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Antibody-independent and dependent infection of human myeloid cells with dengue virus is inhibited by carrageenan

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Highlights

- The λ-carrageenan is a potent and selective inhibitor of primary DENV infection in human myeloid K562 and U937 cells.
- Carrageenan was also effective to block the antibody-dependent infection mediated by Fcγ-RII
- Viral entry is the main antiviral target in the absence or presence of anti-DENVantibodies

Abstract

This study demonstrated that the λ-carrageenan is a potent and selective inhibitor of the primary infection of human myeloid U937 and K562 cells with the four DENV serotypes, achieving a higher than 99% reduction in virus production at the highest tested concentration of 20 µg/ml, without affecting cell viability at concentrations up to 1000 µg/ml. Since antibody-dependent enhancement (ADE) is thought to play a main role in the aggravation of severe DENV disease, we also evaluated the activity of carrageenan against ADE of DENV infection. The λ-carrageenan was also effective to block the antibody dependent infection mediated by Fcγ-RII in

both cell lines, causing 96-99% inhibition in virus production from cells infected with immune complexes of DENV-2 and DENV-3. Moreover, the inhibitory effectiveness of carrageenan was similar against prM-mediated ADE or E-mediated ADE. Mechanistic studies indicated that DENV-2 entry is the main antiviral target for carrageenan in DENV or DENV-Ab infected human myeloid cells since a strong inhibitory effect was observed when the carrageenan was present only during adsorption at 4°C or internalization at 37°C, whereas the infection was not altered when the compound was added after virus internalization. Thus, our findings have shown that carrageenan may be considered an interesting antiviral agent able to block DENV entry during both primary and antibody-dependent infection of human myeloid cells.

Key words: dengue virus, carrageenan, antiviral, myeloid cells, antibody-dependent enhancement, virus entry

1. Introduction

Dengue infection has evolved in the last decades to turn it the most prevalent arthropod-borne viral disease transmitted to human and a serious health threat in tropical and subtropical regions of the world, particularly in America and Asia (Guzmán and Harris, 2015). Approximately 2.5 billion people are living in endemic areas and estimations reported that about 350 million infections occur each year (Bhatt et al., 2013), with a wide variation of clinical manifestations that include inapparent infection or mild febrile illness as well as severe forms of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) with high level of mortality Guzmán and Harris, 2015). Despite the increasing incidence of dengue infections, no specific chemotherapy is available and supportive medical care is the only treatment for patients.

The virion is an enveloped particle containing a single positive-stranded RNA genome that is translated as a polyprotein then cleaved into three structural proteins (the capsid protein C, a membrane protein M, which matures from the precursor prM, and the envelope glycoprotein E) and seven nonstructural proteins. Four dengue virus (DENV) serotypes designed DENV-1 to DENV-4 cocirculate and sequential infections may occur in the same individual. The primary infection with one serotype is lifelong protective against the infecting virus, but the secondary infection with another serotype appears to increase the chance of developing severe dengue apparently through an immunopathological process known as antibody-dependent enhancement (ADE) of infection (Guzmán et al., 2013). In the secondary heterologous infection, antibodies (Ab) triggered during the primary infection bind to the reinfecting virus and the virus-Ab complexes are able to enter into Fc-receptor positive cells leading to an increase in DENV production and pathogenesis (Flipse et al., 2013). The formation of infective immune complexes can also occur in reinfections with the same serotype when low levels of homologous Ab are

present, for example in infants passively provided with maternal Ab that decline with time passing from neutralization to enhancing virus growth (Kliks et al., 1988). Furthermore, the concern about an incomplete protection against the four DENV viruses and the consequent possibility to trigger ADE and severe dengue has been one of the main challenges for effective and safe vaccine development (Halstead, 2017). Then, it must be considered the need of search for agents able to block primary DENV infections as well as ADE-related infections.

Carrageenans are sulfated galactans well characterized as potent DENV inhibitors in monkey Vero cells and human HepG2 and PH cells with very high selectivity indices (Acosta et al., 2014; Talarico et al., 2005; Talarico and Damonte, 2007; Tischer et al., 2006) The antiviral action of carrageenans, as well as other sulfated polysaccharides, against DENV has been shown to be exerted by blockade of virus entry (adsorption and uncoating) (Ichiyama et al., 2013; Talarico and Damonte, 2007, Vervaeke et al., 2013), due to the structural similarities with heparan sulfate (HS) residues present in membrane proteoglycans and proposed as main initial receptors for DENV in mammalian cells (Chen et al., 1997; Dalrymple and Mackow, 2011; Germi et al., 2002; Okamoto et al., 2012).

As mentioned, the antiviral activity of carrageenans has been evaluated in different types of mammalian epithelial cells, similarly as reported in antiviral studies with diverse classes of molecules. The aim of the present study was to evaluate if carrageenans are also active against DENV in human myeloid cells that are more representative of the human natural infection. The inhibitory effect of λ-carrageenan (Fig. 1A) was determined in the primary infection with DENV and also in the infection with DENV particles pre-opsonized with Ab. To this end the human myelomonocytic U937 cell line and the human erythroleukemic K562 cells, both bearing Fcγ-Ab receptors and previously reported to support in vitro ADE of DENV (Carro et al., 2018; Goncalvez et al., 2007; Littaua et al., 1990; Puerta-Guardo et al., 2010), were used as model systems to test the effect of carrageenan under both Ab-independent and dependent conditions of infection.

2. Materials and Methods

2.1. Compound and antibodies

Lambda-carrageenan (Fig. 1A) was purchased from Sigma-Aldrich. Stock solution was prepared in distilled water at 2 mg/ml.

The mouse anti-DENV monoclonal Ab 3H5 produced by the hybridoma HB46 (ATCC, USA), kindly supplied by Dr. Irene Bosch (University of Massachussets Medical School, USA) and 2H2 (Chemicon, USA) were used. 3H5 is an IgG1 Ab that reacts with DENV-2 E protein and binds to IgG Fc receptor II (FcγRII) whereas 2H2 is an IgG2a Ab reactive with the prM

protein of all members of DENV complex and binds to both FcγRI and FcγRII [Henchal et al., 1985].

2.2. Cells and viruses

The human myelomonocytic cell line U937 and the human myelogenous erythroleukemic K562 cells (both provided by Dr. R. Gamberale, Academia Nacional de Medicina, Buenos Aires, Argentina) were grown in RPMI medium 1640 supplemented with 10 % fetal bovine serum (FBS) and 50 μg/ml gentamycin. Medium was supplemented with HCl/NaHCO3 when incubated at 37°C under 5 % CO2. The C6/36 mosquito cell line from Aedes albopictus, adapted to grow at 33ºC, was cultured in L-15 medium (Leibovitz) supplemented with 0.3 % tryptose phosphate broth, 0.02 % glutamine, 1 % MEM non-essential amino acids solution and 10 % FBS. Vero (African green monkey kidney) cells were grown in Eagle's minimum essential medium (MEM) supplemented with 5 % FBS. For maintenance medium (MM), the serum concentration was reduced to 1.5 %.

DENV-2 strain NGC, DENV-1 strain Hawaii, DENV-3 strain H87, DENV-4 strain 8124 and ZIKV clinical isolate INEVH116141, provided by the Instituto Nacional de Enfermedades Virales Humanas, Pergamino, Argentina, were propagated in C6/36 cells and viral titers were determined by a standard plaque assay in Vero cells as described previously [Talarico and Damonte, 2007].

2.3. Establishment of ADE conditions

The conditions for establishment of in vitro Ab-mediated enhancement of infection in U937 and K562 cells were previously established [Carro et al., 2018]. Briefly, serial dilutions of the DENV-specific Ab or media (control without Ab) were incubated with 1.5 x 10^5 PFU of DENV-2 or DENV-3 for 1 h at 37°C. Then, cultures of U937 or K562 cells grown in 24-well microplates were infected with the mixtures and incubated at 37°C. Supernatant samples of each infected culture were titrated by PFU at 72 h p.i. Also control experiments were performed by adding specific blockers of FcγRI and FcγRII.

2.4. Cell viability assay

To evaluate the effect of compound on cell viability, U937 and K562 cells were incubated with MM containing 2-fold serial dilutions of carrageenan. After 72 h of incubation at 37°C, cell viability was determined by staining with 0.4% tryplan blue. The numer of trypan blue positive and negative cells were counted in a hemocytometer under light microscope.

2.5. Virus yield reduction antiviral assays

Antiviral activity was evaluated by a virus yield inhibition assay. For Ab-independent assay, U937 and K562 cells grown in 24-well microplates (3.0 x 10^5 cells/well) were infected with DENV at a multiplicity of infection (m.o.i.) of 5 in the presence or absence (virus control) of different concentrations of carrageenan, 2 wells per concentration. After 2 h of incubation at 37°C, the cell cultures were washed with phosphate buffer saline (PBS) by centrifugation, MM containing or not (virus control) carrageenan was added and cells were further incubated at 37°C. Virus yields were determined by PFU in Vero cells at 48 h p.i.. For Ab-dependent-inhibition, samples of 3H5 or 2H2 Ab (final dilution 1/500) were incubated with 1.5 x 10^5 PFU of DENV-2 or DENV-3 in MM during 1 h at 37°C. Then, cultures of U937 and K562 cells grown in 24-well microplates (3.0 x 10^5 cells/well) were inoculated with the virus-Ab mixtures in the presence or not of serial dilutions of carrageenan and incubation followed at 37°C for 2 h. Thereafter, cell cultures were washed with PBS and resuspended in MM containing or not carrageenan. These experimental conditions of virus opsonization before compound addition were used to better reproduce the expected situation in a therapeutical in vivo ADE intervention. After 72 h of infection at 37°C, virus yields in the supernatants were titrated by PFU in Vero cells. All determinations were performed thrice and each in duplicate.

2.7. Effect of time of treatment with carrageenan on antiviral activity

U937 and K562 cells were infected with DENV-2 or mixtures DENV-2-Ab under different treatment conditions with 20 µg/ml of carrageenan. Adsorption: cells were exposed to virus in the presence of compound and after 2h of adsorption at 4°C, both compound and unadsorbed virus were removed by washing with cold PBS and MM was added. Internalization: cells were infected in compound-free MM and after 2 h adsorption at 4°C, unadsorbed virus was removed by washing with cold PBS, and cells were further incubated at 37°C during 2 h in MM containing compound. Thereafter, cells were washed with PBS and overlaid with MM. Post-internalization: cells were infected and maintained in MM without compound during 2h adsorption at 4°C and 2 h internalization at 37°C; thereafter cells were incubated in MM containing carrageenan and at 10 h after infection medium was removed and compound-free MM was added. As controls, cells were infected and maintained in the absence or presence of compound during the whole period of infection. For all treatments, cell supernatants were collected at 48 h (virus infection) or 72 h (virus-Ab infection) after infection and virus yields were determined by plaque assay in Vero cells.

2.8. Statistical analyses

Statistical analyses were performed using GraphPad Prism software. Comparison of means was tested by ANOVA analysis and Dunnett´s multiple comparison post hoc test, using data obtained from three independent experiments. Statistical significance is depicted in figures.

3. Results

3.1. Antiviral activity of λ-carrageenan in human myeloid cells

The anti-DENV activity of λ-carrageenan in human myeloid cells was evaluated by a virus yield reduction assay in suspension cultures of U937 and K562 cells. Since some studies have reported differential antiviral susceptibility to sulphated polysaccharides among DENV serotypes in mammalian epithelial cells (Ichiyama et al., 2013; Lin et al., 2002; Pujol et al., 2012; Talarico et al., 2005; Talarico and Damonte, 2007), the four DENV serotypes were initially tested. As can be seen in Fig. 1B and 1C, λ-carrageenan exerted a dose-dependent inhibitory effect against the infection of both U937 and K562 cells with DENV. The four serotypes were susceptible to the carrageenan, attaining a reduction in virus production higher than 99% at the highest tested concentration of 20 µg/ml.

Viability of U937 and K562 cells was not affected by the compound as determined by viable cell counting at concentrations up to 1000 µg/ml (Fig. 1D). From the relationship between cytotoxicity and antiviral activity data shown in Fig. 1, the inhibitory action of λ-carrageenan against DENV serotypes in human myeloid cells can be considered very selective in both cell cultures.

The susceptibility to carrageenan of ZIKV, a human pathogen flavivirus phylogenetically related to DENV, was also evaluated in myeloid cells. In contrast to the observed anti-DENV activity, there was no significant inhibition of ZIKV infection (Fig. 1B and 1C).

3.2. Antiviral activity of λ-carrageenan against the Ab-mediated DENV-2 infection in human myeloid cells

To evaluate the ability of λ-carrageenan to inhibit the in vitro DENV ADE we used initially DENV-2 as model serotype and the DENV Ab 3H5, reactive with DENV-2 E protein and able to bind to FcγRII, and 2H2 that reacts with the prM protein of all DENV serotypes and binds to FcγRI and FcγRII. In vitro experiments proved that both anti-E and anti-prM Ab can enhance DENV infectivity (Ayala-Nuñez et al., 2013; Dejnirattisai et al., 2010; Huang et al., 2006) and,

particularly, our previous studies have demonstrated that 3H5 and 2H2 Ab increase DENV-2 infectivity in U937 and K562 cells (Carro et al., 2018). U937 cells express both the high affinity Fcγ-RI and the low affinity Fcγ-RII receptors but K562 cells express only Fcγ-RII. Consequently, it was previously shown that the infection of K562 cells with the complexes DENV-2-3H5 or DENV-2-2H2 is mediated by FcγRII whereas in U937 cells the augmented infection with DENV-2-3H5 is also mediated by Fcγ-RII but Fcγ-RI is the receptor involved in the entry of the complex DENV-2-2H2 (Carro et al., 2018).

Then, the ability of λ-carrageenan to block ADE of infection with opsonised DENV-2 was evaluated with the different virus-Ab mixtures under the optimal conditions of increased virus production in U937 and K562 cells previously established (Carro et al., 2018). The infection of K562 cells with DENV-2-3H5 or DENV-2-2H2 was significantly inhibited by treatment with λ-carrageenan and infective virus production in the presence of both Ab was highly blocked at the maximum concentration of 20 µg/ml (Fig. 2A). These results are comparable to those obtained when myeloid cells were infected with DENV-2 in the absence of Ab (Fig 1A and 1B). By the contrary, the response of U937 cells to the treatment with λ-carrageenan was dependent on the Ab used for opsonization. In U937 cells infected with DENV-2-3H5, a strong inhibition of virus yield was observed after carrageenan treatment (Fig. 2B) as occurred in U937 cells infected with DENV-2 in the absence of Ab. Surprisingly, the λ-carrageenan did not produce any inhibitory effect against the infection of U937 cells with the complex DENV-2-2H2 (Fig. 2B). The same results were obtained when we analyzed the viral RNA by real time PCR. A lack of inhibition in the number of viral RNA copies when U937 cells were treated with λ-carrageenan and infected with DENV-2-2H2 mixtures and a strong inhibition in the viral RNA when U937 cells were infected with DENV-2-3H5 in the presence of λ-carrageenan presence (data not shown). Then, the ADE of DENV-2 infection appears to be susceptible to carrageenan inhibition when the entry of the virus-Ab complex is mediated by Fcγ-RII (infection of U937 cells with DENV-2-3H5 and infection of K562 cells with both mixtures) but there is no inhibition if entry is mediated by Fcγ-RI (DENV-2-2H2 in U937 cells).

To discard that the differential susceptibility to carrageenan observed when the in vitro ADE of infection is mediated by either Fcγ-RI or Fcγ-RII was not exclusive for DENV-2, we decided to test another serotype. Since the Ab 3H5 is reactive exclusively with DENV-2, we only tested the effect of carrageenan against the infection of other serotype with the serotype cross-reactive Ab 2H2. When infection was performed with the mixture DENV-3-2H2 in the experimental conditions for ADE with DENV-3 previously determined (Carro et al., 2018), the susceptibility to carrageenan was similar to that observed for DENV-2. The λ-carrageenan significantly inhibited virus yields from K562 cells infected with DENV-3-2H2 whereas virus

production from U937 cells infected with this complex was not affected by the compound (Fig. 3).

3.3. Influence of time of treatment with λ-carrageenan on DENV infectivity

Mechanistic studies were performed with DENV-2 and K562 cells as model system to elucidate if the antiviral target of λ-carrageenan in myeloid cells is virus entry, as reported in Vero cells (Talarico and Damonte, 2007). K562 cells were infected with DENV-2 and exposed to the carrageenan for different treatment periods: a) compound was present only during virus adsorption for 2 h at 4°C, b) compound was present only during virus internalization for 2 h at 37°C after virus adsorption at 4°C in compound free-medium, c) carrageenan was added after virus adsorption and internalization in compound-free medium, d) compound was present both during virus adsorption and internalization and throughout all the incubation period thereafter (Fig. 4A) For all treatments, virus yields were determined at 48 h p.i. As can be concluded from data presented in Fig. 4B, a strong inhibitory effect was observed when the carrageenan was present only during adsorption at 4°C (90-95% reduction) or internalization at 37°C (99-99.5% reduction). Under these treatment conditions, the level of inhibition was comparable to that observed when the compound was maintained during all the period of infection. Furthermore, the addition of carrageenan after DENV-2 adsorption and internalization did not alter virus yield in comparison to untreated virus control, indicating that DENV-2 entry is the main antiviral target for λ-carrageenan in human myeloid cells. Similar results were obtained when infection of K562 cells was performed with the complex DENV-2-3H5 under similar treatment conditions (Fig. 4C) suggesting that virus entry is also affected by carrageenan in myeloid cells infected in the presence of Ab.

4. Discussion

The studies reported herein have conclusively demonstrated that λ-carrageenan is a potent and selective inhibitor of the Ab-independent infection of U937 and K562 cells with DENV, apparently by a direct interference with virus entry. These results suggest that, as reported in other types of mammalian cells, HS proteoglycans also play any role for DENV entry in human myeloid cells. By contrast, the carrageenan did not inhibit the infection of both U937 and K562 cells with ZIKV. At present, the HS-dependence for ZIKV infection in diverse mammalian cells is debated due to controversial data. A study using surface plasmon resonance showed the binding interaction between ZIKV E protein with HS, chondroitin sulfate and heparin, suggesting a possible function of HS for ZIKV binding (Kim et al., 2017). However, when

infective virus was used instead of the isolated E protein, heparin was found to be protective against ZIKV infection in human neural progenitor cells not through entry blockade but affecting apoptosis pathways (Ghezzi et al., 2017). On the other hand, a promoting activity of heparin was reported for ZIKV replication in Vero cells (Kim et al., 2019), as we observed for carrageenan in ZIKV infected K562 cells. Although the precise role of HS in ZIKV infection and/or pathogenesis still requires further investigation, at present it is clear that HS is not required for ZIKV viral entry into vertebrate cells (Gao et al., 2019), as previously identified for DENV and other flaviviruses.

More interestingly, the inhibitory activity of carrageenan against DENV was also effective in the Ab-dependent infection of human U937 and K562 cells mediated by Fcγ-RII, causing 96-99% inhibition in virus yields from cells infected with immune complexes of DENV-2 and DENV-3. Then, partial opsonization of virus apparently occurs in presence of carrageenan since virus production was not totally suppressed , but the strong inhibitory effect of the polysaccharide may indicate that HS also contributes to DENV entry in Ab-mediated infection. In particular, the antiviral evaluation under different ADE conditions as to Fc-bearing cells and enhancing Ab allowed to demonstrate that the inhibitory effectiveness of carrageenan was similar against prM-mediated ADE (promoted by 2H2 Ab) or E-mediated ADE through 3H5 Ab in K562 cells. Thus, the carrageenan was able to block the internalization of both mature and inmature virus particles, according to their content of precursor prM or cleaved M proteins, usually present in DENV suspensions (Junjhon et al., 2010; Zybert et al., 2008). Apparently only the Fcγ-R involved in ADE may alter the capability of carrageenan to block Ab-mediated DENV infection. Although we do not have a complete explanation for the dissimilar response related to the Fcγ-R, there is an apparent relationship between antiviral susceptibility and the endocytic pathway of internalization for DENV-Ab complex into myeloid cells. Our previous studies demonstrated a role of the Fcγ-R for determining the route of internalization of the DENV-Ab complex in K562 and U937 cells: the entry mediated by Fcγ-RII was dependent on clathrin-coated vesicles for endocytosis, but when ADE was mediated by Fcγ-RI the uptake was clathrin-independent (Carro et al., 2018). Then, the blockade of ADE by carrageenan may be effective when the clathrin-mediated endocytosis is employed for the infective cellular penetration of the inmmune complex, as occurs with DENV-2-3H5 infection of K562 and U937 cells and DENV-2-2H2 infection of K562 cells. Concomitantly, differences in the influence of the signalling cascades after binding of DENV-Ab complexes to myeloid cells have been described between Fcγ-RI and Fcγ-RII (Rodrigo et al., 2006). Moreover, the participation of other cell receptors after the original interaction with the Fcγ-R cannot be discarded to fulfil the productive infection with the immunecomplex (Chotiwan et al., 2014).

Although several compounds have been evaluated as entry inhibitors with therapeutic potential, only a limited number of molecules have been shown to be active against ADE of DENV infection. Ichiyama et al. (2013) reported the inhibitory action of curdlan sulfate, to restrict ADE-mediated DENV-2 infection in human monocytic THP-1 cells in presence of 3H5 Ab, in coincidence with our results about the ability of sulfated polysaccharides to inhibit the Fcγ-RII-dependent entry of the DENV-Ab complex. The other virus entry inhibitors proved to block in vitro DENV-2 ADE include two peptides mimicking different domains in E protein of DENV-2 opsonized by human serum in K562 cells (Nicholson et al., 2011); an aglycon analogue of the antibiotic teicoplanin that interfered with the Ab-mediated entry of DENV-2 particles into mouse macrophage P388D1 cells (De Burghgraeve et al., 2012); and SA-17, a derivative of the antineoplasic antibiotic doxorubicin active against ADE of both mature and immature DENV-2 particles (Ayala-Nuñez et al., 2013). Here we are extending to the λ-carrageenan the ADE inhibitory properties cells against infections with immune complexes of DENV-2 and DENV-3 in human cells.

Even though the assays of possible clinical use of carrageenan formulations with other human viruses were not conclusive about their chemotherapeutic potential, the antiviral efficacy of carrageenans was demonstrated in clinical trials with topical microbicide usage against human papilloma virus (Marais et al., 2011; Ugaonkar et al., 2015) or when applied as nasal sprays for the treatment of respiratory infections (Eccles et al., 2015). Furthermore, it was reported that carrageenans can induce the activation of immunity through oral administration in murine models (Frossard et al., 2001, Kalitnik et al., 2017). This immunomodulatory activity may also contribute to improve their effectiveness against viral infections. At present, carrageenans have no been evaluated in vivo against DENV infection. Based on our results about carrageenan ability to block ADE in vitro, it would be interesting to test their administration in an immunocompetent murine model of secondary DENV infection recently established in C57BL/6 mice where heterotypic $CD8^+$ T lymphocytes played a crucial role in pathogenesis (Talarico et al., 2017). Based on the mode of action of carrageenan against primary and Ab-dependent DENV infection here shown, the interference of carrageenan with viral entry in vivo may prevent virus dispersion and control the dissemination of infection normally occurring in the successive cycles of infection in the absence of treatment. Given the exacerbation of DENV-induced disease in secondary infections, drugs with the chance to block ADE like carrageenans provide interesting perspectives for intervention strategies and deserve further studies to confirm their therapeutic potential.

Author statement

All the research data are included in the manuscript.

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Figure legends

Fig. 1. Antiviral activity of λ-carrageenan against DENV and ZIKV in human myeloid cells. (A) Chemical structure of λ-carrageenan. (B) U937 and (C) K562 cells were infected with DENV-1, DENV-2, DENV-3, DENV-4 or ZIKV (m.o.i. of 5) in the absence or presence of different concentrations of λ-carrageenan. After 2 h at 37ºC the virus inocula were removed and cultures were further incubated at 37ºC in MM with or without compound. At 48 h p.i. extracellular virus yields were quantified by PFU in Vero cells. Results are expressed as % inhibition in treated infected cells with respect to untreated infected cells. (D) Cytotoxicity was measured by incubation of U937 and K562 cells in MM containing serial concentrations of λ-carrageenan. After 72 h of incubation at 37°C, cell viability was determined by staining with 0.4% tryplan blue. In all cases each value is the mean of three independent experiments ± standard error of the mean (SEM).

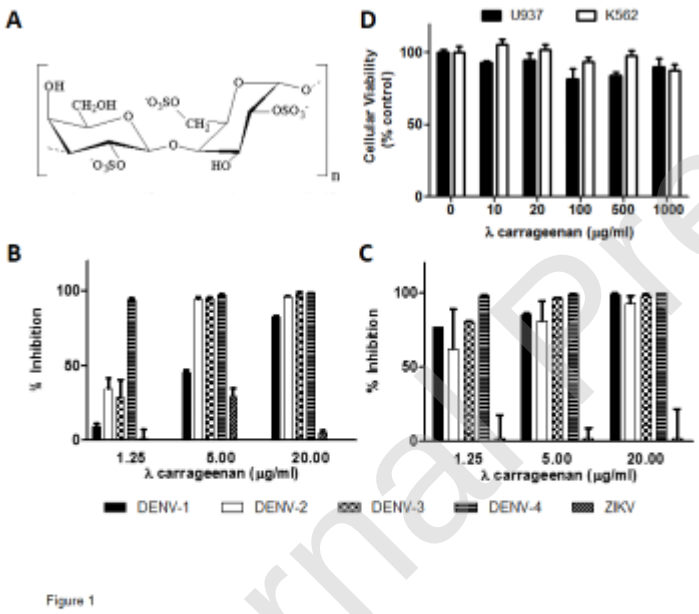

Fig. 2. Antiviral activity of λ-carrageenan against DENV-2 in human myeloid cells in presence of Ab. (A) K562 and (B) U937 cells were inoculated with the virus-Ab mixtures at a m.o.i. of 0.5 PFU/cell in the presence or not of serial dilutions of carrageenan. After 2h at 37°C, inocula were removed and cultures were refed with MM containing or not carrageenan. After 72 h of infection at 37°C, virus yields in the supernatants were titrated by PFU in Vero cells. All

determinations were performed thrice and each in duplicate. Results are expressed as % inhibition in treated cells relative to untreated cells ± standard error of the mean (SEM).

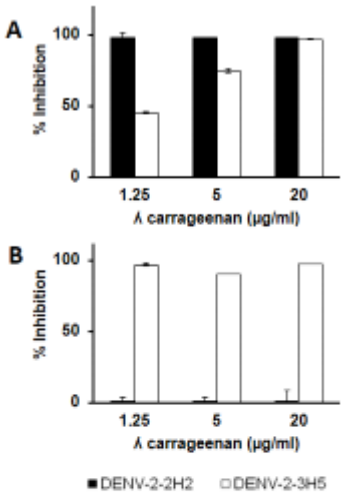

Figure 2

Fig. 3. Antiviral activity of λ-carrageenan against DENV-3 in human myeloid cells in presence of Ab. K562 and U937 cels were inoculated with the DENV-3-2H2 mixture at a m.o.i. of 0.5 PFU/cell in the presence or not of serial dilutions of carrageenan. After 2h at 37°C, inocula were removed and cultures were refed with MM containing or not carrageenan. After 72 h of infection at 37°C, virus yields in the supernatants were titrated by PFU in Vero cells. All determinations were performed thrice and each in duplicate. Results are expressed as % inhibition in treated cells relative to untreated cells ± standard error of the mean (SEM).

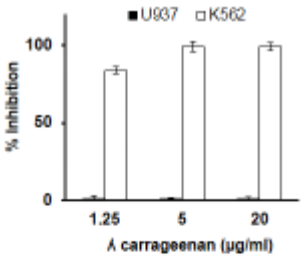

Figure 3

Fig. 4. Effect of time of treatment with λ-carrageenan on antiviral activity. (A) Scheme of assay protocol. (B) K562 cells were infected with DENV-2 (time 0) and treated with 20 µg/ml of carrageenan at various time points or untreated (VC). Virus yields were determined at 48 h after infection by PFU in Vero cells. (C) K562 cells were infected with the mixture DENV-2-3H5 and treated with 20 µg/ml of carrageenan under the same treatment conditions as in (B). Virus yields were determined at 72 h after infection by PFU in Vero cells. Values are the mean of three independent experiments ± standard error of the mean (SEM).

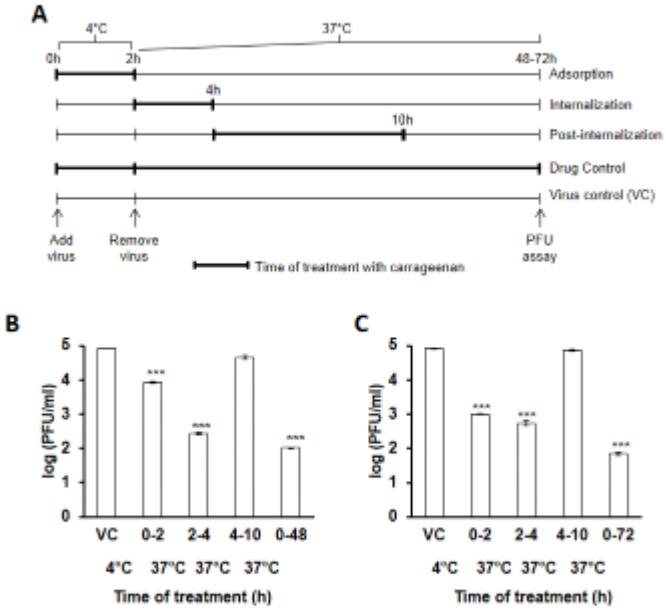

Figure 4