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INVESTIGATION OF THE VIRAL PHYSIOLOGY: APPROACHES TO RECOGNITION INVOLVING ENZYMES, LECTINS AND GLYCOCONJUGATES AGAINST INFECTIONS

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ABSTRACT

In the overview, based mainly on own data, the current knowledge of protein/lectin-glycoconjugate general principles and relationships that can be applied to constituents of the "machine" target recognition units by viruses including examples of phages is accented. The multilevel and hierarchical nature of such system interactions is noted. The functional interrelationships of enzymebiotics, lectinbiotics, enzyme lectins, lectin enzymes and postbiotics are considered. Enzymes and lectins co-function as important network communicators and signals due to the multiple roles and functions in recognition of glycoconjugates, binding and coordinated action, influencing effectiveness of viral submachine blocks of recognition and further penetration into microbes. The prospects of using the contribution of affine recognition types with the participation of both bacterial and viral glycoconjugates in microecological control in terms of microbiocenoses, pro/post/synbiotic compartment and mucosal biotopes are accented. The data expand the possible ways of using the glycoconjugates and proteins recognizing them, as well as viruses (phages) and pro/post/symbiotic combinations in the fight against pathogens and infections for the prevention and therapy of diseases.

KEYWORDS: Glycoconjugates; reception; assembling; machinery; lectinbiotics;

enzymebiotics; enzyme lectins; lectinbiotics, lectin enzymes, synergy, communications; viruses; phages; probiotics; postbiotics; synbiotics; prophylaxis; therapy.

ABBREVIATIONS

Bf	Biofilms
CB	Communication bodies
CBM	Carbohydrate-binding modules
Gc	Glycoconjugates
Gp	Glycoprotein(s)
Ig	Immunoglobulin(s)
LPS	Lipopolysaccharides
PAA	Polyacrylamide
PG	Peptidoglycans
PS	Polysaccharides

1. INTRODUCTION

Glycoconjugates (Gc) of viral, microbial, animal and plant nature include glycoproteins (Gp), glycolipids [including lipopolysaccharides (LPS)], peptidoglycans (PG) [containing polysaccharides (PS) in extracellular layers and capsules], others representing important glycoantigens. The recognition of Gc by viruses, including bacteriophages (phages), is an important key early stage of interaction and binding with cellular targets, initiating penetration into cells and their degradation and lysis, including the destruction of microbial arrays and biofilms (Bf).[1-8] Phages can be considered as probiotic agents for the regulation of microbiocenoses and decontamination of food, animals and plants by microorganisms.[9]

The search and recognition of bacteria by means of phages is a well-established mechanism for the use of a biosensor - a viral reception machine. Such interactions involve lectins/lectinbiotics, lectin-like agents, as well as enzymes/enzymebiotics, which contribute to an increase in the pool of antimicrobial peptides in biotopes.[3-16]

We have formulated the basic principles of the organization and functioning of lectins, systems of Lectins--Gc and Lectins—Gc relationships involved in the interaction of the human body and its biotope microbiocenosis microflora.[2,4,10-25]

The **aim** of the review is, based mainly on our own data, to assess the potential of the

principles of Lectins, enzymes and Gc relationships in the study of viruses, including examples of the "Phages—Bacteria" systems, for further expanding views of their application in the whole protection of the body.

2. THE MAIN CONTENT

2.1. General structural and functional relationships of the Lectins--Gc type interactions

Lectins includes "peptide/protein of non-immunoglobulin (non-Ig) nature"-containing structures and complexes that recognize and bind carbohydrates, their derivarives or other Gc. Lectins have spatial 3D areas/epitopes of Gc recognition. Lectins often function as di- and oligomeric, in supramolecular complexes. Lectins may have Ig-like domains and/or epitopes co-functioning with the lectin site.

Lectins and enzymes can cofunction in defense systems.[23, 26, 27] More over, lectins reveal enzyme activities in complexes.[10-14] In some cases lectinic enzymes can be characterized with the site of lectin activity that is not depended from the catalytic center (lectin site as spatially separated).[16] The presence of the non-catalytic carbohydrate-binding modules (CBM) in enzyme allows to coinsider such enzymes as true lectins: enzymes as lectins (enzymatic lectins) and lectins as enzymes (lectinic enzymes) with the mutual influence of Gc-recognizing sites (domains, epitopes, antennary clusters, 3D modules).[16, 23, 24]

Lectins participate in network immunity. They exhibit functions organizing the infrastructure in biotopes, including those based on "building" biomaterials (PG, layers of cell membranes and extracellular matrices, ordered mucus, organelle surfaces and cytoplasmic "skeleton"), are carrier/delivery agents. Lectins - signaling factors, inform-somal agents (including as part of phenotypic inform-somes); they are auxiliary, corrective and stabilizing agents in relation to the dominant/ pronounced activities of all (sub)classes and groups of protein effectors (enzymes, cytolysins with a modular independent arrangement of functions/ activities, hormones, cellular receptors including enzyme nature, cytokines, defensins, antibiotic peptides, peptides recognizing Gc (for example, including shortened or truncated originally long lectin molecules). Active lectin molecules are asymmetric ones, with built-up exposed or latent/masked activities. Lectins have hydrophobic sites and co-functioning sites with lectin activity, making them susceptible to ordered Gc series (ordered in affinity to the choiced lectin form). Lectin activities often depend on Ca^{2+} cations and/or other metals such as Mg^{2+}, Li^{+}, etc. Lectins can include additional recognition sites (2 or more Gc recognition sites). Lectins can exhibit polyvalence, especially in supramolecular complexes, ensembles and

nano/micro-particles, including in the contact/ interfacial regions. Lectins are characterized by their ability to direct assembly, especially in the interphase/ surface areas of cells, organelles and other solid face structures.

The lectin molecule is usually represented by a system of multiple forms (assembly, shortened, with highly varying glycosylation, hydrophobicity and 3D landscape) with different functions and activities capable of modulation by external factors. The lectin system is aimed at a system of Gc targets, which can be ranked according to the degree of accessibility and affinity (early reversible or later possibly irreversible) to the lectin molecule (the possibility of simultaneous recognition of several different Gc targets separated in space). Lectin systems are mosaic (for example, their distribution during visualization depends on the type of Gc); they are characterized by synergy (for example, with non-competing systems from the same source) when achieving the final result, including antimicrobial and antiviral ones when combined with other antimicrobial substances and compounds.

Lectins are able to assemble into nano/micro particle, latex and filamentous Gc-recognizing lectinic multivalent forms, which are also formed in the case of cell sensitization by means of lectins (particles and cells as model macrolectins, suspension particle and cell forms of lectins). They can exhibit lytic, degrading and regulating/ modulating effects in relation to microbial arrays - microbiocenoses in biotopes and as part of a single symbiotic/probiotic compartment of the organism.

2.2. The keys to the use of natural and polymer synthetic Gc in the study of targets involving Lectins—Gc interactions

Linear Gc polymer based on polyacrylamide (PAA) soluble in water and aqueous salt solutions were used by us. Such Gc polymers are characterized with lateral/ side branches of carbohydrates/ carbohydrate residues as clusters of short glycans (25% carbohydrates within PAA-Gc by weight) (www.lectinity.com), imitating "mucins"-like polyvalent compounds with multiple exposed clusters/ squairs of exposed identical carbohydrate residues in the form of terminal side antennas of 1-3 or more residues. Such Gc adapt their initial conformation of the "random tangle in the mother liquor" in accordance with the features of the carbohydrate-accepting sites of the affine surface of solid-phase proteins, Gp, other natural Gc and their assembly complexes on the solid phase (porous hydrophobic and hydrophilic membranes), simulating the cell surface. The Gc used in the work imitated (could serve as functional

mimetics) natural polymer compounds of varying origin (PS, PG, typical widespread glycoantigens) and could be used, including as models of the components of recognition and natural reactions and processes in the body.

We obtained the results and made conclusions (used as keys) about the patterns of interactions involving lectins, Gp and other Gc, as well as the above-mentioned synthetic polymer analogues of Gc - reaction partners in solutions and on membranes simulating surface cell events.

Such affine interactions are indicated below and are considered promising in the study of phages (potential is indicated in parentheses):

***synergy of the Gc action**

- visual, positional, mosaic synergy; synergism in time, combined, in the reactions of Gc-staining of the assembly polymer complexes on the solid phase (fully refers to phages when considering them as macroGc machine - see also below);
- synergy of Gc among themselves within the framework and in terms of the designated localized Gc systems, in the reactions of comparing pictures of separated multiple forms of therapeutic Gp on the solid phase, "colored" by each of the types of Gc being compared (detailing the options for the preferential action of phages as macroGc with the resulting choice of desired cases);
- synergy of Gc with antibodies, including monoclonal ones, in the process of directed step-by-step solid-phase assembly into a multimolecular "sandwich" ensemble (synergism of the phage/ macroGc [phage acting like macroGc] with antibodies);
- synergy between surfaces with different mosaics of point/spot Gc and proteins/Gp/Lectins (between phages as systems of macroGc and macroLectin);

*the ability of synergistic sets of Gc to recognize and bind protein factors and cytokines in the compositions of complexes, nano- and microparticles (synergy in the effectiveness of modulation of the selected activitties of the phage);

*the ability of a set of Gc to sequentially/step-by-step binding various sites of lectin-like assembly nanoparticles (phages as lectin-like assembly nanoparticles):
-to diagnose forms with the maximal investigated activity associated with the involvement of type/types of Gc;

-to visualize as much as possible the forms specific to Gc and their synthetic and natural analogues in the characteristic packaging:
--including assembly units (cell reception submachine units linked to the penetration of phage blocks, assemblies as assembly units for further assembling) for:
--- directional assembly;
--- predictable/expected assembly on a hydrophobic porous material - membrane;
--- predictable/expected assembly/directed coagulation of human cells affinally sensitized by probiotic lectins and Gc in a micropanel on a hydrophobic material - polystyrene;
-to map the features of the subunit, molecular and supra-molecular structures (in relation to the components of the phage, phage submachine modules);

*the ability of 1-2 types of Gc preliminary selected from a series tested in reactions with Gp as protein factors and therapeutic agents to distinguish differencesbetween "effectors with preserved activities":
-in molecular complexes;
-in supra-molecular ensembles;
-in nano/micro-functioning particles, particles with preset/programmed functions;
-in receptors and receptor-like assembly structures;
-on the cell surface;
-on Bf, inside porous Bf;
-in compositions with oriented recognition molecules (lectins and Gc of other [additional] types);
*the presence of Gc in the spatial field as a system when there is at least one parameter of the coordination of the components of Gc (coordination of a set of phages as Gc macroforms in cocktails):
-major and minor forms as systems and subsystems:
--ordered;
--mosaic systems of multiple forms of Gp, including as complexes, with unequal intensity of modulation of activities;
-asymmetry with pronounced generalized directionality and vectorality of the Gc system;

*representations of cells, nano/microparticles of Gp-Gp, Gp-Gc, Gc-Gc as multivalent/multifunctional/dispatcher/hub (macro)forms of recognition and binding of predominant Gc types (phages as effector forms with Gc-types-predominant actions):

-testing of lectins or Gc in a micropanel with respect to the effect on color cellular reactions, for example, in the hemagglutination, in variants of modulation (additional stimulation) of cellular reactions as a result of adhesion of lectins or Gc in lower titre concentrations when determining agglutination titers by sensitized cells by lectins or Gc (testing of additional stimulation by phages of the studied cell activities);

*preservation of polymer effectors (Gp, other Gc, and their complexes) as part of functionally active Gc complexes and nanoparticles, the ability to further sequential and selective cascade binding of different/non-repeating Gc types (using two or more types of signaling Gc in cascad in modulation/switching of phage action);

*identification of previous and detection of new sites of Gc target recognition under conditions of sequential subunit/molecular protein/Gp-assemblies into supramolecular complexes, ensembles and particles (regulation in phages of the resulting major specificity of particles, their regions and fragments to Gc of the desired type and sets of desired Gc as signals):
-expansion of the potential of Gc recognition by therapeutic protein/ Gp (solving the problems of modulation of activity in phages, loss or acquisition of new ones, including in cases of partial degradation or directed aggregation during storage, aging, in the conditions of the life cycle):
--the ability to form new recognition sites at 3D junctions in complexes and supramolecular ensembles based on interactions between proteins;
-the ability of Gp to recognize the availability and spatial distribution of exposed cluster and individual types of glycan antennas - mapping sensitivity to the mosaic of nanoGc (signal Gp and their [glyco]peptides in modulating the activity of signaling sites of the 3D phage map):
--navigation and signal sensitivity of Gp-containing target complexes and nanoparticles to the topography of exposed constituents:
---clusters/repeats as cmbinative sites for the mosaic of natural and synthetic glycan antennas exposed in the Gc (in the management of phage systems and system individual phages);
---Gc mosaics as"dots on a two-dimensional map"/spots on the extended porous hydrophobic or hydrophilic surface of membranes and others (nano)materials and structures (preparation of affine replicas for targeted communications with phages);
---pronounced 3D macrocharacteristic informational new structures in contacts between Gp and Gp, Gp and Gc, Gc and Gc - depressions, seams, folds, faults (establishment and

standardization of features of characteristic/specific/directed aggregation of phages, for example, in the tail region of possible interphage crystallization/ conservation , as well as effects on viability, aging as phage cycles; 3D modulation of recognition and other functions of phages such as lectinic-like and enzymatic-like);

*visual pattern correspondence between the systems of Gc and "Gc recognition Proteins" as “One Gc--A set of proteins/ lectins”, “One protein/ lectin --a Set of Gc”; the presence of correspondence between the subsystems of “protein/ lectin and Gc”, the establishment of ranking orders (sub)systems of “Gc and protein/ lectin”, depending on the implementation of the types of activities (building a network of these relationships involving representations of the phages as the dominant type/types of Gc or the dominant type/types of protein/ lectin):
-one type of Gc can simultaneously interact with several lectins/ lectin (sub)systems that can be ranked according to the degree of affinity and accessibility to the type of Gc selected;
-one type of lectin can simultaneously interact with several different Gc/ “Gc (sub)systems” that can be ranked according to the degree of affinity to the type lectin selected;

*prediction of the potential of Gc-immunomodulating, prebiotic, postbiotic, antimicrobial and/or antiviral action, for example, in case of fucan analogues, other pseudo-PS and pseudo-PG, is needed to support biotope pro/post/syn-biotic microbiocenoses and compartments, including those that increase the survival of indigenous, symbiotic and probiotic lactobacilli or bifidobacteria, as well as mixtures of bifidobacteria with lactobacilli:
-the use of test method modulation (for example stimulation) of massive growth of bifidobacteria on heparinization with the cations of metal surfaces in the presence of type Gc selected (application of phages as Gc- and lectin-macroforms - as [nano]particle forms of Gc instead of molecular and complex non-[nano]particle Gc):
-the development of the most efficient affine biocompatible with biological fluids and habitats bio[nano]materials and conditions for the implementation of the desired activity:
---increased survival of pro/post/syn-biotic strains and consortia;
---testing of biocompatibility of effector combinations in relation to the effects on Bf microbiocenoses as communication bodies (CB);
---establishment of effective multi-stage efficiency in the (sub)network of reactions of the unified protection of the body;
-establishing the adequacy of Gc interactions involving lectins and enzymes, causing irreversible destruction and lysis (establishment of analogous patterns when using phages):

--opsonized labeled pathogenic bacteria;
--containing pathogenic bacteria Bf;

*pro/post-biotic mosaic organized strategic attack against pathogen CB and Bf (phages instead of antibiotic in the center spot or other selective and wishable place of the agar plate seeded with target bacteria to identify biocompatibility and synergy with lectins and Gc):
-achievement of multicenter CB through temporal characteristic patterns of succession;
-simulation of 2D [taking into account disks] or 3D [taking into account wells]-probiotic functionally coupled microbiocenosis and probiotic biotope compartment (taking into account the phage cocktail as another additional component of probiotic biocenosis on hydrophilic porous gels);
-establishment of a residual diagnostic and prognostic predominantly localized center of CB pathogenicity in a pro/post/synbiotic environment and/or under conditions of a pro/post/synbiotic attack in the biotope of interest as a series of predicted expected visual patterns;

*impaired Bf forming in the presence of probiotic preparations (including Gc and step-by-step associated with Gc combinations) can lead to increased lysis of Bf (modulation of Bf degradation and lysis during Bf assembly using individual type phage or phage cocktails).

2.3. Phages-Bacteria systems with Lectins—"Gc type(s)" interaction

On the one hand, there are a lot of Gp, both exposed and involved in internal assembly and packaging (between proteins and Gp and between proteins and nucleic acid Gc) in asymmetric with a target-oriented complex-configuration recognition machine of the *Caudovirales* order (their body including head/capsid, neck, trunk and, especially, tail, involved in the correct oriented landing/reception on the cell surface), which provides for the principles of lectins—Gc recognition and compact binding. On the other hand, in the shell and cell wall of target bacteria there are sets of receptor/acceptor Gc for phages (see below).

The phage systems synthesized in the parent bacteria enter the extracellular space and interact with target bacteria (for example, pathogens that need to be neutralized), distributed unevenly and mosaically across the cell surface, due to reversible binding and relocation (weakly Ca^{2+}-dependent in the case of phage SPP1 *B.subtilis*) on the cell surface, depending on its characteristics[28]: pole, septic, with an increased level of metabolism (opportunities for phages to more fully use active resources of bacteria). The "uncertainty" or limited/regulated

definiteness of the behavior of a system of multiple phage particles using weakly affine bonds characteristic of lectins—Gc interactions and the characteristics of the target bacterium is coevolutionarily justified and provides the necessary choice of effective and adequate pairs in sufficient quantity, which ultimately increases the reliability of achieving the result - covering the type/subspecies-specific cytolysis.

During reception, the tail components of phages (proteins and their associates as replicas for intracellular 3D printers) find adequate affinity sites for sorption (initially reversible {for example, binding of phage SPP1 with Glc-teichoic acid *B.subtilis* or endolysin PlyP35 phage *Listeria* - with GlcNAc-teichoic acid[29,30]} and later irreversible [subsequent binding of SPP1 with receptor protein YueB as a secondary receptor][29], more broadly – the involvement of oxidoreductases (EC 1.) and esterases (EC 3.1.) in the formation of covalent bonds). Core oligosaccharides (phages as typical suspension macro-Lectin) from the composition of LPS of *Gram* negative bacteria (*E.coli, Y.pestis* {receptor for the phage phi-A1122 – LPS region Hep/Glc-Kdo/Ko}[31], others); capsular galactosylated phosphoramidate (*Campylobacter jejuni*) (receptor for phage F336 - GalNAc MeOPN)[32], PG and glycosylated (a prerequisite for entering the cell in comparison with the phages of the *Myoviride* family) teichoic acids (for example, *S.aureus* in the case of recognition by phages of family *Siphoviridae*[33]).

The creation of unique or highly specific receptors based on LPS, PG, teichoic acids and other Gc with a high affinity for the type of phage, is a way to increase both the phage selectivity (against pathogens) and the speed (accelerated cytolysis). Fc-Ig-receptor-mediated (Ig-dependent) entry of phages into cells is possible.[34] Ig technologies are supported by a wide spread of Ig-like domains (I-set, FN3, and BIG-2) among representatives of *Caudovirales,* families *Myoviridae* (for example, phage T4), *Podoviridae* (for example, phage T7) and *Siphoviridae* (for example, phage lambda, phages of lactococci) infecting *Gram* positive and *Gram* negative bacteria.[35]

2.4. Prospects of Gc in the investigation of enzymes including enzymes from the composition of phages

Within phage body, the composition of the acute tail protrusions contains proteins of primary and secondary reception and signalling. These proteins include sets of enzymes of different classes, namely, endolysins[36] (for example, hydrolase class 3, according to International Enzyme Classification [EC],[10]), recognizing and destroying the layers masking bacterial cell surface and membrane PG, LPS, glyco/lipo]teichoic acids and other constituents, which are

necessary for the subsequent introduction of packed nucleic acids of the capsid into the cell. These include endoglycan hydrolases such as muramidases (EC 3.2.1.) and lysozyme (EC 3.2.1.17)-like PG-ases; endo-N-acetyl-D-neuraminidases (EC 3.2.1. endosialidases, for example phage K1F endosialidase F]), endoramnosidases (EC 3.2.1.), other hydrolases (for example, EC 3.5. amidases), depolymerases and polysaccharide lyases (for example EC 4.2.2. pectin lyases/ pectinases/ pectic transeliminases), lytic transglycosylases and inverted other glycosyl hydrolases (EC 3.2.1.). Phage endolysins exhibit typical properties of primary signals, since as a result of limited hydrolysis of biopolymers, a spectrum of secondary shortened signals is formed and acted.[36] For endolysins, phages are characterized by modular organization including the presence of CBM with a highly pronounced thermostability (the presence of a spatial module of recognition and binding to bacterial receptors; often within C-terminal amino acid sequence in the form of several repeats) and a separate module of lytic activity (usually in the N-terminal region), destroying the cell wall at the binding site(s). Thus, in the case of phage Cp-7 (*S.pneumoniae*), CW_7 repeats bind GlcNAc-MurNAc-L-Ala-D-isoGLN muropeptides to PG.[35=35] The activity of the subsequent introduction of nucleic acids is paired in cascades in space and time with both of the mentioned above endolysin activities (in the process of biosynthesis and assembly of phage at the genetic and final structural-packaging levels). The variety of phage modules allows, by combining them, to create new components of nanomachines - key mechanical nodes for reception and further injection into cells using the phage tail.[37] In this sense, phages can be considered as nanorobots co-functioning together with target bacteria. The use of the phage cocktail expands the spectrum of endolysins and increases the reliability of achieving the final result – selective and intensive cell lysis (for example, the destruction of PG can be achieved by a set of PG hydrolases of highly varying different types acting as cascades).

Phages are able not only to change the phenotype of bacteria and determine the abnormal formation of films, but also, due, for example, to the disturbed assembly of own machine, to cause enhanced bacterial lysis involving spectrum of enzymes.[36,38] In combination with antibiotics of the aminoglycoside group (gentamicin, others), phages exhibit a synergistic effect against *S.aureus* Bf.[39] The mechanism of synergy is to enhance the degrading effect of phages against the formed aggregates of bacteria (gentamicin can induce a pronounced aggregation phenotype of staphylococcus populations). Pectin lyase-like domains of antistaphylococcal tails (including anti-*S.aureus*) of phages are considered as promising for the lysis and degradation of Bf.[40]

Other possible combinations of the pro/post/synbiotic protector factors with phages can be perspective. This is especially true for the potential construction of mixed combinations of probiotic lactobacilli and bifidobacteria as well as phytopreparations, probiotic and postbiotic metabolites together with phages.[19-21,41-45] In addition, the potential of the pro/post/synbiotic preparations to manage bacterial compartments at the level of leader microorganisms[46] may allow reducing the load on phage protective cocktails in the direction of restricting their choice against highly effective and most dangerous pathogens. In the whole, the use of pro/post/synbiotics in combination with phages should lead to a decrease in the diversity of phage targets while increasing phage effectiveness.

Technologies using phages allow the development of peptide imitators and mimetics of carbohydrates/(oligo)saccharides and PG, are promising for sensory and therapeutic uses as antiviral agents, including as inhibitors of cell adhesion and in the development of vaccines.[47-50]

3. CONCLUSION

The data presented indicate the perspective of ideas and principles regarding macro/ micro/ nano/ molecular hierarchic levels of interactions between lectins/enzymes and Gc, which can be applied for a comprehensive assessment of the features of receptor-recognizing interactions involving viruses, as well as for deeper understanding the events during Phages-Bacteria system interactions in biotope microbiocenoses, which is important for biocontrol and the construction of therapeutically significant viruses and their submachine constituents in combinations with pro/post/symbiotics.

Disclosure of conflict of interest

The authors declare no conflict of interest.

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