Antiviral activity obtained from aqueous extracts of the Chilean soapbark tree (*Quillaja saponaria* Molina)

Michael R. Roner, Jennifer Sprayberry, Matthew Spinks and Salima Dhanji

Department of Biology, The University of Texas Arlington, Arlington, TX 76019, USA

Natural, aqueous extracts of *Quillaja saponaria*, the Chilean soapbark tree, contain several physiologically active triterpenoid saponins that display strong adjuvant activity when used in either human or animal vaccines. In this paper, we describe studies that demonstrate a novel antiviral activity of *Quillaja* extracts against six viruses: vaccinia virus, herpes simplex virus type 1, varicella zoster virus, human immunodeficiency viruses 1 and 2 (HIV-1, HIV-2) and reovirus. We demonstrate that microgram amounts of extract, while exhibiting no cell cytotoxicity or direct virucidal activity, prevent each of the six viruses tested from infecting their host cells. In addition, the presence of residual amounts of extract continue to block virus infection and render cells resistant to infection for at least 16 h after the removal of the extract from the cell culture medium. We demonstrate that a *Quillaja* extract possesses strong antiviral activity at concentrations more than 100-fold lower than concentrations that exhibit cell cytotoxicity. Extract concentrations as high as 100 μg ml⁻¹ are not cytotoxic, but concentrations as low as 0.1 μg ml⁻¹ are able to block HIV-1 and HIV-2 virus attachment and infection.

**INTRODUCTION**

Aqueous extracts of the Chilean soapbark tree (*Quillaja saponaria* Molina) contain several physiologically active triterpenoid saponins (Guo & Kenne, 2000). These saponins have been shown to exhibit strong adjuvant activity that has been exploited for use in animal and human vaccines (Dalsgaard, 1974; Kensil, 1996; Wu et al., 1992). Additionally, triterpenoid saponins from other sources such as *Maesa lanceolata*, *Maesa chisia* and *Maesa indica* have been reported to exhibit direct virucidal activity against Newcastle disease virus, vaccinia virus and herpes simplex virus (Jassim & Naji, 2003).

A number of antivirals attempt to block or inhibit virus replication by interfering with virus attachment to cells, interfering with viral enzymes or suspending viral genome replication. To be effective, compounds working at each of these points within the virus replication cycle must target the viral process while avoiding similar ongoing cellular processes. Preventing a virus from reaching its cellular receptor should not inadvertently activate the cell, as most receptors used by viruses are also functional cellular receptors being hijacked by the virus. Most cells in a virus-infected individual will be uninfected, and antivirals acting on these cells should not interfere with the normal functions of these cells if they are to be safe and effective. Many of the current antiviral drugs target persistent viruses, such as herpes viruses and human immunodeficiency virus (HIV), where therapy will be long-term.

Saponins offer some novel mechanisms of antiviral action, including interactions with viral envelopes leading to their destruction, interactions with host-cell membranes leading to a loss of virus binding sites and coating of cells to prevent virus binding (Amoros et al., 1987; Apers et al., 2001; Gosse et al., 2002; Tokuda et al., 1988; Verma & Raychaudhuri, 1970). In this paper, we examine the ability of a *Quillaja* extract to block virus infection and reduce virus spread.

**METHODS**

**Virus and cell lines.** The viruses used in this study were reovirus serotype 3 (ST3) strain Dearing (Ramos-Alvarez & Sabin, 1958), vaccinia virus strain WR (Mackett et al., 1982), herpes simplex virus type 1 (HSV-1) strain GHSV-UL46 (ATCC VR-1544); varicella-zoster virus (VZV) strain Ellen (ATCC VR-1367); HIV-1 (NIH-AIDS Research and Reference Reagent Program, HIV-1 89.6) and HIV-2 (NIH-AIDS Research and Reference Reagent Program, HIV-2 CDC310248). Mouse L929 fibroblasts (ATCC CCL-1) were used to support the growth of reovirus, human 143 cells (Patel & Pickup, 1987) to support the growth of vaccinia virus, Vero cells (ATCC CCL-81) to support the growth of HSV-1, BS-C-1 cells (ATCC CCL-26) to support the growth of VZV and CEMx174 and LuSIV cells (NIH-AIDS Research and Reference Reagent Program) to support the growth of HIV-1 and HIV-2. All cell lines were propagated in monolayer cultures using minimal essential medium (MEM) with Earles’ salts, supplemented with 10% fetal bovine serum (FBS). Cells were passaged at 1:2 to 1:10 dilutions according to conventional procedures using 0.05% trypsin with 0.02% EDTA.

**Preparation of Quillaja extract.** The *Quillaja* extract used was obtained from Desert King International (San Diego, CA, USA). The
material, UltraDry 100-Q, is the spray-dried, purified, aqueous extract of the Chilean soapbark tree (Quillaja saponaria Molina), consisting of >93 % Quillaja solids and with a moisture content of less than 7 %. The extract was prepared by dissolving the dried material into MEM at a concentration of 1.07 g 100-Q per 100 ml and filter-sterilized to yield a 10 mg ml⁻¹ stock. From this working stock, material was transferred to MEM with 10 % FBS to yield the indicated final concentrations and the medium was added to the cell cultures.

The extract is a complex mixture of triterpenoids (saponins) (van Setten et al., 1995, 1998; van Setten & van de Werken, 1996). It has been established that these terpenes are built around a common quillaic acid which is decorated with oligosaccharides at C3 and in most cases C28. The differences in the members of this family arise from the level of oxidation around the quillaic acid skeleton (typically at C23 and C30), the type, location and number of sugars and the number, location and types of acyl moieties (most often on the C28 fucose, at C 3′ and C 4′). Presumably, the quillaic acid moiety simply serves as a scaffolding element, which then presents the oligosaccharides in the appropriate orientation and spatial distribution for interaction with the cellular target(s). The general structure of the Quillaja saponins is illustrated in Fig. 1.

**Determination of Quillaja extract cytotoxicity.** Quillaja extract cytotoxicity was determined by plating the indicated cells at a concentration of 5 x 10⁴ cells per well (6-well plate) in MEM with 10 % FBS and incubating the plates at 37 °C in 5 % CO₂ for approximately 24 h or until the cells divided to yield 1 x 10⁵ cells per well. At this time, the medium was removed and replaced with MEM with 10 % FBS and the indicated concentration of Quillaja extract and the cells were incubated for 96 h in the presence of the extract. Cytotoxicity was measured microscopically after 96 h by counting the cells in three individual wells of a 6-well plate, 500 cells per well, and using the trypan blue dye exclusion procedure (Bjorkerud & Bondjers, 1972). Assays were performed in triplicate to generate nine measurements per time point. The results are presented as the concentration of the candidate drug that results in the death of 50 % of the host cells. This value is commonly referred to as the median cellular cytotoxicity concentration and is identified as the CCIC₅₀.

**Direct virucidal effect of Quillaja extract.** The ability of the Quillaja extract to inactivate each of the six viruses directly was examined by using a standard plaque-reduction assay or, for HIV-1 and HIV-2, an infectious centre assay. This technical difference was required because the CEMx174 cells do not attach to tissue culture flasks. Briefly, 1 x 10⁶ p.f.u. [or infectious units (i.u.) for HIV-1 and HIV-2] of each virus was incubated in the presence or absence of Quillaja extract ranging from 0 to 10 mg ml⁻¹. Viruses were suspended in 200 µl Quillaja extract-containing MEM with 1 % FBS for 1 h at 37 °C. Virus was pelleted at 100 000 g for 15 min, resuspended in fresh MEM with 1 % FBS and repelleted; the process was repeated three times to remove any remaining Quillaja extract. After final centrifugation, the virus was resuspended in 200 µl MEM with 1 % FBS and virus infectivity was assayed by plaque assay or infectious units as described. Each assay was performed in triplicate.

**Virus attachment to cells.** The ability of these viruses to attach to their host cells was determined by adding 1 x 10⁶ p.f.u. or i.u. to 1 x 10⁵ of the appropriate host cells in 6-well plates in MEM with 10 % FBS. After 5, 10, 15, 20, 30, 45 or 60 min, the virus-containing medium was removed and the cells were washed three times with 1 ml fresh MEM with 10 % FBS. The three washes were combined and the ‘free’ virus, i.e. virus that did not remain attached to the host cells, was determined by plaque assay or infectious unit assay. The assays were repeated using three individual wells of a 6-well plate. Assays were performed in triplicate to generate nine measurements per time point. We selected this experimental design because it allowed us to follow the role of both unattached virus and virus able to attach to cells in the presence of Quillaja extract. Virus that was unable to attach was assayed for infectivity to examine the possibility that the Quillaja extract inactivated the free virus rather than preventing it from attaching to the cell monolayers as we had hypothesized. For virus that did attach, we examined the cells to see whether the attached virus was still able to initiate an active infection or was destroyed and/or released but damaged and was subsequently not detected in the assays for infectious virus that we carried out on the supernatant and cell washes. This assay mimics conditions that would exist when viruses are ingested, inhaled, injected or otherwise introduced into the human body, conditions under which saponins could be used to protect various body surfaces.

To validate this method, we also used the standard technique of adding radioisotope-labelled virus (³⁵S-methionine/cysteine) to cells and measuring cell-associated radioactivity. Briefly, radioisotope-labelled virus was prepared by infecting the indicated host cell line at an m.o.i. of 20 in the presence of ³⁵S-methionine/cysteine (20 μCi ml⁻¹) for 48 h and the viruses were extracted from cell debris as described by Balazs & Caldarella (1981). The ability of each virus to attach to their host cells was determined by adding 1 x 10⁵ c.p.m. of each virus to 1 x 10⁶ of the appropriate host cells in 6-well plates in MEM with 10 % FBS. After 5, 10, 15, 20, 30, 45 or 60 min, the virus-containing medium was removed and the cells were washed three times with 1 ml fresh MEM with 10 % FBS. The cells were then harvested and the radioactivity (bound virus) was determined by liquid scintillation counting of triplicate samples. The amount of virus bound is expressed as a percentage of the total input 1 x 10⁶ c.p.m.

**Infectious centre assay to measure cells infected following treatment with Quillaja extract.** The ability of each virus to infect their host cells following Quillaja extract treatment was determined by incubating 1 x 10⁵ of the appropriate host cells in 6-well plates in MEM with 10 % FBS supplemented with the indicated concentration of Quillaja extract. Following incubation for 1 h at 37 °C in 5 % CO₂, the Quillaja extract-containing medium was removed, the cells were washed three times with MEM without extract and the cells were then infected by adding 1 x 10⁵ p.f.u. or i.u. of the indicated virus to duplicate wells of the appropriate host cells in 6-well plates in MEM with 10 % FBS. After 5, 10, 15, 20, 30, 45 or 60 min, the virus-containing medium was removed and the cells were washed with 1 ml fresh MEM with 10 % FBS. After washing three times to remove unbound virus, the cells were harvested into 1 ml MEM without serum and then pipetted forcefully to generate a single cell suspension and serial 10-fold dilutions were prepared. The diluted cell suspensions were plated onto cell monolayers as

---

**Fig. 1.** General structural features of the Quillaja saponins. Var, Variable group.
described for the standard viral plaque assays or for HIV-1 and HIV-2 i.u. assays. Cells infected in the presence of the *Quillaja* extract that are able to support virus replication and produce infectious progeny virus will release this new virus and it will infect cells of the cell monolayers and generate plaques or, in the case of HIV-1 and HIV-2, spread to adjacent cells. Assays were performed in triplicate to generate six measurements per time point. These assays examined the ability of these viruses to establish a productive infection in *Quillaja* extract-treated cells and for the infection to spread to adjacent cells to produce a visible ‘plaque’ or, in the case of HIV-1 and HIV-2, increased numbers of infected cells.

**Infectious centre assay to measure the lasting effect of *Quillaja* extract treatment on cells.** The lasting effects of *Quillaja* extract treatment were examined using a standard infectious centre assay as described above. Cells (1 × 10⁶) were treated with *Quillaja* extract as described above for 1 h. The *Quillaja* extract-containing medium was removed, the cells were washed three times and fresh MEM with 10 % FBS was added. Immediately (t = 0) or after 1, 2, 4, 8, 12, 16 or 24 h, the cells were infected by adding 1 × 10⁵ p.f.u. of the indicated virus to duplicate wells of the appropriate host cells in 6-well plates in MEM with 10 % FBS. After an additional 60 min, the virus-containing medium was removed and the cells were washed with 1 ml fresh MEM with 10 % FBS. The remaining procedure was identical to that described above for the infectious centre assay.

**Reovirus and vaccinia virus plaque assays.** L929 or 143 cells were grown to confluence in 6-well plates in MEM with 10 % FBS. The medium was removed and the cells were infected with either reovirus or vaccinia virus at serial 10-fold dilutions ranging from 1 × 10⁶ to 10 p.f.u. per well in 250 μl MEM without serum. After 60 min, the medium was removed and replaced with 2 ml MEM containing medium continued to divide during the 96 h treatment and, when the *Quillaja* extract was removed and replaced with *Quillaja* extract-free medium, the cells suffered no long-term effects and were maintained in the laboratory for at least 3 months. Based on these results, we tested for antiviral activity at *Quillaja* extract concentrations of less than 1.0 mg ml⁻¹.

**RESULTS**

**Highest concentration of *Quillaja* extract tolerated by cell cultures**

Growth of L929, 143, Vero, BS-C-1 and CEMx174 cells was unaffected by concentrations of 0.1 mg *Quillaja* extract ml⁻¹ or less in MEM with 10 % FBS (Table 1). The CCIC₅₀ values for these cells were 10-fold higher, at 1.0–0.9 mg ml⁻¹. Cells maintained in this *Quillaja* extract-containing medium continued to divide during the 96 h treatment and, when the *Quillaja* extract was removed and replaced with *Quillaja* extract-free medium, the cells suffered no long-term effects and were maintained in the laboratory for at least 3 months. Based on these results, we tested for antiviral activity at *Quillaja* extract concentrations of less than 1.0 mg ml⁻¹.

**Direct inactivation of viruses by *Quillaja* extract**

Also summarized in Table 1 are the results of tests to examine the ability of *Quillaja* extract to inactivate each of these viruses directly at concentrations from 0 to 1 mg ml⁻¹. The results are presented as the ED₅₀, the dose of a drug that is effective at inactivating 50 % of the treated virus within 1 h at 37 °C. After incubation for 1 h at 37 °C, the 143 cells were infected in the presence of the extract and assayed in CEMx174 cells (Stefano *et al.*, 1993) and/or LuSIV cells (Roos *et al.*, 2000). After adsorption for 2 h at 37 °C, 4 ml low-melting-temperature agarose (0.75 %) in MEM with 2 % FBS was added. After 4 days for HSV and 6 days for VZV, the agarose was removed and cells were rinsed with PBS, fixed for 7 min with a solution of methanol/acetic acid (3:1), stained with crystal violet (1 %) in methanol (50 %) and rinsed with water (Cole & Grose, 2003). Assays were performed in triplicate.

**Table 1. Cytotoxicity of extracts and direct inactivation of viruses**

The results are presented as means of four experiments using triplicate samples in each experiment. See Methods for assay conditions. CCIC₅₀ is defined as the amount of extract (mg ml⁻¹) that leads to loss of cell viability of 50 % of treated cells after 96 h. ED₅₀ is defined as the amount of extract (mg ml⁻¹) that leads to inactivation of 50 % of virus within 1 h.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>CCIC₅₀</th>
<th>Virus</th>
<th>ED₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>L929</td>
<td>1.0±0.1</td>
<td>Reovirus</td>
<td>&gt;1.0±0.1</td>
</tr>
<tr>
<td>143</td>
<td>0.9±0.1</td>
<td>Vaccinia virus</td>
<td>0.11±0.02</td>
</tr>
<tr>
<td>Vero</td>
<td>0.9±0.1</td>
<td>HSV-1</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>BS-C-1</td>
<td>0.9±0.1</td>
<td>VZV</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>CEMx174</td>
<td>1.0±0.1</td>
<td>HIV-1</td>
<td>0.15±0.02</td>
</tr>
<tr>
<td>CEMx174</td>
<td>1.0±0.1</td>
<td>HIV-2</td>
<td>0.14±0.02</td>
</tr>
</tbody>
</table>
Can Quillaja extract be used to treat cells and to reduce infection efficiency?

To determine whether Quillaja extract-treated cells were now resistant to virus attachment, we treated cells with Quillaja extract for 1 h, replaced the medium with Quillaja extract-free medium and then measured the amount of virus that could bind to treated or untreated cells within an additional 60 min. We tested Quillaja extract concentrations ranging from the highest that demonstrated no cell cytotoxicity (0.1 mg ml$^{-1}$) across a 4-log reduction to 0.00001 mg ml$^{-1}$. The ability of each virus to attach to their host cells following treatment of the cells was determined as described above. We measured virus binding using two methods, the first an assay for infectious virus, the second an assay to measure binding of radiolabelled virus to the treated cells. The infectious assay measures the removal (binding to cells) of virus from the medium during the 5–60 min test period. In each panel of Fig. 2, graphs (i) and (ii) show the results of the infectious virus assay and panels (iii) and (iv) show the binding of radiolabelled virus. The data demonstrate that treatment of cells with Quillaja extract renders them resistant to virus infection even in the absence of continuous Quillaja extract in the medium. Quillaja extract at concentrations as low as 0.001 mg ml$^{-1}$ for reovirus, vaccinia virus, HSV-1 and VZV and as low as 0.0001 mg ml$^{-1}$ for HIV-1 and HIV-2 was able to block virus binding completely as measured using both the infectious virus and radiolabelled virus binding assays.

As shown in Fig. 2, within 45 min of virus addition to untreated cells, more than 95% of each of the six viruses tested attached to their host cells (i.e. were removed from the test medium) in the absence of Quillaja extract. Following treatment with non-cytotoxic amounts of Quillaja extract (0.0001 to 0.1 mg ml$^{-1}$), less than 0.25% of each of the six viruses was able to attach to their host cells after 60 min and remained free and infectious in the cell culture medium. The Quillaja extract-treated cells demonstrated no cytotoxicity yet remained highly resistant to virus binding during both of the virus binding assays. More than 99.75% of the original 1 × 10$^6$ p.f.u. of added infectious virus remained free and infectious in the supernatant and less than 1% of the radiolabelled virus was able to bind to these cells. It should be noted that the infectious assay measures 'loss' of virus from the test medium during the incubation period. This is because the test medium is removed, the cells are washed to remove unbound virus and the combined rinse medium is tested for infectious virus. If any residual Quillaja extract is acting on the viruses directly and rendering them non-infectious, they would not be detected in the plaque assay of the medium performed at the end of the treatment period. This is not what we found; virus added to cells treated with Quillaja extract does not absorb to cells but remains free in the supernatant and infectious during the 60 min test period. In separate experiments, we have demonstrated that the concentrations of Quillaja extract that we are using have no direct effect on virus infectivity. The results using the radiolabelled virus assay in combination with the infectious virus assay support our hypothesis that the Quillaja extract prevents virus binding to/association with treated cells.

Quillaja extract-treated cells do not demonstrate active virus infection

We were also interested in the fate of the few cells that appeared to be binding virus following treatment with Quillaja extract. To pursue this, we examined Quillaja extract-treated and infected cells using an infectious centre assay. This technique, as described in Methods, involves recovering treated and potentially infected cells and plating them onto new cell monolayers or adding them to uninfected cells (HIV-1 and HIV-2) to assay for infectious virus produced and released by these treated cells. This assay allows us to examine the fate of the adsorbed virus during the 60 min absorption period following treatment of the cells with Quillaja extract. If the viruses were able to attach, infect the cells and initiate an infection, the progeny virus would infect cells of the new monolayer and generate a plaque. As shown in Fig. 3, allowing virus to attach to untreated cells for 60 min followed by incubation for an additional 6 h results in each of the six virus–host cell systems demonstrating active viral infections in 16–55% of the cells. Following infection of cells pretreated with the smallest active amounts of Quillaja extract (0.001 or 0.0001 mg ml$^{-1}$), less than 0.005% of each of the six virus–host cell systems displayed active viral infections. Quillaja extract treatment appears to alter the cells and to suppress virus replication even in the rare event that the viruses were able to attach to and infect the cells. Quillaja extract has not been shown to induce an interferon response, but we were unable to detect a response in our treated cells (data not shown). We demonstrated that only 0.25% of the added virus was able to attach to Quillaja extract-treated cells. This low percentage should result in at least 0.125% of the cells displaying active viral infections based on our results with untreated cells. We found less than 0.005% of the cells producing infectious virus.
**Maintenance of virus protection following removal of Quillaja extract**

We were also interested in the length of time that cells would remain resistant to virus attachment/infection after the Quillaja extract was removed. To examine this, cells (1 × 10^6) were treated with Quillaja extract as indicated previously for 60 min. The Quillaja extract-containing medium was removed, the monolayers were washed three times and fresh MEM with 10% FBS was added. Immediately (t=0) or after 1, 2, 4, 8, 12, 16 or 24 h, 1 × 10^6 p.f.u. or i.u. of each virus was added to the appropriate cell line. As described previously, the virus inoculum was removed after 1 h and, 6 h later, the cells were harvested and plated onto new cell monolayers or with uninfected cells (HIV-1 and HIV-2) as described for the infectious centre assay or infectious unit assay. As shown in Fig. 3, cells treated with the smallest amounts of Quillaja extract (0.001 or 0.0001 mg ml\(^{-1}\)) remain resistant to infection by each of the six viruses tested for at least 16 h after the Quillaja extract is removed. Twenty-four hours after treatment with Quillaja extract, the six cell lines tested returned to near normal in terms of virus susceptibility and virus infection rates were nearly identical to those of untreated cells, indicating no long-term alteration of the cells following treatment with Quillaja extract.

**DISCUSSION**

Natural, aqueous extracts of Quillaja saponaria Molina, the Chilean soapbark tree, contain a number of physiologically active triterpenoid saponins that have antiviral activity. Our results demonstrate that a Quillaja extract can disrupt viral envelopes and capsid proteins at concentrations 10-fold below cytotoxic levels. Additionally, following treatment of cells with this extract, a ‘block’ of the host cells occurs that prevents virus attachment and reduces virus spread to uninfected cells from cells that do manage to become infected, at concentrations 100- to 1000-fold below cytotoxic levels. The Quillaja extract used here is only cytotoxic at high concentrations (> 1 mg ml\(^{-1}\)) and is currently approved for use in food and beverages by the FDA (under CFR 172.510, FEMA number 2973 NON-GMO) and is allowed for use in organic foods (under N.O.P. 205.605). Based on the results presented here, we believe that this Quillaja extract can readily be combined with existing spermicidal agents and delivery systems to produce a product that is both spermicidal and virucidal, blocking virus attachment to cells in the treated area. The extract is well suited to such an application as it is pH stable, from pH 2 to 11, it is already approved as a food additive, it foams easily, is inexpensive and readily available and is soluble in water. The Quillaja extract is very effective at blocking...
HIV-1 and HIV-2 infection of cells at concentrations as low as 0.0001 mg ml\(^{-1}\). This blocking activity is active for cells in the presence of the Quillaja extract and for up to 16 h after its removal, making it an excellent candidate to prevent the spread of sexually transmitted viruses when applied prior to exposure.

Other saponin extracts have demonstrated similar activity. The antiviral activity of a triterpene saponin isolated from Anagallis arvensis (Primulaceae) was studied \textit{in vitro} against several viruses including HSV-1 and poliovirus type 2 (Amoros \textit{et al.}, 1987). These authors demonstrated that the antiviral activity was not due to a virucidal effect but appeared to involve an inhibition of virus–host cell attachment. The antiviral effects of triterpene glycosides and monoterpene glycosides were demonstrated by their ability to prevent viral activation of Raji cells by Epstein–Barr virus binding (Tokuda \textit{et al.}, 1988).

We selected these six viruses because they cover a broad range of virus structures, cell receptors utilized, membrane transport and cellular localization. Based on our results, the most likely mechanism of action of the extract is through disruption of cellular membrane proteins and/or virus receptors, preventing virus infection of these cells. In support of this conclusion, we present a brief summary of what is known about the molecular events that control attachment of each of these viruses to their host cells.

Reovirus is a dsRNA, non-enveloped virus that utilizes cellular receptors containing sialic acid and normally uses endocytosis into vesicles to gain access to the cell cytoplasm. Within the cytoplasm, the infecting particle is not broken down but is activated as a transcription machine, producing large amounts of mRNA in infected cells (Tyler \textit{et al.}, 2001). Reovirus is a model virus for rotavirus, a virus that, when ingested via contaminated water, results in diarrhoea and approximately 500 000 deaths worldwide each year (Parashar \textit{et al.}, 2006). Reovirus was more resistant to direct inactivation by the extract than the enveloped viruses, but was just as sensitive to being ‘blocked’ from infecting extract-treated cells.

Vaccinia virus is the vaccine strain for smallpox virus. The virus is enveloped and enters the cell via membrane fusion. No unique viral attachment protein or cell-surface receptors have yet been identified. Cell-surface heparan sulfate and chondroitin sulfate act as primary receptors and help vaccinia virus to adsorb to cells (Smith & Law, 2004). The viral genome is dsDNA but, unlike most dsDNA viruses, it replicates in the cytoplasm of infected cells. Treatment of vaccinia virus with a number of proteases increases the likelihood that it will infect a cell. This virus served as a model to test whether saponins might have a similar enhancement effect. This was not what we found, however; we only measured a reduction in infectivity at high extract concentrations and no enhancement was seen with any of the viruses.

HSV-1 is a neurotropic dsDNA virus that initially replicates in epithelial cells of the oral cavity. Amplified progeny virus enters sensory neurons and is transported to the nuclei located in the trigeminal ganglion, where a latent infection is established (Burkhart, 2005). The entry of HSV into cells begins with binding of viral glycoproteins gB or gC to heparan sulfate proteoglycans on the cell surface. After this binding, a third glycoprotein, gD, binds one of its receptors. The gD–receptor binding triggers fusion of the virus envelope with the cell membrane. Once fused, viral capsid and some tegument proteins are released into the cytoplasm of the cell. The cellular receptors for gD are very diverse in nature. They come from three different classes of cell-surface receptors: (i) HVEM, the first known gD receptor, is a member of the tumour necrosis factor receptor family, (ii) two others (nectin-1 and -2) are members of the immunoglobulin superfamily related to the poliovirus receptor and (iii) some unique sites in heparan sulfate resulting from the action of specific 3-O-sulfotransferases also produce HSV-1-specific receptor. Interaction of gD with any one of those receptors triggers the membrane fusion reaction, which, by an unknown mechanism, also requires a concerted action of three other envelope glycoproteins, gB and gH–gL (O’Donnell \textit{et al.}, 2006; Scanlan \textit{et al.}, 2003; Tiwari \textit{et al.}, 2004, 2005; Valyi-Nagy \textit{et al.}, 2004; Xia \textit{et al.}, 2002; Xu \textit{et al.}, 2005). The Quillaja extract may disrupt any of these glycoproteins, resulting in a ‘block’ of virus infection. Future work will examine the attachment process in detail following Quillaja extract treatment.

VZV is transmitted as airborne virus particles shed from the skin of an infected person. The new host breathes in the virus, which enters the mucous membrane in the respiratory tract and begins to spread without its envelope from cell to cell. The virus invades T cells of the blood, and these T cells carry the virus to the skin. There, the virus can recreate its envelope, because the top layer of the skin lacks the endosomal pathway that removes glycoproteins from the envelope (Cole & Grose, 2003). Studies with VZV have revealed that oligosaccharides derived from glycoproteins of the VZV envelope contain mannose 6-phosphate (Man6P) (Gabel \textit{et al.}, 1989); moreover, Man6P and other phosphorylated monosaccharides protect cells from the cytopathic effect of VZV. Treatment of cells with chloroquine, which reduces the expression of the cation-independent Man6P receptor (MPRCi) at cell surfaces, also protects against infection by VZV. Electron microscopic observations have revealed that enveloped virions are associated with MPRCi at the cell surface and that newly enveloped virions are incorporated into MPRCi-containing vesicles in the Golgi network (Gershon \textit{et al.}, 1994). Such a mechanism may also influence the infection of cells by HSV; HSV gD acquires Man6P residues and binds to Man6P receptors (Brunetti \textit{et al.}, 1994). Binding of either VZV or HSV to a Man6P receptor, however, has yet to be demonstrated. This virus was selected because of its reliance on cell-to-cell spread to remain in its host. We found that cells treated with...
saponins did not support the replication of this virus via spread within cell cultures; the Quillaja extract reduced the spread of this virus and resulted in a reduced number of plaques compared with mock-treated cells.

CD4 is a primary and necessary receptor for HIV-1, HIV-2 and SIV (Dalglish et al., 1984; Klatzmann et al., 1984). Several groups have shown that CCR5 is a necessary co-receptor for monocytotropic (M-tropic) HIV-1 isolates (Deng et al., 1996; Doranz et al., 1996; Dragic et al., 1996). Additionally, the chemokine receptor CXCR4 (fusin) was described as being the co-receptor used by T cell-tropic (T-tropic) HIV isolates (Feng et al., 1996). M-tropic HIV-1 isolates are classically those viruses that are most easily propagated in macrophage cultures and are unable to infect T cell lines (i.e. immortalized T cells), but are able easily to infect primary T cells from peripheral blood samples. Conversely, T-tropic HIV-1 isolates have classically been identified as being those that are easily propagated in T cell lines and grow poorly in macrophages, but are also able to infect primary T cells from peripheral blood samples. These T-tropic HIV-1 isolates are able to propagate in macrophage cultures and are unable to infect T cell lines (i.e. immortalized T cells), but are able easily to infect primary T cells from peripheral blood samples. Conversely, T-tropic HIV-1 isolates have classically been identified as being those that are easily propagated in T cell lines and grow poorly in macrophages, but are also able to infect primary T cells from peripheral blood samples. The Quillaja extract, which is a necessary co-receptor for M-tropic HIV isolates (Feng et al., 1996), has been shown to be effective in reducing the spread of this virus and resulted in a reduced number of plaques compared with mock-treated cells.

ACKNOWLEDGEMENTS

We are grateful for the excellent technical assistance of Sandra Caldwell and Kristie Ibarra, discussions and interactions with Jack Wiersma and the support of the UTA Office of Research and Development and the Department of Biology. The use of saponin as a ‘pathogen-filling foam’ is protected by US patent 6,022,545 [Inventors; Schmittmann; H. B. (Velbert, DE); Dietrich; J. (Monheim, DE)].

REFERENCES


