling tool are not designed for toxic effects on cells, otherwise trophozoites would have no opportunity to attach. They use EVs precisely to establish contact with the epithelial cell, which allows them to further attach. But when cells are incubated with active trophozoites of *Tritrichomonas* sp. we can see how the ratio of live/dead cells changes (**Fig. 1c**), which is 79% dead cells and 21% live cells. At the same time, a sharp decrease in the titer of live cells occurred already in the 1st hour of cocultivation (**Fig. 1c**). Also, after staining with Calcein AM and 7AAD (**Fig. 1d**), we observe many cells stained with 7AAD in the field of view, which indicates their death. From this we can conclude that EVs themselves have no toxic effect but are only an adhesion factor to host epithelial cells and have only signaling functions. However, the trophozoites themselves, obviously, after attachment contribute to cell death, since they actively use them as an energy substrate for reproduction.

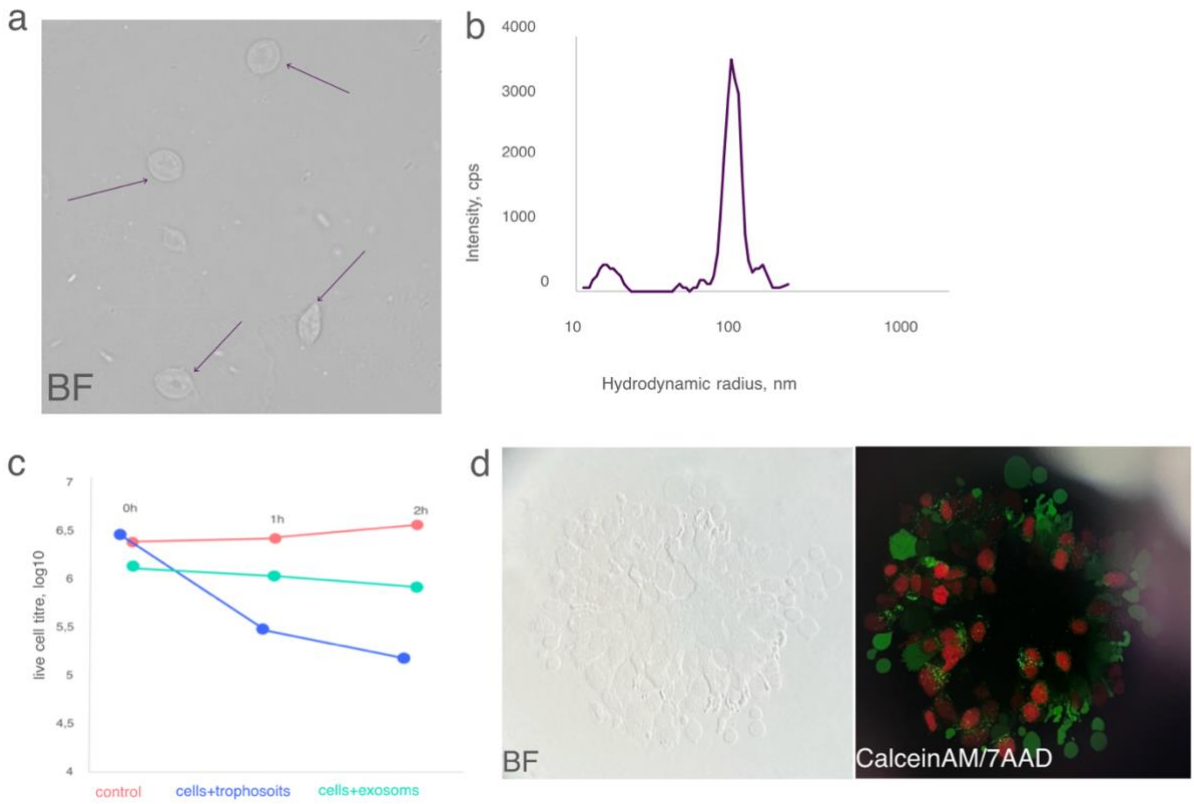

Figure 1. Determination of cytotoxicity of *Tritrichomonas* sp. trophozoites on CaCo-2 cells. **a.** Live trophozoites of *Tritrichomonas* sp. in culture medium (indicated by arrows). **b.** Titer of live CaCo-2 cells after incubation with exosomal vesicles of the parasite and its active trophozoites 1 hour and 2 hours. **c.** Determination of the hydrodynamic radius of *Tritrichomonas* sp. exosomal vesicles using Photocor Complex nanosizer. **d.** Staining of CaCo-2 cells with fluorescent dyes Calcein AM and 7AAD, where it is shown that 7AAD passes into dead cells with damaged membrane and stains them orange. Living cells glow green (Calcein AM).

To determine the glycan profile of CaCo-2 cells, they were treated with lectins (**Table 1**). Since adenocarcinoma cells have differentiation stages, lectin treatment was performed at different points of differentiation, before (immediately after the 3rd passage) and after (14 days).

Table 1. Specific binding of lectins.

LECTIN	DESCRIPTION	SPECIFIC BINDING
UEA	*Ulex europaeus*	fucose
SNA	*Sambucus nigra*	sialic acid, *N*-acetylgalactosamine or galactose
MAL	*Maackia amurensis*	galactose
SBA	*Soybean agglutinin*	N-acetylgalactosamine
JAC	*Jacalin*	galactosyl (β-1,3) N-acetylgalactosamine
RCA 120	*Ricinus communis* agglutinin	galactose or N-acetylgalactosamine
ECL	*Erythrina Cristagalli Lectin*	Sialic acid
PNA	*Peanut agglutinin*	galactosyl (β-1,3) N-acetylgalactosamine
GSL I	*Griffonia (Bandeiraea) Simplicifolia Lectin I*	α-galactose
GSL II	*Griffonia (Bandeiraea) Simplicifolia Lectin II*	N-acetylglucosamine
AAL	*Aleuria Aurantia Lectin*	fucose linked

We obtained a high cell coverage with RCA120 lectin (>60%) for both differentiated and undifferentiated cells (**Fig. 2a**). Whereas GSL I showed only 39.96% coverage for differentiated cells and 18% for undifferentiated cells. Also, GSL II did not show much coverage (12.84% for undifferentiated and 42.69% for differentiated cells). Here we can see that differentiated cells increase binding to galactose-containing lectins, which likely makes them more susceptible to interacting with vesicles. In addition, we observed that fucose-binding lectin showed high cell coverage (81.48% undifferentiated and 81.67% differentiated cells), suggesting that glycans capable of binding fucose-containing lectins are present on cell membranes, at different stages of the life cycle. In addition, the fucose-binding lectin UEA also showed a significant representation on cells (75.78% undifferentiated and 87.13% for differentiated cells). The cloud of cells stained with fluorescent labelling was clearly visible on cytometry because binding of biotinylated lectin on the cell surface to fluorescent labelled streptavidin occurred (**Fig. 2b**).

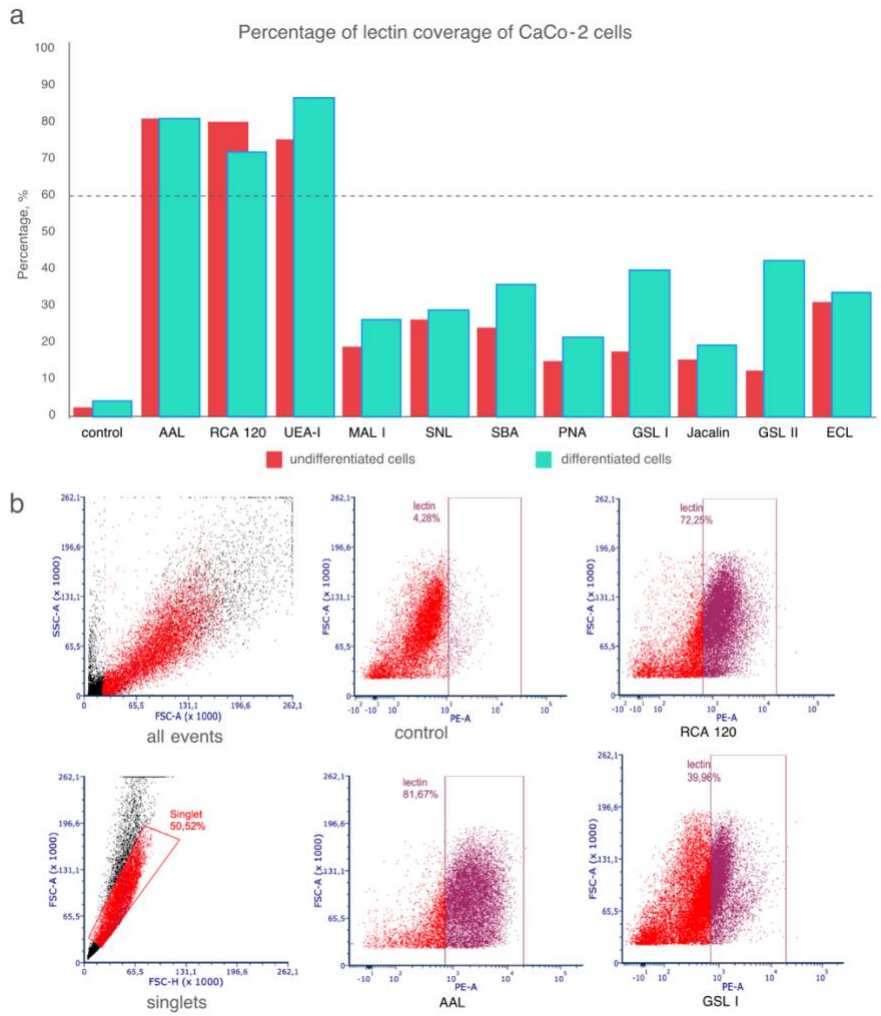

Figure 2. Coating of CaCo-2 cells with lectins to determine binding to specific glycans on the surface. **a.** Percentage of lectin representation bound to the cell surface; the dotted line highlights the boundary of significant cell coverage (>60%). **b.** Gating of cells on flow cytometry, the percentage of cells glowing with the fluorescent label is shown. If glycans are present on the cell surface that can bind to a specific lectin, the cloud of cells falls into the stained zone; if there are no lectin-defined glycans on the cells, the cloud of cells does not fluoresce.

We identified several candidate cell surface glycans for binding and further identified lectins on the surface of exosomal vesicles to understand through which glycan-lectin sites the interaction might occur.

By imaging vesicles on chips with over 800 glycans, we obtained data on the presence of lectins binding fucose-containing structures on the vesicle surface (**Fig. 3**)

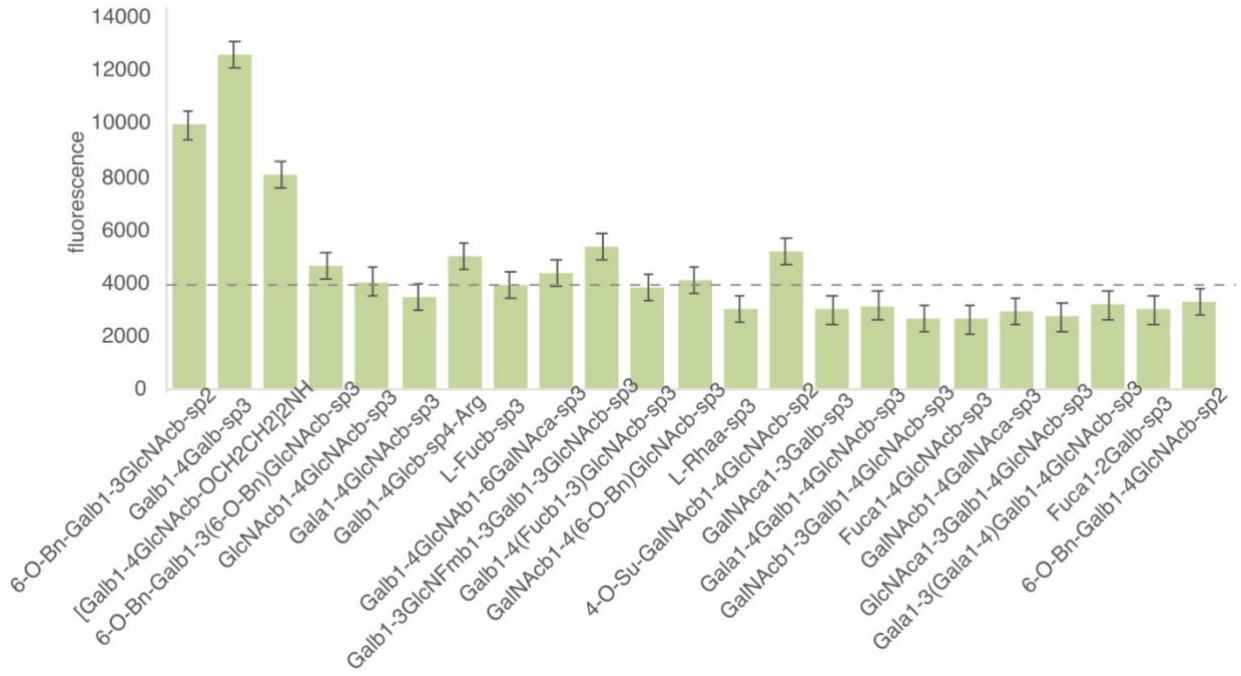

Figure 3. Glycans on chips that showed binding to exosomal vesicles from scanned using a fluorescence reader, the dotted line indicates the minimum threshold of the considered luminosity.

Thus, we observed the binding of vesicles to glycans including alpha-Gal beta-Gal and GalNAc, as well as Fuca1. This is consistent with data on the glycan profile of CaCo-2 cells that bind to galactose- and fucose-binding lectins. We suggest that these glycans may be candidate glycans for trophozoite and vesicle adhesion. Therefore, it is hypothesized that glycans with a similar structure may block the adhesion of vesicles and trophozoites. Since on the surface of CaCo2 cells there are glycans with fucose, which is recognized by UEA I, and earlier in Achasova's work[24] it was shown that fucose eliminates trichomonads from the intestinal lumen, the second candidate for blocking would be a glycan with fucose in the terminal position.

Conclusion

The study of glycan-lectin bonds is a promising method for the search of modern drugs against protozooses. Pathogenic protists are resistant to antibiotics that act directly on the parasitic cell. However, if we can find ways to inhibit the adhesion of the parasite to the host cell, we can deprive the parasite of reproduction. This will allow the protozoa to be safely eliminated from the host organism.

Conflict of interest. The authors declare that there is no conflict of interest.

Acknowledgements. The study was supported by a grant from the Russian Science Foundation (project No. 23-26-00270).

Reference

1. Artuyants, A. *et al.* Extracellular vesicles produced by the protozoan parasite Trichomonas vaginalis contain a preferential cargo of tRNA-derived small RNAs. *Int J Parasitol* 50, (2020).
2. Fei, L. *et al.* Trichomonas vaginalis triggers the release of THP-1 extracellular traps. *Parasitol Res* 118, (2019).
3. Mahajan, G. *et al.* Vaginal microbiome-host interactions modeled in a human vagina-on-a-chip. *Microbiome* 10, (2022).
4. Ong, S. C. *et al.* The core exosome proteome of Trichomonas vaginalis. *Journal of Microbiology, Immunology and Infection* 57, (2024).
5. Twu, O. *et al.* Trichomonas vaginalis Exosomes Deliver Cargo to Host Cells and Mediate Host:Parasite Interactions. *PLoS Pathog* 9, (2013).
6. Paschinger, K. *et al.* The N-glycans of Trichomonas vaginalis contain variable core and antennal modifications. *Glycobiology* 22, (2012).
7. Lopez-Escalera, S. & Wellejus, A. Evaluation of Caco-2 and human intestinal epithelial cells as *in vitro* models of colonic and small intestinal integrity. *Biochem Biophys Rep* 31, (2022).
8. Han, H. *et al.* Multi-Species Probiotic Strain Mixture Enhances Intestinal Barrier Function by Regulating Inflammation and Tight Junctions in Lipopolysaccharides Stimulated Caco-2 Cells. *Microorganisms* 11, (2023).
9. Darling, N. J., Mobbs, C. L., González-Hau, A. L., Freer, M. & Przyborski, S. Bioengineering Novel *in vitro* Co-culture Models That Represent the Human Intestinal Mucosa With Improved Caco-2 Structure and Barrier Function. *Front Bioeng Biotechnol* 8, (2020).
10. Lin, H., Han, R. & Wu, W. Glucans and applications in drug delivery. *Carbohydrate Polymers* vol. 332 Preprint at https://doi.org/10.1016/j.carbpol.2024.121904 (2024).
11. De Felice, B. *et al.* Effect of beta- and alpha-glucans on immune modulating factors expression in enterocyte-like Caco-2 and goblet-like LS 174T cells. *Int J Biol Macromol* 153, (2020).
12. De Coninck, T. & Van Damme, E. J. M. Plant lectins: Handymen at the cell surface. *The Cell Surface* vol. 8 Preprint at https://doi.org/10.1016/j.tcsw.2022.100091 (2022).
13. Ivashenka, A. *et al.* Glycolipid-dependent and lectin-driven transcytosis in mouse enterocytes. *Commun Biol* 4, (2021).
14. Yang, W. & Huang, G. Extraction methods and activities of natural glucans. *Trends in Food Science and Technology* vol. 112 Preprint at https://doi.org/10.1016/j.tifs.2021.03.025 (2021).
15. Chang, H. L. & Lin, J. C. SRSF1 and RBM4 differentially modulate the oncogenic effect of HIF-1α in lung cancer cells through alternative splicing mechanism. *Biochim Biophys Acta Mol Cell Res* 1866, (2019).
16. Konozy, E. H. E. & Osman, M. E. M. From inflammation to immune regulation: The dual nature of dietary lectins in health and disease. *Heliyon* 10, e39471 (2024).

17. Bala Subramaniyan, S. & Veerappan, A. Lectins as the prominent potential to deliver bioactive metal nanoparticles by recognizing cell surface glycans. *Heliyon* 10, (2024).

18. Cobo, E. R., Campero, C. M., Gimeno, E. J. & Barbeito, C. G. Lectin binding patterns and immunohistochemical antigen detection in the genitalia of tritrichomonas foetus-infected heifers. *J Comp Pathol* 131, (2004).

19. Kochanowsky, J. A. *et al.* Trichomonas vaginalis extracellular vesicles up-regulate and directly transfer adherence factors promoting host cell colonization. *Proc Natl Acad Sci U S A* 121, (2024).

20. Szempruch, A. J., Dennison, L., Kieft, R., Harrington, J. M. & Hajduk, S. L. Sending a message: Extracellular vesicles of pathogenic protozoan parasites. *Nat Rev Microbiol* 14, 669–675 (2016).

21. Mantel, P. Y. & Marti, M. The role of extracellular vesicles in Plasmodium and other protozoan parasites. *Cell Microbiol* 16, 344–354 (2014).

22. Lin, H. C. *et al.* Proteomic signatures of metronidazole-resistant Trichomonas vaginalis reveal novel proteins associated with drug resistance. *Parasit Vectors* 13, (2020).

23. Saito-Nakano, Y. *et al.* Prevalence and metronidazole resistance of Trichomonas vaginalis among Japanese women in 2021. *IJID Regions* 7, (2023).

24. Achasova, K. M., Kozhevnikova, E. N., Borisova, M. A. & Litvinova, E. A. Fucose ameliorates tritrichomonas sp.-associated illness in antibiotic-treated Muc2−/− mice. *Int J Mol Sci* 22, (2021).

25. Slivka EV, Shilova NV, Obraztsova EA, Kapustkina DS, Khaidukov SV, Nokel AY, Ryzhov IM, Henry SM, Bovin NV, Rapoport EM. Surface Glycans of Microvesicles Derived from Endothelial Cells, as Probed Using Plant Lectins. International Journal of *Mol Sci* 25, (2024)