Vaccine xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Target cells for antibodies detection in rabies vaccine control

Diego Fontana, Claudio Prieto*, Ricardo Kratje, Marina Etcheverrigaray

Laboratorio de Cultivos Celulares, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Ciudad Universitaria, Paraje "El Pozo" – C.C. 242, S3000ZAA Santa Fe, Argentina

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Rabies
Vaccine
Neutralizing antibodies
Rabies virus glycoprotein

ABSTRACT

Rabies causes one of the most lethal zoonotic diseases, with more than 55,000 deaths reported annually. Prevention is based on pre-exposure vaccination of individuals at high risk of contracting rabies, and mass vaccination of dog, which are the main vector for transmission to humans. Post-exposure prophylaxis includes vaccination and rabies immunoglobulins treatment. The measurement of neutralizing antibodies in sera of vaccinated individuals is a primary concern to determine the efficacy of immunization schedules or the potency of new vaccines. Antibodies against rabies glycoprotein are considered an ideal indicator. In this work we showed the development of a VERO clone that is able to detect by fluorescence microscopy and flow cytometry, the presence of antibodies against the rabies glycoprotein, specifically in its native conformation anchored in the plasma membrane. These cells could trigger the development of a new rapid method for the detection of rabies virus neutralizing antibodies in vaccinated individuals.
© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Rabies is a viral zoonosis caused by *Rhabdovirus*, a virus that is responsible for a mortality rate of almost 100% when not detected or treated. Nowadays, there are more than 10 million post-exposure treatments in humans [1].

Neutralization is the primary function of antibodies in virus infections, and the evaluation of virus neutralizing antibodies (VNA) levels is essential to determine the efficacy of immunization schedules or the potency of new vaccines. VNA are directed to the epitopes of the rabies virus glycoprotein (G), the only one that is exposed on the viral surface.

Currently, measurement of neutralizing antibodies is performed *in vivo* using the mouse neutralization test (MNT) [2] that requires a great amount of animals. The standard *in vitro* assays are the rapid fluorescent focus inhibition test (RFFIT) or the fluorescent antibody virus neutralization test (FAVN) [3,4], which demand live rabies virus (RV) and can be performed only in restricted reference laboratories. The enzyme-linked immunosorbent assay (ELISA) is the only commercially available test for the prediction of VNA [5].

The objective of this study was to develop an alternative assay for the detection of G protein specific antibodies to be validated against the standard described methods that detect VNAs, in order to avoid the use of animals and active virus for vaccine control.

2. Results

By using lentivirus to transduce cells, we constructed a recombinant Vero cell line that expresses the G protein in the plasma membrane. Lentivirus particles were produced by simultaneous co-transfection of HEK 293 cells with four different plasmids: the packaging construct (pMDLg/pRRE) [6], the VSV-G-expressing construct (pMD.G) [7], the Rev-expressing construct (pRSV-Rev) [6], and the self-inactivating (SIN) lentiviral vector construct containing the glycoprotein sequence (pLV–G). The medium containing lentiviral particles was collected 48 h after transfection, clarified by centrifugation 10 min at 2000 rpm and then stored at −80 °C. VERO cells were seeded at 3×10^4 cell/ml in 6-well plates and maintained for 18 h. The supernatant was replaced by 1 ml of lentiviral particles, followed by incubation overnight. Then, the supernatants were replaced with fresh medium. 96 h post-transduction cells where incubated with the puromycin selection reagent, using a multi-step gradual selection protocol based on previous descriptions [8]. The obtained cell line was named VERO-G.

The protein expression was detected by flow cytometry using a G protein specific monoclonal antibody, obtaining 85% of positive cells (Fig. 1A). The presence of the G protein in the membrane of the cells was confirmed by immunofluorescence microscopy using an FITC conjugated antibody (Fig. 1B). After that, VERO-G cell line was cloned using the dilution limit method and the obtained 50 clones were analyzed by flow cytometry. Over 50% showed a percentage of positive cells higher than the initial cell line. Clone P1B6 was chosen for the following experiments because it showed the highest protein expression.

* Corresponding author. Tel.: +54 342 456 4397; fax: +54 342 456 4397.
E-mail address: cprieto@fbcb.unl.edu.ar (C. Prieto).

http://dx.doi.org/10.1016/j.vaccine.2014.02.030
0264-410X/© 2014 Elsevier Ltd. All rights reserved.

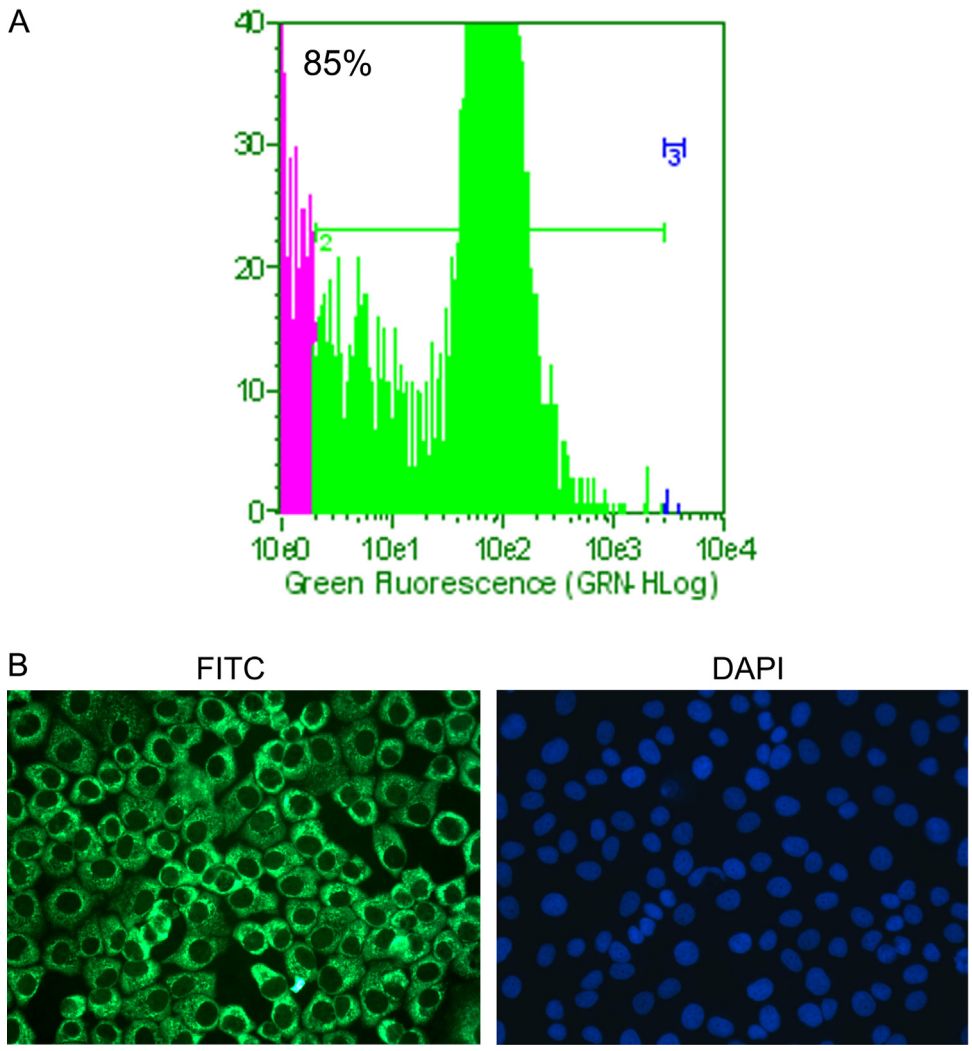

Fig. 1. Analysis of the expression of G protein by VERO-G cell lines. (A) Flow cytometry assay using a G protein specific monoclonal antibody. (B) Fluorescence microscopy of VERO-G cell line monolayer using an FITC conjugated antibody. DAPI was used for nuclei localization.

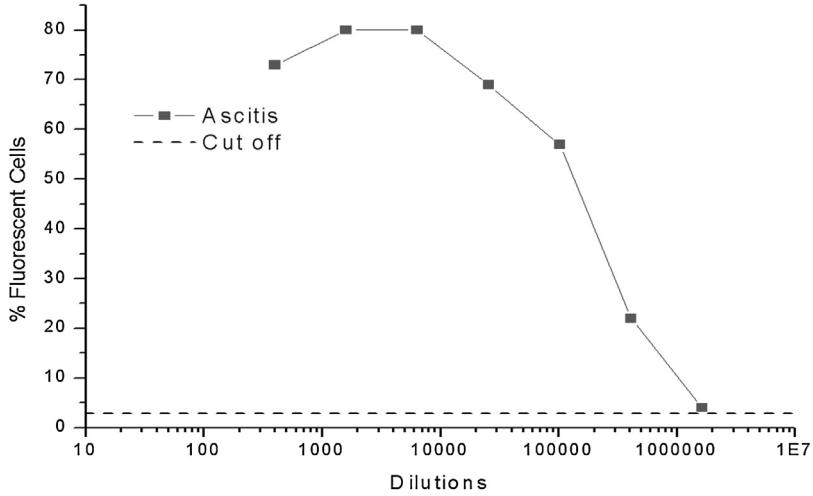

Fig. 2. Flow cytometry analysis using a monoclonal ascites. Fourfold dilutions were incubated with P1B6 clone, followed by incubation with an AlexaFluor488® conjugated antibody. The cut off value was established using basal mice sera.

Please cite this article in press as: Fontana D, et al. Target cells for antibodies detection in rabies vaccine control. Vaccine (2014), http://dx.doi.org/10.1016/j.vaccine.2014.02.030

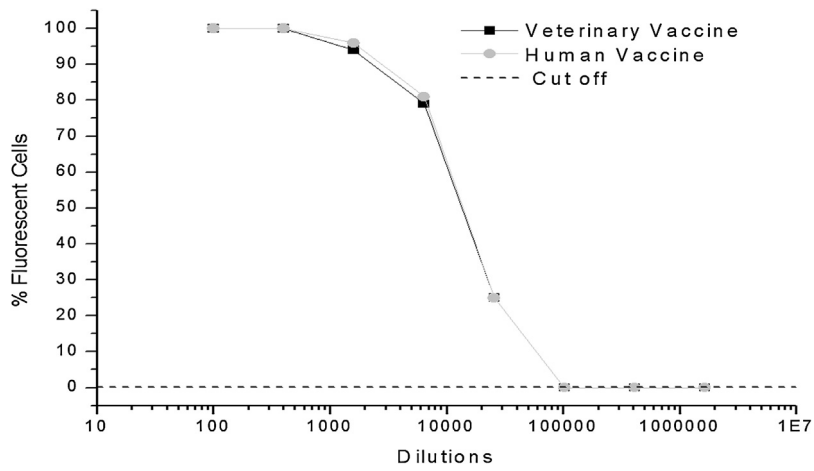

Fig. 3. Flow cytometry analysis of sera from vaccinated animals with commercial human and veterinary vaccines. P1B6 clone was used to analyze the presence of G protein specific antibodies.

To evaluate if recombinant cells were suitable to be used for the quantitation of specific serum antibodies, a flow cytometry assay using P1B6 was performed as follows: a suspension of 1×10^5 cells were incubated for 20 min at RT with 4-fold serial dilutions of a monoclonal ascites, followed by 20 min incubation with AlexaFluor488® conjugated antibody (Invitrogen, USA). Cells were analyzed using a GUAVA EasyCyte cytometer (Millipore, France) acquiring 5000 events during 3 min. The obtained results show a good correlation between dilutions and percentage of fluorescent cells (Fig. 2), and we could calculate antibody titers against G protein. (cut off = % + 2 SD; being % the percentage of fluorescent cells obtained with basal mice sera and SD the standard deviation).

After that, P1B6 was used to evaluate serum pools of animals immunized with human (VERORAB, Sanofi Pasteur, France) and veterinary (Bagovac Rabia, BIOGÉNESIS-BAGÓ, Argentina) commercial vaccines. Cells incubated with these sera were analyzed by flow cytometry and titers of 1/25,600 were obtained for each pool (Fig. 3).

3. Conclusions

These results show that G-expressing cells are able to detect antibodies that recognize the immunogenic protein in its native conformation and anchored in the cell membrane. The results obtained here should be compared with the standard methods used to determine NVA in order to determine whether this new assay distinguish NVA from not neutralizing anti-G antibodies. In the case that both methods correlate adequately, it should provide a new system for NVA antibodies titration, to be applied in the control of production batches of rabies vaccine as well as to the control of vaccinated individuals.

This assay has the benefit that can be performed in a very short time, without the use of active virus or animals.

On the other hand, as it was described [9], anti-G Mab is reactive to paraformaldehyde fixed cells. Thus, the recombinant cells presented here could be the basis for the development of a commercial kit with low cost, complexity and accessible in unconventional settings.

References

[1] Schnell MJ, McGettigan JP, Wirblich C, Papaneri A. The cell biology of rabies virus: using stealth to reach the brain. Nat Rev Microbiol 2010;8:51–61.
[2] Webster LT, Dawson JR. Early diagnosis of rabies by mouse inoculation measurement of humoral immunity to rabies by mouse protection test. Proc Soc Exp Biol Med 1935;32:570–3.
[3] Smith JS, Yager PA, Baer GM. A rapid fluorescent focus inhibition test (RFFIT) for determining rabies virus-neutralising antibody. In: Meslin F-X, Kaplan MM, Koprowski H, editors. Laboratory techniques in rabies. 4th ed. Geneva, Switzerland: World Health Organization; 1996. p. 181–92.
[4] Cliquet F, Aubert M, Sagne L. Development of a fluorescent antibody virus neutralisation test (FAVN test) for the quantitation of rabies-neutralising antibody. J Virol Methods 1998;212:79–87.
[5] Feyssaguet M, Dacheux L, Audry L, Compoint A, Morize J, Blanchard I, et al. Multicenter comparative study of a new ELISA PLATELIA RABIES II, for the detection and titration of anti-rabies glycoprotein antibodies and comparison with the rapid fluorescent focus inhibition test (RFFIT) on human samples from vaccinated and non-vaccinated people. Vaccine 2007;25:2244–51.
[6] Dull T, Zufferey R, Kelly M, Mandel RJ, Nguyen M, Trono D, et al. A third-generation lentivirus vector with a conditional packaging system. J Virol 1998;72:8463–71.
[7] Naldini L, Blomer U, Gallay P, Ory D, Mulligan R, Gage FH, et al. In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science 1996;272:263–7.
[8] Prieto C, Fontana D, Etcheverrigaray M, Kratje R. A strategy to obtain recombinant cell lines with high expression levels. Lentiviral vector-mediated transgenesis. BMC Proc 2011;5(8):P7.
[9] Fontana D, Kratje R, Etcheverrigaray M, Prieto C. Rabies virus-like particles expressed in HEK293 cells. Vaccine 2014, http://dx.doi.org/10.1016/j.vaccine.2014.02.031.