The Brazilian Journal of
INFECTIOUS DISEASES

www.elsevier.com/locate/bjid

Original article

Development of a prototype immunochromatographic test for rapid diagnosis of respiratory adenovirus infection

Inarei Paulini[a,*], *Joselma Siqueira-Silva*[b], *Luciana Thomaz*[c], *Leticia Rocha*[d], *Charlotte Harsi*[b], *Nancy Bellei*[a], *Celso Granato*[a]

[a] *Universidade Federal de São Paulo, Departamento de Medicina, Laboratório de Virologia, Disciplina de Infectologia, São Paulo, SP, Brazil*
[b] *Universidade de São Paulo, Instituto de Biociências, Departamento de Microbiologia, Laboratório de Adenovírus, São Paulo, SP, Brazil*
[c] *Instituto de Biociências, Departamento de Microbiologia, Laboratório de Micologia, São Paulo, SP, Brazil*
[d] *Instituto Butantan, Laboratório de Bacteriologia, São Paulo, SP, Brazil*

ARTICLE INFO

Article history:
Received 9 December 2016
Accepted 29 March 2017
Available online 15 June 2017

Keywords:
Adenovirus
Monoclonal antibodies
Immunochromatographic test

ABSTRACT

Human adenoviruses comprise an important group of etiologic agents that are responsible for various diseases in adults and children, such as respiratory, ocular, gastroenteric, and urinary infections. In immunocompromised and organ-transplanted individuals, these agents can cause generalized infections. Rapid diagnostic methods for detecting these infectious agents are not widely available.

The aim of this work was to produce monoclonal and polyclonal anti-adenovirus antibodies to be used in a rapid diagnostic test for respiratory infections.

Adenovirus hexons were satisfactorily purified by ultracentrifugation and chromatography. After virus purification, anti-hexon monoclonal antibodies were produced and characterized, following classical methods. Antibodies were specific for adenoviruses 2, 3, 5, and 41. The proposed immunochromatographic test was standardized using colloidal gold.

The standardization of the rapid test was sufficient to detect adenovirus antigens (in nasopharyngeal lavage samples) with sensitivity of 100% and specificity of 85% when compared to direct immunofluorescence.

The immunochromatographic assay prototype was sufficiently sensitive to detect B (3), C (2 and 5), and F (41) adenovirus samples. Although based on preliminary data, the test demonstrated the same performance as direct immunofluorescence, but with the advantage of being a point-of-care test. Further studies are still needed to confirm its effectiveness in clinical practice.

* *Corresponding author.*
E-mail address: inareip@yahoo.com.br (I. Paulini).
http://dx.doi.org/10.1016/j.bjid.2017.03.023

Introduction

Human adenoviruses (HAdVs) are important agents that cause serious infections in children and immunocompromised patients. HAdVs were first detected in military personnel with acute febrile respiratory disease. Later, clinical manifestations such as gastroenteritis, cystitis, hepatitis, keratoconjunctivitis, meningoencephalitis, and myocarditis were also related to these viruses.[1–10]

The epidemiological studies of adenovirus infections have limitations due to the relatively high incidence of doubtful and inconclusive results obtained in the tests currently in use. Direct immunofluorescence (IFD) and Enzyme Linked ImmunonoSorbent Assay (ELISA) assays are techniques that are not very sensitive for the diagnosis of adenovirus infections, compared to cell culture methods and molecular diagnosis.[3–5,10]

Virus isolation in cell cultures is a sensitive method for adenovirus detection, but this method is costly and time-consuming, taking several days to perform the isolation. As some adenovirus serotypes are difficult to culture, in order to maximize sensitivity the virus should culture on at least two or three different cell lines.[10] The turn-out of culture results are immediately provided to the clinician, and are impractical in studies with a large number of clinical samples.[3,10]

Molecular diagnosis by PCR (Polymerase Chain Reaction) is a relatively quick and sensitive tool, enabling direct laboratory diagnosis in the clinical sample.[10] Depending on the DNA region amplified by PCR, it is possible to distinguish between species and serotypes.[10] However, this technique still has a high cost and requires a specialized technical team to perform it, and it is therefore not very accessible.[3,9,10]

In this study, the purified adenovirus antigen was used in the production of monoclonal and polyclonal antibodies, which in turn were applied to a nitrocellulose membrane. The rapid test prototype consisted of anti-hexon polyclonal antibodies coupled to the glass fiber superimposed on high-flow nitrocellulose 180.

The combination of the 6D-1G monoclonal antibody on the test line (capture) with gold-conjugated rabbit polyclonal antibodies (fiber glass/detection) provided better specificity and sensitivity. When 6D-1G (concentration 1.2 mg/mL) was tested with sucrose (3%) and trehalose (2.5%), there was successful detection of adenoviruses 2, 3, 5, and 41.

In the configuration of the proposed adenovirus detection test the monoclonal antibody was bound to a nitrocellulose membrane and the polyclonal antibody to a glass fiber membrane, and this proved to be a promising format in the standardization of an immunochromatographic assay for detecting all adenovirus serotypes.[29]

Material and methods

Production of adenovirus hexons/purification stocks, electron microscopy and virus concentration

Production of adenovirus stocks and purification, electron microscopy, and virus concentration were performed as previously described.[11–19] The protein content was measured by bicinchoninic acid method.[20]

Production of polyclonal antibodies

Immunization of BALB/c mice

Virus antigen was purified using cesium chloride gradient centrifugation and dialysis against 10 mM HEPES buffer, pH 7.4, 10% glycerol and 1 mM EDTA. Nine male BALB/c mice of approximately six weeks were immunized intraperitoneally (i.p.) as follows[20]: injection of 50 μg adenovirus hexon protein (pure antigen) diluted in Freund's adjuvant. The booster dose was performed with i.p. injection of approximately 50 μg antigen in Freund's incomplete adjuvant. The third dose was performed i.p. injection of approximately 50 μg antigen in Freund's incomplete adjuvant. The fourth dose was administered with injection with 50 μg antigen in Freund's incomplete adjuvant. Finally, spleen cells were used for the fusion.

Production/purification of polyclonal antibodies in rabbit was performed as previously described.[20–22]

Indirect ELISA of polyclonal antibodies from BALB/c mice

Selection of positive mice, i.e., those that produced adenovirus antigen-specific antibodies, was based on the result of indirect ELISA test. The plate was coated with purified "adenovirus hexon" diluted in 0.05 M carbonate/bicarbonate buffer, pH 9.6 (50 μL/well), at a concentration of approximately 10 μg/well. The plate was incubated overnight at 4 °C and subsequently washed once with 0.05% PBST, and blocking solution (1% tryptone, 1% BSA, 3% sucrose (150 μL/well)) in PBS for 1 h at 37 °C was then added. The plate was washed once with 0.05% PBST. Next, 50 μL of sera were added to the ELISA plate, which was then incubated at 37 °C for 1 h. The negative control was PBS. The plate was washed 3 times with 0.05% PBST and then incubated with secondary antibody, horseradish peroxidase-conjugated goat anti-mouse IgG (H+L) (Immunopure Pierce), diluted 1/10,000 in 0.05% PBST. Peroxidase activity was revealed by the addition of ELISA substrate TMB BD OptEIA. The reaction was stopped by adding 50 μL/well of 3 N H_2SO_4 and the plate read on a TP Reader Thermo Plate at a wavelength of 450 nm.[20]

Monoclonal antibodies production

Hybridoma technology

Fusion was performed according to the protocol proposed by Dr. Leticia Barboza's group (Bacteriologia – Instituto Butantan). Myeloma cells from the Sp2/0-Ag14 line (ATCC CRL 1581-Köhler and Milstein, 1976) were purchased from the Rio de Janeiro Cell Bank (BCRJ). Cells were previously thawed, grown in pre-fusion medium (RPMI 1640 medium (Invitrogen) plus 10% NCTC (Invitrogen) and 10% FBS) and kept in an incubator at 37 °C in 5% CO_2.

For the preparation of hybridoma conditioned medium (feeder), mouse macrophages of the BALB/c strain were obtained by the intraperitoneal lavage procedure. Macrophages were obtained by introducing 5 mL of RPMI 1640 medium (4 °C) into the peritoneum. The medium was