Med Microbiol Immunol (2006) 195: 85–92
DOI 10.1007/s00430-005-0004-8

ORIGINAL INVESTIGATION

G. Paglini · E. del C. Pereyra · S. Paglini

Cellular receptor for pixuna virus in chicken embryonic fibroblasts

Received: 6 June 2005 / Published online: 12 October 2005

Abstract In this study, we describe the isolation and partial characterization of a Pixuna virus receptor, which is a component of a plasma membrane fraction of chicken embryo fibroblast (CEF). Polyclonal antiserum was prepared from rabbits immunized with the membrane fraction. Said polyclonal antiserum reacted in a similar way as monoclonal antibodies raised against the membrane fraction. Both antisera were able to prevent CEF and Vero cells from infection with Pixuna virus. Immunofluorescence studies suggested that the receptors found in the fibroblasts and in the Vero cells shared at least some epitopes. The Western blot analysis of the purified membrane fraction antigens, which reacted with the monoclonal and polyclonal antibodies, detected a double band with a molecular mass of approximately 60 kDa. Not only immunofluorescence staining but also electron and immunoelectron microscopy studies evidenced the receptor localization in the plasma membrane. In this manner, we reported the isolation and partial characterization of a new Pixuna virus receptor in the plasma membrane of chicken embryo fibroblasts in culture. The data obtained demonstrated the receptor significance for the penetration of Pixuna virus into fibroblasts and mammalian cell and the related importance of designing new antiviral drugs by blocking the mechanism of receptor penetration of the virus into the cells.

Keywords Pixuna virus · Virus receptor · Fibroblast receptor · Vero receptor · Alphavirus receptor · Pixuna virus receptor

G. Paglini · E. del C. Pereyra · S. Paglini (✉)
Laboratorio de Receptores Celulares, Instituto de Virología, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Ciudad Universitaria, 5000 Córdoba, Argentina
E-mail: spaglini@cmefcm.uncor.edu
E-mail: spaglini@fcm.unc.edu.ar
Tel.: +54-351-4334022
Fax: +54-351-4617150

Introduction

Our laboratory is involved in the study of virus–cell interactions taking Togaviruses as models. In the family *Togaviridae*, the genus *Alphavirus* consists of a large number of viruses closely related with regards to their molecular characteristics and structure [25]. Hemagglutination-inhibition (HI) and neutralization tests have been used to subdivide the genus into six antigenic complexes. One of these complexes, the *Venezuelan Equine Encephalitis* (VEE), has been subdivided into six subtypes. Pixuna virus belongs to subtype IV [11] and it has been included in the evolutionary line of the history of the Alphaviruses [25]. As a matter of fact, Alphaviruses infect both invertebrate and a variety of vertebrate including humans [34]. Thus these viruses are associated with a wide variety of clinical syndromes in humans and/or animals. Besides, that it has been suggested that Alphaviruses may use distinct strategies for either binding to different host cells or attaching to conserved cell components [14]. Furthermore, the number of viral receptors that have been isolated and characterized has increased in the past few years. The receptors include: CD4 for HIV [12, 28], CD46 [6, 20] and SLAM [35] for measles virus, and CAR for adenovirus and coxsackievirus [1]. The cellular receptors have a physiologic role but there are no receptors exclusive of the viruses. Hence, it has been recently demonstrated that CD46 is used by four viruses and bacteria [3], and by adenovirus type 11 [29]. Several cellular receptors for Alphaviruses have been pointed out; however, no receptors for Pixuna virus have been included among these [33].

In previous studies, we reported the isolation and characterization of a mouse brain fraction that could inhibit eastern equine encephalitis virus hemagglutination [21]. Therefore, due to the involvement of gangliosides in the activity of the fraction, we performed experiments to assess the role of gangliosides in the hemagglutination-inhibition reaction [23, 40, 42]. At that time, a theory about gangliosides and their

function was postulated [41] and a biophysical study was performed [22]. Then, Pixuna virus (family *Togaviridae*, genus *Alphavirus*) was selected in order to carry out electron microscopy and morphogenesis studies in our laboratory [9, 16, 17, 27]. As a result, a fraction of a mouse brain as well as 1-day-old chicken erythrocytes, which function as receptors for Pixuna virus, have been isolated and characterized in our laboratory [15, 24]. Consequently, this paper reports the isolation and partial characterization of a new receptor for Pixuna virus in the plasma membrane of chicken embryo fibroblasts.

Materials and methods

Cells and viruses

Chicken embryo fibroblasts (CEF) were obtained from 10-day-old chicken embryos. Heads, wings, legs, and internal organs were removed from the embryos that were minced, trypsinized (0.25% Trypsin Difco 1:250 in phosphate buffered saline-PBS, pH 7.4), and then they were filtered through gauze and centrifuged at 1,000 *g* for 10 min. Cells were seeded and grown at a concentration large enough to reach confluence after 3 days in 60 mm plates in autoclavable Eagle's minimum essential medium with Earle's salts (Gibco Lab Grand Island, NY, USA) supplemented with L-glutamine and 10% fetal calf serum (FCS) (Natocor-Córdoba, Arg.), 100 U of penicillin and 100 μg of streptomycin both per ml (MEM). The incubation was performed at 37°C in 5% humidified CO_2. When necessary, the cells were maintained in the same medium with 1% FCS. Vero cells were grown under similar conditions.

CEF and Vero cells were infected with Pixuna virus BeAr strain [31], which had undergone 21 passages in newborn mouse brains. The inoculum was 20% of brain suspension in PBS. After having performed two passages and after 3 days of postinfection, the cells exhibited cytopathic effects. The infected cells were harvested, frozen and thawed followed by centrifugation at 3,000 *g* at 4°C for 20 min to discard cellular debris. This supernatant fluid was the virus stock that was aliquoted and frozen at −130°C. Virus titers were assayed through plaque formation on CEF or Vero cell monolayers grown in 60 mm plates. After 1 h absorption at 37°C of serial tenfold virus dilutions in MEM with 1% FCS, the inoculum was removed and the cells were overlaid with 1.5% ultra pure agarose (Gibco BRL) in 4% FCS MEM 2x. Three days later, the cells were fixed with 10% formaldehyde in water and the overlaid medium was removed. Then, the monolayers were stained with 1% crystal violet in water and the plaques were counted. Sometimes, after 3 days have passed, the cells were overlaid with a second overlay of 1.5% agarose in water with 8% neutral red. After overnight incubation, the plaques were counted. Infectivity titers were expressed as PFU per milliliter.

Hemagglutination and hemagglutination-inhibition (HA–HI)

The hemagglutinating antigen was prepared with Pixuna virus by using the acetone–sucrose technique [4]. The HA and HI were performed in microscale at pH 6.2 [4] and the titers were expressed as the reciprocal of the dilution. Eight hemagglutinating units were used to perform the microscale HI test using 25 μl of the hemagglutinating antigen plus 25 μl of each one of the four receptors, which were incubated with shaking for 1 h at room temperature. Next, 50 μl of 1/50 red blood cells were added in buffer pH 6.2 [4] from 1-day-old chicken. The HI titers were calculated in 25 μl.

Receptor solubilization, isolation and purification

The following steps were performed at 4°C. Monolayers of CEF were washed three times with PBS, scrapped with a rubber policeman and pelleted at 1,000 *g* for 10 min in a 10 ml graduated tube. Pellets were suspended V/V in 1% octyl glucoside from Sigma in PBS (pH 7.4) in a proportion of 0.1:2 and they were gently shaken for 1 h. The suspension was centrifuged at 15,000 *g* for 2 h and the supernatant fluid was dialyzed overnight against 100 times its volume of PBS with magnetic stirring. The dialyzed fluid (crude extracts: CE) was precipitated with 50% $(NH4)_2SO_4$ and left to stand at 0°C overnight. After centrifugation at 15,000 *g* for 15 min, the pellet was brought to its original volume and it was suspended in PBS and dialyzed overnight as above (precipitated receptor: PP). The precipitated receptor was centrifuged at 100,000 *g* for 8 h in the SW 41 (Beckman ultracentrifuge) Ti rotor at 4°C, and a pellet (PL) and a supernatant (SN) were obtained. The pellet was brought to its original volume and it was suspended in PBS and it was sonicated. We initially measured the capacity of the four receptor preparations to inhibit Pixuna virus hemagglutination as well as protein concentration (commercial kit from Bio-Rad Dc Protein Assay-Bio-Rad Laboratories, CA according to the manufacturer instructions). After the purification steps, we also performed a control by using PAGE and the samples from the different fractions were aliquoted and frozen at −132°C.

Generation of antireceptor antibodies in rabbit antisera and monoclonal antibodies

PL was used as an antigen. In order to prepare the rabbit polyclonal antibodies we carried out the following process: 2-year-old New Zealand male rabbits were used. Each rabbit was injected with 1 ml of the receptor (80 μg protein) emulsified in an equal volume of Freund's complete adjuvant. These 2 ml were distributed subcutaneously in ten distinct locations in the back of the rabbits using 0.2 ml each time. This immunization was

repeated twice at intervals of 3 weeks. Finally, another immunization without the adjuvant was carried out after 3 weeks. Seven days later, the rabbits were bled by cut of the ear vein. This polyclonal serum provided a positive control [5] and served to perform the experiments in those cases in which we needed to compare a polyclonal antibody with a monoclonal antibody [26]. Both antibodies had the same outcome.

The monoclonal antibodies were obtained using mice (Balb C strain), which were immunized with PL. The hybridoma cells were prepared as previously described [38] to obtain the hybridomas. The production of antibodies was checked by means of dot blot. Briefly, pellet samples of 5 µl were dot-blotted by triplicate on Nitrocellulose membranes and then blocked with TBS with 5% BSA. The dot-blotted membranes were incubated overnight with 50 µl of hybridoma cell culture supernatant at 4°C. Then, the membranes were washed three times with TBS-0.05% Tween 20, and incubated at 37°C for 1 h with a secondary alkaline phosphatase-conjugated antibody (anti-mouse) (Photo Blot Western Blot Alkaline Phosphatase System; Promega Corporation, Madison, WI, USA). After five washings with TBS-0.05% Tween 20, the blots were developed with Bromochloroiodylphosphate (15 µl of a 50 mg/ml stock solution) and nitroblue-tetrazolium (2.5 µl of a 75 mg/ml stock solution) in 10 ml of alkaline phosphate-detection buffer (100 ml mM Tris, 100 mM NaCl, 5 mM $MgCl_2$, pH 9.5).

Western blot analysis

Equal amounts of protein (30 µg) from chicken embryo fibroblasts and the different fractions of the four receptors—the crude extract (CE), precipitated (PP), pellet (PL) and supernatant (SN)—were boiled for 5 min in a volume of 2× buffer sample (125 mM Tris, pH 6.8; 600 mM 2-mercaptoethanol; 6% sodium dodecyl sulfate, 20% glycerol; 0.005% bromophenol blue). Then, they were analyzed on 12% SDS PAGE and transferred to polivinylidene difluoride (PVDF) membranes in a Tris-glycine buffer containing 20% methanol. The membranes were washed several times with TBS (10 mM Tris, pH 7.5, 150 mM NaCl) and blocked in TBS containing 5% BSA for 1 h and incubated overnight at 4°C with the primary antibodies (polyclonal or monoclonal antibodies) in TBS containing 1% BSA. The membranes were then washed three times (10 min each) in TBS containing 0.05% Tween 20, and incubated with a secondary alkaline phosphatase-conjugated antibody (PhotoBlot Western Blot Alkaline Phosphatase System; Promega Corporation, Madison WI, USA) at 37°C for 1 h. After five washings with TBS-0.05% Tween 20, the blots were developed with bromochloroiodylphosphate (15 µl of a 50 mg/ml stock solution) and nitroblue-tetrazolium (2.5 µl of a 75 mg/ml stock solution) in 10 ml of alkaline phosphate-detection buffer (100 ml mM Tris, 100 mM NaCl, 5 mM $MgCl_2$, pH 9.5).

Immunofluorescence

Cells grown on coverslips were fixed with 4% (wt/vol) paraformaldehyde and 4% sacharose in PBS for 20 min at 37°C and then washed with PBS. Cells without permeabilization were blocked with 5% BSA in PBS during 1 h at room temperature. Primary polyclonal or monoclonal antibodies were diluted in 1% BSA in PBS and incubated overnight at 4°C in different dilutions from 1/50 to 1/1,600 and washed three times with PBS. The secondary antibodies Alexa Fluor 488 and 568 (Molecular Probes) were incubated in 1% BSA in PBS for 1 h at room temperature, washed three times with PBS and mounted using the Fluorsave Reagent (Calbiochem). The cells were analyzed with a Zeiss LSM 5 confocal scanning microscopy. Images were captured using CCD camera (Orca 1000, Hamamatsu Corp., Middlesex, NY). Control preparations included either untreated cells and cells treated with purified gamma globulins [10] from pre immune rabbits or purified gamma globulin from negative hybridoma cell culture supernatant.

Blocking assay of the receptor in fibroblasts

The assay of anti-receptor activity from poly and monoclonal antibodies was performed by determining their ability to protect fibroblasts from virus infection [5]. Briefly, monolayers of CEF in 60 mm plates were treated with 200 µl of different dilutions of purified immune gamma globulin in MEM without FCS for 1 h at room temperature with gentle shaking. Then, these monolayers were challenged with 200 µl containing different Pixuna virus dilutions in the same solvent at 37°C during 1 h with gently shaking and covered with agarose as above. Three days later, the plaques were stained and counted as above. The control consisted of using the purified gamma globulins from the pre immune rabbits and the cell culture supernatant of negative hybridome. A gamma generalized linear model [18] with a linear prediction containing a treatment factor was used in order to evaluate differences between both normal and immune gamma globulin treatments.

Blocking assay of the receptor in Vero cells

Vero cell monolayers in plates were treated with different dilutions of purified gamma globulin from monoclonal or polyclonal antibodies as above. After 60 min incubation at room temperature with gently shaking, the monolayers were challenged by Pixuna virus and the same steps as the ones described above were performed. Controls as the ones described above were used. The

same linear model was followed to calculate significant differences.

Enzymatic treatment

Sigma-Chymotrypsin type II 3x from 4x and trypsin TPCK treatment type XIII from a stock of 10 mg/ml in PBS at different concentrations during 1 h at 37°C were used. The reaction was stopped by immersing the tubes in a water bath at 75°C for 15 min. The crude extracts (CE) and the pellet (PL) were tested for their property of inhibiting viral hemagglutination before and after the enzymatic treatment.

PAGE without SDS

The four fractions (CE, PP, PL, and SN) with the concentration of protein previously determined were analyzed by PAGE [7] without using the sample solubilization solution and SDS. A volume of 100 µl of each fraction was mixed with 20 µl of tracer buffer (glycerol 1 ml, PBS 10 ml, bromophenol blue 1 ml (0.1% w/v solution in water) and then loaded onto a 10.5% polyacrylamide gel. Samples were electrophoresed at room temperature.

Electron and immunoelectron microscopy of the pellet (PL)

3.5 µl of the pellet were placed on a prewashed 300 mesh cooper grid coated with Formvar and carbon. After 2 min of sample absorption, a 2% phosphotungstic acid (pH 7.0) stain was applied. After 30 s of staining, grids were allowed to air dry for a minimun of 30 min. Samples were then viewed under a Siemens Elmiskop 101 electron microscope operated at 80 kv with a magnification of ×12,000. Images were captured on Kodak SO-163 EM film. Pellet was made to react with the gamma globulins from the polyclonal antibodies at room temperature during 30 min with shaking. This was centrifuged at 100,000 *g* for 3 h and processed as described above.

Results

Receptor isolation

In order to extract the receptor from chicken embryo fibroblasts, 1% octyl glucoside in PBS was used which proved to be the best concentration. Then, several steps were performed to purify the fraction with HI properties. We obtained a relatively pure membrane component with HI properties after $(NH4)_2SO_4$ precipitation followed by 100,000 *g* ultracentrifugation as described in Materials and methods. Four fractions were collected during the purification steps: crude extract (CE) before purification; precipitate (PP) after precipitation with 50% $(NH4)_2SO_4$; pellet (PL) and supernatant (SN) after centrifugation at 100,000 *g* for 8 h.

Receptor activity

Hemagglutination-inhibition was used to assess the different preparations interaction with the hemagglutinating Pixuna virus antigen. The CE as well as the $(NH4)_2SO_4$ precipitated fraction (PP) showed an HI titer of 64 per 25 µl. The SN inhibitory titer was negative or four, lesser than the previous values. In addition, PL had an inhibitory titer higher than CE or PP (Table 1). Although the HI titer increased or remained constant in the different fractions, protein concentrations decreased as the fractions were purified (this is shown in Table 1). The results are the mean of ten determinations.

Receptor identification

The fraction called PL was used to prepare rabbit polyclonal and mouse monoclonal antibodies (see Materials and methods), and we used the gamma globulin from both antisera to perform Western blot analysis of the total chicken fibroblasts and the fractions described above. As shown in Fig. 1, the gamma globulin from the monoclonal or polyclonal antibodies showed a double band with a molecular weight of approximately 60 kDa in three of the four samples. Neither band nor HI activity was detected in the supernatant. When PL was treated with proteases, both the HI activity and the double band disappeared (not shown). Indirect immunofluorescence microscopy of the fibroblasts treated with either polyclonal or monoclonal antibodies as the primary antibody detected positive staining on the plasma membrane (Fig. 2a). Positive fluorescence similar to that one of the fibroblasts was also detected in

Table 1 The hemagglutination-inhibition titers of the four receptors and the protein concentration, both measured in 25 µl, and the HI/protein relationship

Receptor	HI titer	Protein concentration	HI/protein
Crude extracts (CE)	64	25	2.56
Precipitated receptor (PP)	64	15	4.26
Pellet (PL)	64	6	10.6
Supernatant (SN)	Negative or <4	1.5	–

HI figures are the reciprocal of the maximal dilution producing hemagglutination-inhibition of eight hemagglutinating units and they constitute the mean of ten determinations. Pellet (PL) at a protein concentration of 6 µg shows an HI titer of 64

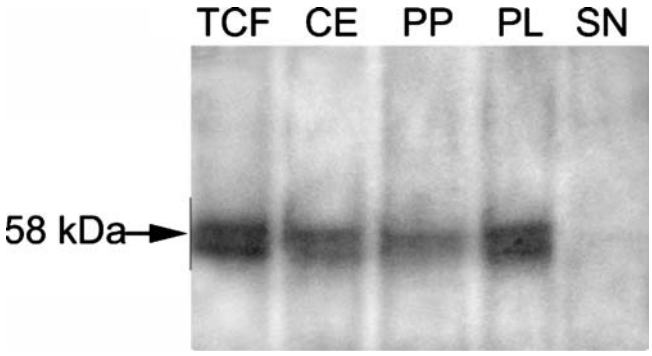

Fig. 1 Western blot analysis of the total chicken fibroblast cells (*TCF*) and the four separations carrying the receptors (*CE* Crude extract, *PP* Precipitated receptor, *PL* Pellet, and *SN* Supernatant). Equal amounts of protein were separated by using SDS-PAGE, transferred to PVDF membranes and blotted with the monoclonal antibodies (see Materials and methods). The receptor was characterized as a protein band of approximately 60 kDa. This protein band was absent in the 100,000 *g* SN. The polyclonal and monoclonal antibodies showed the same pattern

Vero cells treated with monoclonal or polyclonal antibodies and stained with secondary fluorescent antibodies (Fig. 2b).

The receptor: a membranous structure

The fractions analyzed using PAGE without SDS (Fig. 3a) showed that the amount of protein in each fraction decreased after each purification step, even though the HI activity remained high. Three of the fractions showed one portion each that did not penetrate the gel. The other fraction (SN) did not show this portion in the gel (Fig. 3a). These findings as well as the pelleting of the HI substrate suggested that the HI activity is attached to the membranes. We also performed electron microscopy and immunoelectron microscopy of PL. Figure 3 shows that this fraction holds mainly membranes (b) and that these membranes reacted with the antibodies of rabbit antiserum (c).

Cell protection from Pixuna virus infection

Figure 4 illustrates a representative experiment of prevention from Pixuna virus infection of CEF. Dish a has been pretreated with different concentrations of purified gamma globulin from negative hybridoma cell culture supernatant or from pre immune rabbits and then, the dish was infected with Pixuna virus. The monolayer of CEF in dish b was previously treated with gamma globulin purified from monoclonal or polyclonal antibodies (which were obtained as described in Materials and methods), and then, the dish was infected with the same concentration of Pixuna virus. A significant plaque reduction was obtained. Figure 4 is representative of eight experiments from which the fitted model indicated a significant difference of $P = 0.032$ between a and b calculated at the eight experiments. Similar results were obtained with the two antibodies. Besides, a similar significant plaque reduction was also obtained in Vero cells pretreated with monoclonal or polyclonal antibodies (not shown).

Proteolytic treatment of PP and PL

In order to gain knowledge about the chemical nature of the receptor in the membrane fractions obtained, we digested the PP and PL with the proteolytic enzymes Chymotrypsin and trypsin TPCK. Both enzymes fully destroyed the HI activity of both fractions so that the proteic nature of the active site was demonstrated.

Discussion

Other studies reported and described Alphaviruses attachment to lipids as well as liposomes [13, 19, 30, 32]. Accordingly, there have been isolated receptors for the genus prototype, Sindbis virus [2, 37]. However, receptor molecules mediating Pixuna virus entry into CEF have not been characterized until now. In the past, we described receptors for Pixuna virus in mouse brain [15]

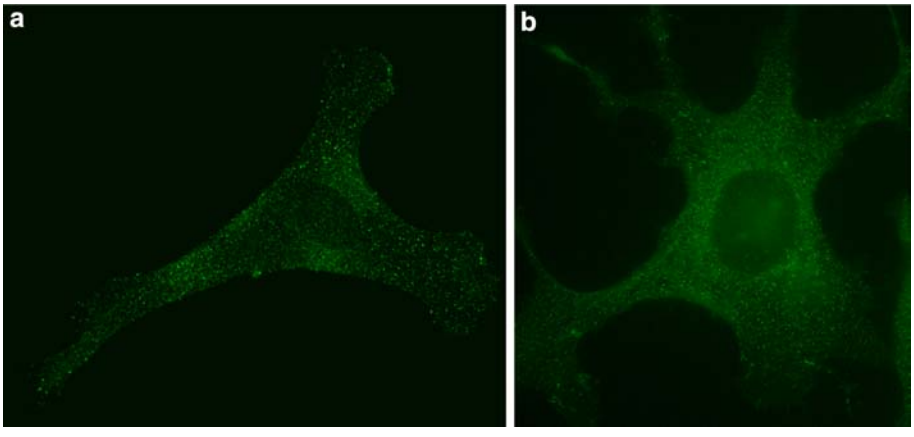

Fig. 2 Indirect immunofluorescent staining with monoclonal or polyclonal antibodies directed against the receptor. The receptor clearly detected in the plasma membrane of the chicken fibroblasts (**a**) as well as in the Vero cells (**b**)

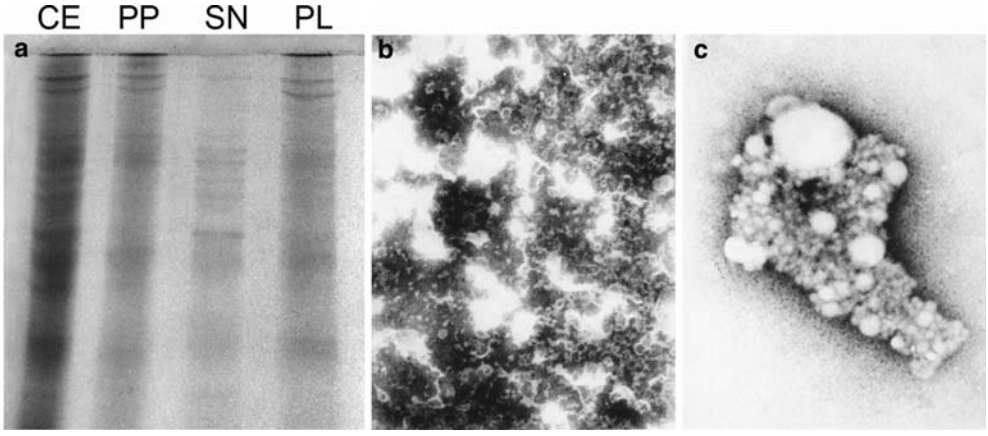

Fig. 3 PAGE without SDS of the total receptor *CE*, precipitated receptor *PP*, receptor supernatant *SN* and purified receptor *P*L (**a**). There is a visible decrease in the number and in the intensity of the protein bands, which show an evident purification when CE and PL are compared. The striking fact is that there was a band in CE, PP and PL which did not penetrate in the gel, but which is absent in the SN gel. This finding suggested that the receptor could be a membranous structure. This was confirmed by electron microscopy (**b**) and immunoelectron microscopy (**c**). Magnification of ×12,000

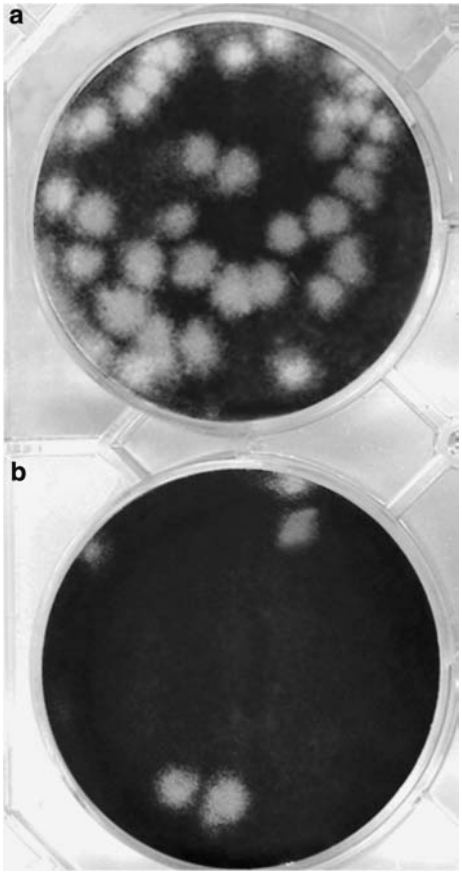

Fig. 4 Fibroblasts protected against the infection with Pixuna virus. Dish a is the plaque controls where the cells were treated with purified gamma globulins from negative hybridome cell culture or preimmunized rabbits. Said dish was infected with 10^4 PFU. Dish b is the monolayer previously treated with purified gamma globulins from monoclonal or polyclonal antibodies. Said dish was infected with the same concentration of Pixuna virus. This figure is representative of eight experiments. A significant plaque reduction was obtained and the gamma generalized linear model showed a significant difference of $P = 0.032$. Vero cells showed the same pattern (not shown)

and in 1-day-old chicken erythrocytes [24] the active group in the mouse brain was a proteolipid membrane with a protein/lipid ratio of 3/1. The receptor activity was fully destroyed by proteolytic enzymes [15]. On the other hand, the receptor described in chicken erythrocytes was not protein in nature. In this case, trypsin and chymotrypsin proteases were able to expose several binding sites in the erythrocytes and to significantly increase the hemagglutinating titer. Cholesterol and phospholipids were involved in the HI activity of these membrane structures.

Here, we have studied a receptor for Pixuna virus in chicken embryonic fibroblast cells in culture. The protein nature of the receptor was established as a result of the loss of activity after exposure to two different proteases. PAGE without SDS showed several bands but the striking feature was a protein band that did not penetrate into the gel. This band disappeared after the enzyme treatment. Besides, the last step performed to purify the receptor, that is the 8-h centrifugation at 100,000 *g*, indicated that there was a band in the pellet with HI activity, which did not penetrate into the gel but on the other hand there was no band or HI activity in the supernatant. These findings suggested that we were dealing with a membranous structure and this was confirmed by electron and immunoelectron microscopy (Fig. 3a–c). Moreover, the immunofluorescence microscopy using poly and monoclonal antibodies was positive with both antibodies: the immunofluorescence was exclusively located in the plasma membrane (Fig. 2a).

We could identify the receptor as a double band protein of approximately 60 kDa with Western blot using poly and monoclonal antibodies (Fig. 1). In the same way, Wang et al [36] isolated a receptor for Sindbis virus with a molecular weight of 63 kDa from chicken cells. Thus, it would be interesting to determine if the receptor for Pixuna virus is the same one as that for Sindbis virus.

In order to confirm that the poly- and monoclonal antibodies blocked the receptor in the plasma membrane of chicken embryo fibroblasts, we performed protection experiments. Both antibodies protected the cells (Fig. 4) and they also inhibited the CPE (not shown). The monoclonal and polyclonal antibodies protected both Vero cells and fibroblasts from Pixuna virus infection. These results suggested that both receptors shared epitopes in spite of belonging to different cell species, namely, bird and mammalian. As confirmation, immunofluorescence procedure in Vero cells showed a reaction similar to the one shown by the fibroblasts (Fig. 2b). Additional studies will be worthy to determine the relationship between both receptors. Another receptor that we characterized was found in 1-day-old chicken erythrocytes [24]. Unpublished data from our laboratory suggested that both receptors share epitopes, but that their chemical compositions are different: lipids are involved in erythrocytes [24] while the receptor in CEF is protein in nature. So far we do not have an explanation for the latter. The receptor in fibroblasts shares epitopes with the receptor in Vero cells. These findings evidenced that the same virus can bind to different or similar receptors to start replication. This had been pointed out in other viruses such as Sindbis virus, which binds to the heparan sulfate surface [2] and to the receptor in mosquitoes and birds, and to another receptor in mammalian cells [36, 37]. Besides that, measles virus can bind to CD46 receptor [6, 20] as well as to SLAM receptor [35]. A third receptor for measles virus the Edmonston strain has been showed on CEF [8]. Although CD46 is used by measles virus, this receptor is also used by adenovirus 37 when this virus produces keratoconjunctivitis [39]. All these findings motivated us to study our receptor in connection with the one for measles virus. Experiments in progress will allow us to show if the Pixuna virus receptor is involved in measles virus binding to CEF.

Elucidating the molecular mechanisms of the binding of virus to a specific receptor is crucial for understanding viral pathogenesis, virus host range, tissue tropism and for the intelligent design of efficient and safe viral vectors for use in basic research as well as in human gene therapy. At the same time, studying the virus interaction with the host cell could help to reveal general mechanisms underlying the development of antiviral and vaccine reagents. In this work we were able to partially characterize another receptor for Pixuna virus, which allows evidence of the virus into the cell.

Acknowledgements GP is a member of the Research Career from the Consejo Nacional de Investigaciones Científicas y Técnicas de la R. Argentina and contributed principally to this work. We gratefully acknowledge to Sondra Schlesinger and Marta Ruben-Campioni for fruitful and excellent critiques of the manuscript. We appreciate the assessment and language editing work carried out by Valeria Mosqueda and Lorena Riquelme. We thank Avicola Bartolucci that provided free the chicken embryo and the 1-day-old chicken. We also thank Cristina Maldonado for assistance with electron microscopy of the receptor as well as to Maria del Pilar Díaz for the statistical data and to Brenda Königheim, Javier Aguilar and Luciana Martinez from our Institute for the supply of Vero cells in the final stage of this study. Part of this work was supported from SECyT, UNC.

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