Chapter 20

Polysaccharides from Red Algae: Genesis of a Renaissance

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Additional information is available at the end of the chapter

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1. Introduction

The Red Queen Effect is an evolutionary hypothesis [1]. This evolutionary concept is named for the Red Queen´s comment to Alice in Through the Looking Glass that "it takes all the running you can do, to stay in the same place". It posits that multicellular organisms with long life cycles must constantly change, adaptation process driven by the changing conditions of the environment, in order to survive the onslaught of potentially lethal pathogens which have much shorter life cycles and can thus evolve orders of magnitude faster.

Viruses play a relevant role in these new evolutionary mechanisms by transferring of genes to and from the hosts they parasite [2]. Over the past three decades, it has become apparent that viruses are ubiquitous, abundant and ecologically important in the environment [3]. As phylogenetic analysis shows, nearly all organisms of all kingdoms have become infected by viruses since the beginning of life. The great impact that viruses can have on the genetic systems is well illustrated by the evolution of mitochondria. In reference [4] have shown, the existence of a strong selection pressure has pushed for the replacement of cellular enzymes by viral ones in mitochondria and chloroplasts. In both organelles, this replacement has been associated with profound modifications in the mechanism of DNA replication and chromosome structure [5]. The fact that viruses are probably very ancient allows better understanding their extraordinary diversity, explaining why most viral proteins inferred from genome sequencing have no cellular homologues [6]. Besides, the existence in the biosphere of an unlimited reservoir of viral proteins has provided opportunities at different steps of the evolutionary process, to introduce new functions into organisms.

At the present, it remains controversial the inclusion of viruses in the "tree of life". Several authors assume viruses are non-living organisms and believe their properties are driven solely by thermodynamically spontaneous reactions while others give priority to the fact that phylogenetic tree is based on the genomic content of its components, not the physical manifestations of these genomes. Moreover, the fact that viral genomes carried inside virions encode gene products that allow for adaptation and response to changing intracellular and extracellular conditions favors the inclusion of these agents in the tree of life [7,8].

The oligosaccharides chains (glycans) attached to cell surface and extracellular proteins and lipids are known to mediate many important biological roles [9,10]. However, for many glycans, there are still no evident functions that are of obvious benefit to the organism that synthesizes them. In 1949, Haldane postulated "Now every species of mammal and bird so far investigated has shown quite surprising biochemical diversity by serological tests. The antigens concerned seem to be proteins to which polysaccharides are attached. We do not know their functions in the organism, though some of them seem to be part of the structure of the cell membrane. I wish to suggest that they may play a part in disease resistance, a particular race of bacteria or virus being adapted to individuals of a certain range of biochemical constitutions, while those of other constitutions are relatively resistant" [11]. In [12], suggested that glycan diversification in complex multicellular organisms is driven by evolutionary selection pressures of both endogenous and exogenous origin. They also argued that exogenous selection pressures mediated by viral and microbial pathogens and parasites that recognize glycans have played a more prominent role, favoring intra-and interspecies diversity.

2. Red algae and carrageenans

Red algae (Division: Rhodophyta) are one of the oldest and largest groups of eukaryotic algae with more than 10000 species described (Figure 1). They are distributed worldwide but grow best in waters of near 15°C. They have the characteristic of all eukaryotes including the nuclei which in some algae are smaller than their plastids. However, their cells lack of flagella so they need the water movement to carry masculine cells to the oocyte. They also have disorganized chloroplasts lacking of external endoplasmic reticulum and containing unstacked thylakoids. Their red colour is due to the presence of the phycoerythrin pigment which reflects red light and absorbs blue/green ones. Since blue light penetrates water to a greater depth than light of longer wavelengths, red algae are able to photosynthesize and live in water of 260 m in deep which receive 0.1% of surface irradiance; this means one thousand times less light than the surface. Those rhodophytes that have small amounts of this pigment might seem green or bluish from the chlorophyll and other pigments present in them.

Over the last 2.45 billion years, algae have been diversifying [13] in order to survive in competitive ecological niches. This adaptation led to evolution of a large and diverse array of biochemical constituents.

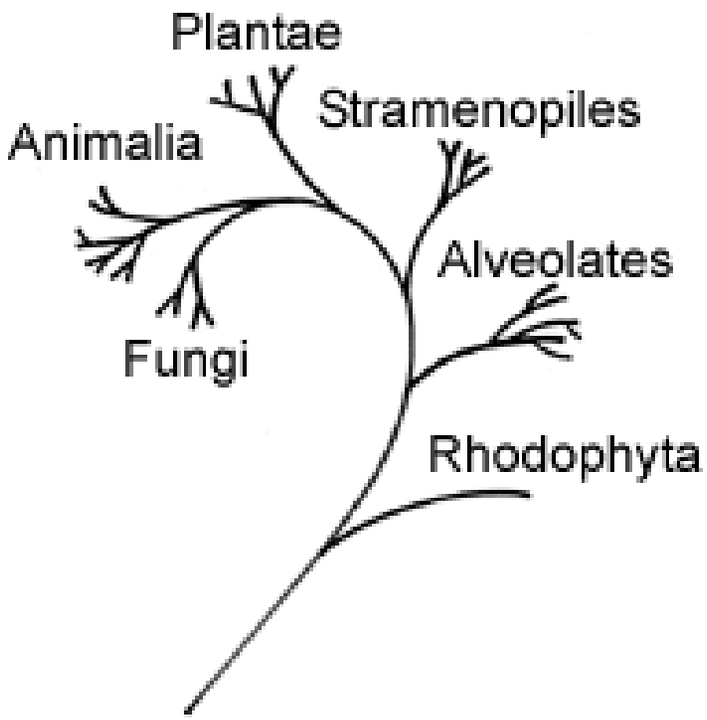

Figure 1. Rhodophyta branched off very early in the tree of life

Red algae contain large quantities of polysaccharides in the cellular wall, thereof, most are sulfated galactans. These galactans are generally constituted by alternatively repeated units of bonds 1,3-α-galactopyranose and 1,4-β-D-galactopyranose can defer in the level and pattern of sulfation, in the substitution by methoxy and/or pyruvate groups and other sugars such as mannose and xylose. They also defer in the 3,6-anhydrogalactose content and the 1,3 -α-galctopyranose residues configuration.

Among these galactans, the carrageenans (CGNs) may be mentioned, which have similar structures to the pattern observed in the galactosaminoglycans. They comprise a wide group of structures that may be divided in two families: the κ-family, defined by the presence of a sulfated C4 group in the unit β-D, and formed by CGNs-κ/ι and the carrageenans-μ/ν, and the λ-family, characterized by a sulfate-C2 group and constituted by all the varieties of λ structures. The λ- and ι-carrageenan types are more strongly sulfated than the most of the heparan sulfate (HS) derived from tissues [14]. In general, this type of carrageenans exhibits a viral inhibitory potential a little greater than the κ-carrageenans.

Polysaccharides are composed of building-blocks that although being not numerous, their almost infinite combination led to an array of polysaccharides with an important structural complexity [15]. The diversity of polysaccharides can be further increased by acetylation,

methylation and, more commonly in the case of many marine algal polysaccharides, sulfation [16]. Moreover, many algal polysaccharides are metabolically active, either as a storage molecule which undergo structural changes during their life cycles or as a structural component [17].

The sulfated polysaccharides are highly abundant and accessible compounds that may be isolated from various natural sources. Micro- and macroalgae are under investigation for numerous commercial food, agri- and horticultural, pharmaceutical, cosmetic and bioenergy applications [18-21]. Polysaccharides are also known for their wide and variable physicochemical properties which make them suitable for different applications in the fields of medicine and pharmacology. They have proved to be useful tools due to their immune-modulator and antitumoral activity, their interference in the clotting system and in the inflammatory processes, in dermatology, in dietary programs and moreover by affecting the viral replication. Among the natural sources where they can be found are the cell walls from algae. Depending on the type of algae, those with similar structures to the glycosaminoglycans (GAGs) and wide antiviral activity can be isolated [22] (Table 1). Antiviral activity has been documented for retrovirus: human immunodeficiency virus type 1 and 2 (HIV-1 and HIV-2), herpesvirus: herpes simplex virus (HSV) type 1 and 2, human cytomegalovirus (HCMV); pseudorabies virus; flavivirus: dengue virus type 2; smallpox virus: variola virus; hepadnavirus: hepatitis B virus (HBV); orthomyxovirus: influenza A virus (inf A); paramyxovirus: respiratory syncytial virus (RSV) and parainfluenza virus; rhabdovirus: vesicular stomatitis virus (VSV); arenavirus: Junin virus, Tacaribe virus and togavirus: Sindbis virus, Semliki Forest virus and against some naked viruses, such as encephalomyocarditis virus, Hepatitis A virus [23] and papilloma virus (HPV) [24], of both DNA and RNA viral types (Table 1). For most of these viruses the initial bond of the virus to the cells would be mainly mediated by the interaction of virus with a GAG of the cellular surface known as HS [14]. In general, sulfated polysaccharides have a chemical structure very similar to the HS. Thus, they might block viral infection by competing against virion attachment to the cell surface.

GAGs are linear polysaccharides constituted of successive repetition of a disaccharide unit which may be sulfated. GAGs can be divided in two groups; Glucosaminoglycans, like HS and galactosaminoglycans like chondroitin sulfate. The initial incorporation of saccharide units of *N*-acetylglucosamine or *N*-acetylgalactosamine, respectively, gives their names. An important difference between these groups is that Glucosaminoglycans are attached by 1,4 union while galactosaminoglycans are attached by 1,3 and 1,4. GAGs are found mainly in the cell surface and in much of the intracellular matrix of the mesodermic tissue as is shown in Table 2 (connective, cartilage, muscle and bone). Frequently, they are linked to a core protein and one or more covalently attached glycosaminoglycan chains, known it as Proteoglycans.

GAGs are negatively charged molecules that can have a physiological significance like hyaluronic acid, dermatan sulfate, chondroitin sulfate, heparin, HS and keratan sulfate.

Algae	**Compound**	**Virus**	**Reference**
	Red algae		
Schizymenia pacifica	λ- carrageenan	HIV-1, AMV	[25]
Schizymenia dubyi	Sulfated galactans with uronic acid	HIV-1, HSV-1, HSV-2, VSV	[26]
Nothogenia fastigiata	(Xylo)mannans	HIV-1,HIV-2, SIV, HSV-1, HSV-2, HCMV, Inf A, RSV, Junin, Tacaribe	[27,28]
Aghardiella tenera	Sulfated Agarans	HIV-1, HIV-2, HSV-1, HSV-2, HCMV, VSV, Inf A, RSV, togavirus, parainfluenza virus, smallpox	[29]
Digenea simples	sp non-characterized	HIV	[30]
Nothogenia fastigiata	Xylogalactans	HSV-1, HSV-2	[31]
Pterocladiella capillacea	Sulfated Agarans and hybrid DL- galactans	HSV-1, HSV-2, HCMV	[32]
Gigartina skottsbergii	λ-,κ/ι- and μ/ν-carrageenans	HSV-1, HSV-2	[33,34]
Cryptopleura ramosa	Sulfated agarans	HSV-1, HSV-2	[35]
Stenogramme interrupta	Carrageenans	HSV-1, HSV-2	[36]
Asparagopsis armata	Sulfated agarans	HIV	[37]
Bostrychia montagnei	Sulfated agarans	HSV-1, HSV-2	[38]
Gymnogongrus torulosus	Hybrid DL- galactans	HSV-2, virus dengue 2	[39]
Gracilaria corticata	Sulfated agarans	HSV-1, HSV-2	[40]
	Brown algae		
Pelvetia fastigiata	Fucans	HBV	[41]
Fucus vesiculosus	Fucans	HIV-1	[42]
Sargassum horneri	Fucans	HSV-1, HCMV HIV-1	[43]
Leathessia difformis	Fucans	HSV-1, HSV-2	[44]
Adenocystis utricularis	Fucans	HSV-1, HSV-2	[45]
Microalga			
Cochlodinium polykrikoides	Extract	HIV-1, RSV, Inf A, Inf B	[46]
Porphyridium sp	Extract	HSV-1, HSV-2	[47]
	Green algae		
Monostroma latissinum	Sulfated Rhamnans	HSV-1, HCMV, HIV-1	[48]

Table 1. Antiviral activity of sulfated polysaccharides extracted from marine algae

GAG	Location	Comments
Hialuronates	Synovial fluid, vitreous humor, extracellular matrix with loss of connective tissue (vasculogenesis).	Long polymers (containing no sulfates), shock-absorbing.
Chondroitin sulfate	cartilages, bone and cardiac valves.	More abundant GAGs.
Heparan sulfate	Basal Membrane and components of the cellular surface.	
Heparin	Components of the intracellular granules of the mastocytes, coating of the lung arteries, liver and skin.	More sulfated than the Heparan sulfate.
Dermatan sulfate	Skin, cardiac valves and blood vessels.	Long polymers (no sulfates), shock-absorbing.
keratan sulfate	Cornea, bone and cartilage.	More abundant GAGs.

Table 2. Normal distribution of GAGs in the body.

3. Relationship among glycosaminoglycans, carrageenans and viruses

The discovery that viruses are highly abundant in natural waters initiated renewed research on the impact of viral infection and lysis on aquatic microorganisms [49]. It is believed that viruses influence the composition of marine communities and are a major force behind biogeochemical cycles. Each infection has the potential to introduce new genetic information into an organism or progeny virus, thereby driving the evolution of both, host and virus [50].

The eukaryotic algae represent the oldest known eukaryote for which there exist clear geological data [51] and all classes of algae have their specific DNA virus. The HSV is an ancient DNA virus which is widespread in nature and has coevolved with its hosts. Many viruses interact with their host polysaccharides present on the cell wall; HSV uses HS.

The basic structural motifs and modifications of HS glycosaminoglycans seem to have been conserved for several hundred million years of evolution [52,53].

One suggested explanation is that endogenous heparan sulfate-binding proteins may have developed different binding specificities with evolutionary time. At first glance, this may seem an exception to the suggestion made here, in view that extensive glycan diversification has accompanied species evolution. However, HS can generate numerous intrinsic structural variations, and there are currently inadequate data about the extent of species-

specific differences in the specificities of the binding proteins and/or the expression of structural motifs in different cell lineages.

HS is highly sulfated and it is thought to be the most biologically active GAG. The sulfated monosaccharide sequences within HS determine the protein binding specificity and regulate fundamental biological functions including growth control, signal transduction, cell adhesion, homeostasis, morphogenesis, lipid metabolism and pathophysiology [14]. Numerous viruses including herpesviruses utilize cell surface HS as receptor to infect target cells.

It has been reported that in the course of an inflammation, an infection or tissue damage, the proteoglycan HS is cleaved causing fragments of soluble HS [54]. On the other hand, in healthy tissues, no significant fractions of soluble HS are found, though they can be found in the fluids of damaged tissues –at concentrations within the required ranges to stimulate dendritic cells [55] and in the infected individuals urine [56].

HSV attaches to cells by an interaction between the envelope glycoprotein C and cell surface HS. The virus-cell complex is formed by ionic interactions between the anionic (mainly sulfate) groups in the polysaccharide and basic amino acids of glycoproteins, and non-ionic ones depending on hydrophobic amino acids interspersed between the basic ones in the glycoprotein-binding zone [57]. This interaction is a decisive step in virus multiplication and may be differentiated but not dissociated from an evolutive point of view.

CGNs resemble to some extent the naturally occurring GAGs owing to their backbone composition of sulfated disaccharides are believed to be of potential therapeutic importance because they can mimic with GAGs present in cell membranes.

Natural CGNs, extracted from red seaweeds, are well known as potent and selective inhibitors of HSV-1 and HSV-2. CGNs chemical structures are similar to that of HS that serves as a primary receptor for adsorption of HSV onto cells. Mode of antiviral action is mediated by the interference with HSV attachment to cells, blocking the interaction virus-HS, a mandatory step during the multiplication cycle to achieve a productive infection that involves viral glycoproteins. In this work were used the κ and ι CGNs, their structures are present in the compound named 1C3 CGN, which is an "hybrid" κ/ι-and partially cyclized μ/ν CGN (Figure 2).

Since most pathogens and the toxins they produce bind to specific sugar sequences to initiate infection and disease, it is reasonable to assume that at least some glycan variation must have arisen from this selection pressure [12]. On this basis, pressure of selection *in vitro* with an antiviral drug like HS in the case of HSV may be employed to shorten the time necessary for attenuation. Moreover, in this last case we may speculate that if herpesviruses which are extensively spread in the environment are exposed to sulfated polysaccharide (its natural receptor), in the form of CGN, the appearance of virus variants would readily occur as a consequence of an intense virus-host interaction. Our results indicate that attenuation is a common trait of HSV obtained under selective pressure of CGN.

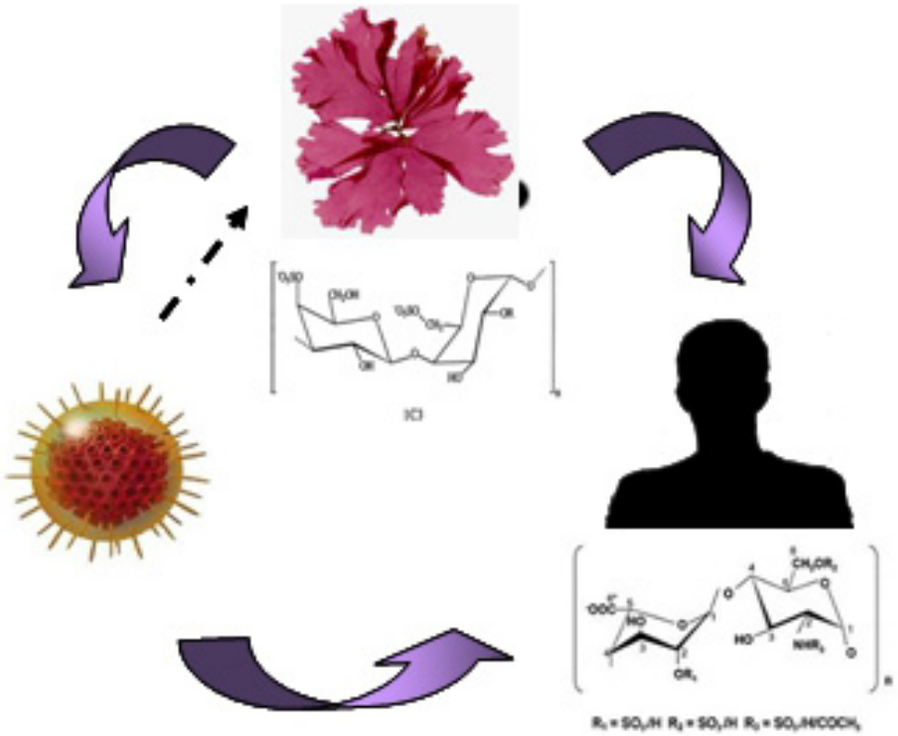

Figure 2. 1C3 CGN isolated from the red algae *Gigartina skottbergii* has a chemical structure similar to cellular HS. Attenuated HSV can be isolated from viral populations grown in the presence of increasing concentrations of CGN. This procedure may reflect an accelerated evolution process for HSV where biological modification of the viral particle can be demonstrated.

4. Viruses: Friend or enemy

It is tempting to postulate that the driving forces of evolutionary novelty are not randomly derived from chance mutations of the genetic text, but from a precise genome editing by omnipresent viruses [58]. For decades, non-coding regions of the genome have been ignored or declared as "junk"-DNA. Recently, scientists have realized that these regions incorporate decisive higher-order regulatory functions. New research has shown that these non-coding repetitive sequences originated primarily from retroviral RNA [59,60].

For a long time, viruses were interpreted as causing acute infections of susceptible organisms, using the host cellular machinery to reproduce, and achieving their lytic nature only in order to infect other cells. Although this narrative remains valid, it merely represents a case of viruses that were unable to reach persistent or chronic status of infection [61]. Most viruses, however, are stable, persistent agents that are able to establish a complex relationship with the host cell and in many cases this interaction lasts for the entire lifespan

of the cell even when a competent immune system is present. The immune system is of crucial importance in defense against infection. It has to cope with a large number of different pathogens that relentlessly develop new ways to avoid recognition or elimination. Yet most infections are cleared. Immune-system genes must evolve to keep pace with increasingly sophisticated evasion by pathogens. To remain effective, defense demands creativity and competence because many pathogens have sophisticated and rapidly evolving evasion mechanisms [62]. This defense response is triggered by the presence of pathogens within the host, however there exists an immune response that comprises a set of autoreactive "natural antibodies" that do not rely on exogenous antigen stimulation to be synthesized by autoantibody-secreting B lymphocytes [63,64]. On the other hand, these autoreactive natural antibodies are reactive against components of the host´s immune system (i.e. cytokines) exacerbating ongoing infectious diseases or predisposing host to infection [65,66].

Individual resistance to pathogens depends on the combination of receptors on cells from the immune system although non-immune genes also influence resistance [67]. Signs of natural selection in a human population are especially illustrative, when a mutation in a certain gene is dangerous in normal conditions but confers resistance to infections widespread in the region. Among the better known are the mutations in hemoglobin and glucose-6-phosphate-dehydrogenase affecting red blood cells and conferring resistance to malaria [68]. Another example is the deletion at the 5´end of the CCR5 chemokine receptor conferring resistance to HIV infection. This molecule serves as the principal co-receptor, with CD4, for HIV-type 1. The allele with the deletion was intensely selected in Europe probably because it also provided resistance to plague and smallpox [69]. More subtle, but nonetheless important, relationship between cell and virus is that associated to changes in cell physiology due to viral infection that regulates cell death, transformation, secretory pathways, cell stress response, etc [70-72]. This panorama may account for a "symbiotic evolution" of cell and virus, although viruses have much shorter generation times than cells. Studies of genomic polymorphism of HSV-1 suggest that the evolution of this virus would be very slow and host-dependent [73].

5. Effect of carrageenans on the virus

In our laboratory, viral variants of HSV-1 (strain F) and HSV-2 (strain MS) were obtained by successive passages in Vero cells under selective pressure with the 1C3 CGN (is an "hybrid" κ/ι-and partially cyclized μ/ν-CGN) and ι or κ-CGNs respectively in order to test the ability of the CGN to generate resistant variants during the selection process and to study the CGN-virus modulation. Different clones were plaque purified and pretested to exclude reversion to wild type (Figure 3). 1C314-1 and 1C317-2 are viral variants derived from HSV-1 strain F (1C314-1 means, "1C3" is the type of CGN that was used for the selection of the variant, "14" number of pasagge that we chose for cloning, and "1" is the number of selected clon). κ22-12, κ22-13, ι22-9 and ι22-12 are variants derived from HSV-2 strain MS. In this case, κ and ι CGNs were used for the selection of these variants, respectively.

After the viral selection and cloning, all the variants showed a syncytial phenotype on Vero cells, this phenotype was also observed on mouse lung and genitals primary cell cultures [74,75].

Figure 3. Variants of HSV were obtained by successive passages in Vero cells under selective pressure with different types of CGNs with increasing concentration of them.

In order to characterize the obtained viral variants, the susceptibility to CGNs was assessed. All the viral variants showed low or middle levels of resistance to CGNs, heparin, Aciclovir (ACV), and Foscarnet (PFA) (Figure 4).

The results are shown as relative resistance (RR): IC50 viral variant/IC50 parental strain.(IC50 : inhibitory concentration 50%, is the concentration in µg/ml required to reduce plaque number by 50%).

Although some variants showed 15 or 17 fold of RR, those values are not significant compared with viral variants selected with ACV (ACVp6-F and ACVp6-MS), that showed higher values, > 50 RR, after only 6 passages with ACV. (unpublished data).

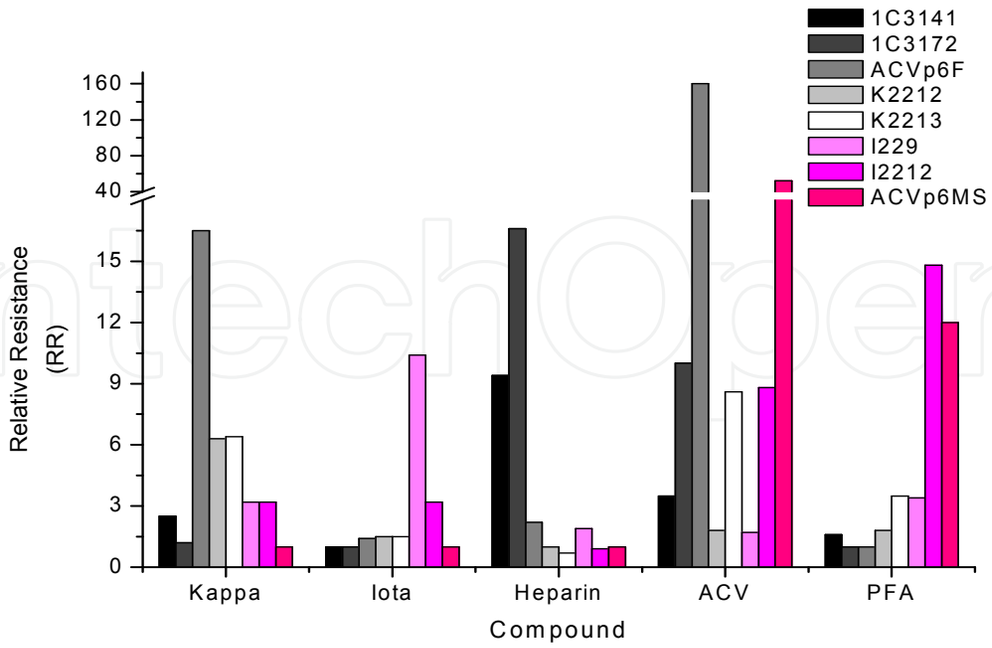

Figure 4. Susceptibility of HSV viral variants to several compounds with known antiherpetic activity. IC_{50}values were determinate on Vero cells by a reduction plaque assay; the RR values were calculated as (IC_{50}: viral variants/ IC_{50} parental strain). Kappa: κ CGN; iota: ι CGN; ACV: Aciclovir; PFA: Foscarnet.

Mucosal surface represents the primary site for replication and spread of several viruses including HSV type 1 or 2. In order to resemble natural routes of infections, virulence of the viral variants was assessed in a mouse model by two routes of infection, intravaginal or intranasal using either BALB/c or C57BL/6 mice.

The HSV-1 variants were avirulent for BALB/c mice infected by intravaginal route, although the parental strain F was highly lethal (Table 3). The attenuation of the variants correlated with low levels of pro-inflammatory cytokines (IL-6 and TNF-α) in vaginal lavages with respect to the parental strain, despite that viral titers were similar between the viral variants and the F strain. Nevertheless, the variants were highly lethal for BALB/c mice inoculated by the intranasal route, with a generalized organ spreading of virus.

On the other hand all the clones of HSV-2 were less virulent for mice intravaginally and intranasally inoculated, particularly for C57BL/6 mice, whereas MS strain produced 100% of mortality. In contrast to HSV-1 variants, the attenuation correlated with high levels of pro-inflammatory cytokines (TNF-α and IL-6) detected in vaginal lavages (Table 3).

Despite the differences in the virulence of variants, the levels of infectivity in the site of inoculation did not differ from those observed for the parental strains. These results suggested that the observed attenuation was not due by a lower viral replication and it could be explained by a differentiated immunological response.

Viral Characterization	HSV-1 (F)		HSV-2 (MS)	
	Parental strain	Viral variants	Parental strain	Viral variants
Cytopathic effect (*in vitro*)	Cell rounding	Syncytial	Cell rounding	Syncytial
Intravaginal virulence	High	Avirulent	High	Low
Intranasal virulence	High	High	High	Low
Levels of IL-6 and TNF-α	High	Low	Low	High

Table 3. Characterization of HSV variants obtained by selective pressure with CGN. Viral variants obtained under pressure of selection with carrageenans were characterized regarding their phenotype, *in vivo* virulence for BALB/c mice and immune response. The parental strains were also assessed for final comparison.

Moreover, by the same methodology, variants of HSV-2 obtained by selective pressure with nonsulfated compounds (from the condensation of mandelic acid) have been obtained. Viral variants were not syncytial and showed resistance to the same drug, heparin and the carrageenan $1C_3$ in the order of 2.6 to 6.7 times with respect to the control virus. However, in vivo, these variants showed no difference of pathogenicity and mortality with the parental strain.

6. The evolutive beliefs

About seven hundred and fifty million years ago, the first multicellular organisms appeared on earth. Since then, cells from multicellular organisms have found a way to become smarter. Multicellular life forms were initially isolated communities or "colonies" of unicellular organisms. However, the evolutionary advantages of living in a community soon led to communities composed of millions and billions of individual cells socially interactive. The evolutionary trend towards increasing complexity in the community is but a reflection of the biological imperative of survival. The better an organism perceives the surrounding environment, the more chances to survive. Unfortunately, we "forget" that cooperation is conveniently necessary for the evolution when Charles Darwin brought out a radically different theory about the origin of life. One hundred and fifty years ago, Darwin concluded that living organisms are involved in a constant "struggle for survival". In the final chapter of The Origin of Species by Means of Natural Selection, or The maintenance of favored races in the struggle for existence, Darwin speaks of an unstable "struggle for existence" and that evolution is conditioned by "the war of nature, from famine and death". This concept is related to the Darwinian notion that evolution occurs at random [76]. While Darwin is the most famous evolutionist, the first scientist to consider evolution as a scientific fact was the distinguished French biologist Jean Baptiste de Lamarck [77,78]. Even Ernst Mayr, the principal agent of the "neo-Darwinism," a modernization of Darwin's theory that incorporates molecular genetics of the twentieth century, admits that Lamarck was the pioneer [79]. Lamarck presented his theory not only fifty years before Darwin, but also offered a far less violent theory of evolutionary mechanisms. Lamarck's theory suggests that evolution is based on a cooperative and “instructive” interaction between organisms and the environment that allows living things to survive and evolve in a dynamic world. His idea was that organisms acquire and pass on the necessary adaptations to survive in a changing environment. One of the reasons why scientists are rethinking the theories of Lamarck is that evolutionists continue to remind the invaluable role of cooperation in maintaining life in the biosphere. In the book Darwin's Blind Spot written in 2002 by British physicist Frank Ryan has been recorded a number of these relationships [80]. Today, the understanding of cooperation in nature is much deeper than that obtained by simple observation. Biologists are increasingly aware that the animals have coevolved with different sets of microorganisms necessary for a healthy and “normal” development [81]. Recent advances in genetics have revealed an additional mechanism of cooperation between species. It has been discovered that genes are shared not only among individual members of a species (sexual reproduction) but also between members of different species. The distribution of information through gene transfer accelerates the process of evolution, in view that organisms can learn lessons "learned" by others [82-85]. Because of this distribution of genes, organisms can no longer be considered as isolated entities, there are no walls between species [86]. The distribution of genes is not a “by chance” mechanism. Is the method that nature uses to increase the survival of the biosphere. Genes are nothing more than a physical memory of the experiences learned by the organisms, the exchange of these genes between species spreads these “memories” and, consequently, influences the survival of all

organisms that constitute a living community. Timothy Lenton has shown that evolution is more dependent on the interaction between species than on the interaction among individuals of the same species [87]. The evolution thus becomes a question of survival of fittest groups, not of individuals better adapted.

7. Genesis of a renaissance – Cooperative biocommunication

Darwinian theory emphasizes competition and selection of the individual as a main guiding force in evolution (not cooperation or group membership). Evidence supports the fact that viruses and other genetic parasites are key elements in the evolution of all living organisms. In "Life: The Communicative Structure", Dr. Witzany suggests that the genesis of new species, genera, and realms of organisms would not occur in any neo-Darwinistic sense via "chance mutations" and selection, but via a kind of innovation code (evolution code, creation code, text generating code), which is capable of DNA/RNA text editing [88]. It turns out that the genetic code that encodes proteins -practically the sole subject of current bioengineering- is only a kind of structuring vocabulary, and not a complete structure in itself, and is subjected to a high-order regulatory code that lies hidden in the nonprotein-coding regions of the DNA, which have been identified as RNA agents many years later [89]. There is increasing evidence that all cellular life is colonized by exogenous and/or endogenous viruses in a nonlytic but persistent lifestyle. A persistent lifestyle in cellular life-forms most often seems to derive from an equilibrium status reached by at least two competing genetic and the immune function of the host that keeps them in balance. If we imagine that humans and one of the simplest animals, *Caenorhabditis elegans*, share a nearly equal number of genes (ca. 20,000) it become obvious that the elements that create the enormous diversity are not the protein coding genes but their higher order regulatory network that is processed by the mobile genetic elements, such as transposons and retroposons and noncoding RNAs [90]. If we consider the important role of the highly structured and ordered regulatory network of noncoding RNAs as not being randomly derived, one of the most favorable models with explanatory power is the virus-first thesis [91].

For many decades it was common practice to speak about the "genetic code" with its inherent language-like features. The concept postulated by Manfred Eigen that nucleic acid sequences are comparable to and function like a real language, coherent with a (molecular) syntax, linguistic and a vocabulary, was commonly used in genetics, cell biology, and molecular biology.

In contrast with the evolutionary paradigm of random assemblies of nucleic acids that constitute the genetic text we do not know any real-life languages or codes which emerged as a randomly derived mixture of characters. Every language is based on signs, whether they are signals or symbols. In humans and other animals they are transported auditively, visually, or tactilely. In nonhuman living beings they are transported by small molecules in crystallized, fluid, and gaseous form. Additionally these signs can be combined coherently with combinatorial rules (syntax). Signs are not generated and used by themselves, but in

real-life languages by living beings. These sign-generating and sign-using agents live *in vivo* in continued changing interactions and environmental circumstances (interaction HSV-cells-carrageenans). This is the context (pragmatics) in which a living being is interwoven. This context determines the meaning (semantics) of the signs in messages that are used to communicate and to coordinate single as well as group behavior (changes in HSV-glycoproteins in contact with cell surface modificate innate response). Therefore, we may understand that the same sentence, or the same syntactic sequence order, of any language or code can have different, and in extreme cases, opposite meanings and therefore transport different messages. The important consequence of this fact is that it is not possible to extract the meaning of an information content solely out of the syntactic structure, but someone has to identify the context within which the living being uses this syntactic structure. The primary agents are not the sequences of signs, nor the rules which determine sequences, but the living agents. Without living agents there are no signs, no semiotic rules, no signalling, and no communication, no living agents could coordinate growth and development. If we assume the genetic code to function language-like, knowing that no language which has been observed functions by itself, then we have to postulate living agents that are competent to use signs coherent with syntactic, pragmatic, and semantic rules. Adapted to the genetic code, this means that there must be living agents competent in generation and integration of meaningful nucleotide sequences, and meaningful nucleotide sequences are not a randomly derived mixture of nucleotides. In accord with Dr. Witzany, this view could change the construction of research projects, that is, shifting the focus from mutational (random) changes of nucleotide sequences to investigating nucleotide sequences from the perspective of viral-derived sequences that now play important roles in the regulation of cellular functions, for example, HSV asymptomatic infection where the virus have reached an equilibrium status balance by the immune response of the infected host to achieve a latent lifestyle. Their status within one of many addiction modules (genetic and genomic innovations) together with the host immune system, each of them a unique culture-dependent habitat can be changed by nonbeneficial circumstance for the cell (e.g., stress) and they may become lytic again, resulting in a variety of diseases [59].

8. Conclusion

This work invites us to think about a possible alternative to attenuate virus for basic science study, therapeutical or prophylatic applications, employing natural compounds with chemical structures already "seen" by the pathogen and present in the host as essential cellular components widely distributed in nature. Besides, selective reexpression of viral ligands (using different type of polysaccharides) in conjunction with pathogenesis experiments will allow the testing of predictions about putative protective roles played by some glycans in certain tissues. Studying the comparative glycobiology of closely and distantly related species should also help, by ascertaining the rates of glycan diversification during evolution.This strategy could be considered as a natural evolutionary process where the virus contributes with valuable “updated” information gathered from previous ancestral infections and making it available for “new” actual hosts, generating a reciprocal benefit between host and virus.

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