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Development of a rapid and sensitive latex agglutination-based method for detection of group A rotavirus ☆

Ana Carolina Magalhães Andrade de Góes [a], Marcia Terezinha Baroni de Moraes [b,*], Waldemir de Castro Silveira [c], Irene Trigueiros Araújo [d], Jean-Claude de H'alluin [e], Waldemar da Silva Souza [f], José Godinho da Silva Junior [g], José Paulo Gagliardi Leite [d]

[a] *Biopharmaceuticals Program, Bio-Manguinhos, Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil*
[b] *Laboratory of Recombinant Technology, Bio-Manguinhos, Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil*
[c] *Biodevices – Industry and Commerce Ltda, R&D Department, São João de Meriti, Rio de Janeiro, Brazil*
[d] *Laboratory of Comparative Virology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil*
[e] *INSERM U817, IMPRT, University of Lille 2, 1 Place de Verdum, 59045 Lille Cedex, France*
[f] *Laboratory Accreditation Division, National Institute of Metrology, Standardization and Industrial Quality (INMETRO), Rio de Janeiro, Brazil*
[g] *Laboratory of Macromolecules, Bio-Manguinhos, Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil*

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Abstract

Considering the background of morbidity and mortality caused by human rotavirus, detection methods that use rotavirus group antigen (VP6) in either enzyme immunoassay (EIA) or latex agglutination test (LAT) has been employed routinely in clinical diagnostic and epidemiological studies. In order to develop a rapid and sensitive rotavirus group A LAT, part of segment 6 corresponding to conserved N-terminal portion of the VP6 (1–245 aa) was cloned in *Escherichia coli* expression pGEX vector (glutathione S-transferase-GST gene fusion system) that has been modified previously containing a histidine tail at C-terminus. The immunological propriety of the recombinant VP6 having a total molecular weight of 52 kDa was evaluated by Western blot and by the ability of inducing anti-recombinant VP6 polyclonal antibodies in rabbit. The polyclonal serum produced was conjugated to a latex support to detect rotavirus in stool specimens. The percentage values for sensitivity and specificity of the rotavirus group A LAT were 98.5% and 100%, respectively.

Keywords: Gastroenteritis; Group A rotavirus; Recombinant protein; VP6; Latex

1. Introduction

Rotaviruses comprise a genus within the family *Reoviridae* and are responsible for a significant number of gastrointestinal diseases affecting primarily children under 5 years of age. The World Health Organization (WHO) estimates 611,000 of rotavirus-related deaths and according to Parashar et al. (2006), from 2000 to 2004 human group A rotaviruses were responsible for approximately 39% of childhood diarrhea hospitalizations. Rotavirus infection burden underscores a need for interventions such as vaccines. The development of group A rotavirus vaccines that started in the mid-1970s has followed a long and difficult path towards a safe vaccine, to prompt incorporation into the immunization programs of countries, especially those in the poorest areas of the world where these vaccines are most needed. At present, there are two rotavirus vaccines licensed and are now used widely in several countries. The pentavalent human-bovine (WC3) reassortant (G1, G2, G3, G4 and P1A[8]) live-attenuated, oral vaccine (RotaTeq™) developed by Merck and the attenuated human rotavirus (G1 P1A[8]) developed by Avant Immunotherapeutics and modified further and licensed to

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* Corresponding author at: Bio-Manguinhos, Instituto de Tecnologia em Imunobiologicos, Av. Brazil, 4365-Manguinhos, 21045-900 Rio de Janeiro, Brazil. Tel.: +55 21 3882 9471; fax: +55 21 2260 4727.
E-mail address: baroni@bio.fiocruz.br (M.T.B. de Moraes).

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GSK Biologicals, resulting in the vaccine RIX4414 (Rotarix®) (Dennehy, 2007; Angel et al., 2007; Buttery and Kirkwood, 2007). A realistic goal for a rotavirus vaccine is to duplicate the degree of protection against the disease that follows natural infection. The development and introduction of rotavirus vaccines for children in the resource poor countries of the world has been given high priority by WHO.

The rotavirion is a non-enveloped particle and possesses a triple layered capsid structure that surrounds the genome composed of 11 segments of double-stranded RNA (dsRNA). There are six structural proteins and six nonstructural proteins, each protein encoded by a unique genome segment except for two nonstructural proteins (NSP5 and NSP6), which are encoded by an overlapping reading frame of a single segment. The antigenic properties of group, subgroup and serotype/genotype of rotaviruses are determined by the viral capsid proteins (VP), named VP6, VP4 and VP7. Rotaviruses have seven major groups (A–G) classified according to the VP6 antigenicity. Most human strains belong to group A, although groups B and C have been associated with human illness.

VP6 participate in the architecture of the virus and is the most abundant intermediate-layer capsid viral protein. It forms spontaneously trimers and is extremely stable. These characteristics and the presence of conserved epitopes among many virus strains explain why VP6 is the major antigen targeted in diagnostic assays. Immunodominant sites that contain group-specific epitopes for the group A rotaviruses have been localized in four regions on VP6 (amino acid residues-aa: 32–64, 155–167, 208–294, and 380–397 (Estes, 2001)). Furthermore, VP6 classify rotavirus into subgroups (SG) I, II, I + II, non-I and non-II, which have been defined according to the presence or absence of two distinct epitopes reactive with one, both, or neither of the monoclonal antibodies (MAbs) 255/60 and 631/9 (Greenberg et al., 1983). The SG specificities are located at aa positions 305 (region between positions 296 and 299)-SG I and position 315-SG II (Tang et al., 1997; Lopes et al., 1994).

The VP6 from group A rotaviruses is 397 aa long corresponding to a molecular mass of 41 kDa. Two domains, termed the base and the head, form the hydrophobic, non-glycosylated and highly antigenic and immunogenic VP6. While the base is rich in α-helices, the head domain mainly contains β-sheets. A zinc ion is located on the threefold axis of the trimer and is coordinated to histidine residue 153 of each of the VP6 monomers (Erk et al., 2003).

Different methods have been used for the group A rotavirus detection in stool specimens. Initially, direct visualization of stool material by electron microscopy (EM) was employed for rotavirus detection. However, an electron microscope has not been available in most of the laboratories. Various automated methods such as enzyme immunoassay (EIA), passive haemagglutination (PHA), latex agglutination test (LAT) and lateral flow test (LFT), are available and have been used as confirmatory method. In addition reverse transcriptase–polymerase chain reaction RT-PCR as a molecular method has also been employed (Dewar et al., 2005; Roman and Martinez, 2005).

In this paper we reported the construction of the pGEX2T-HisVP6 that expresses in *Escherichia coli* (*E. coli*) system the N-terminal portion of the VP6 (1–245 aa). Also an easy purification method of this recombinant VP6 with an acceptable purification degree to produce polyclonal antiserum has been developed. The anti-VP6 policlonal antibodies produced was suitable for detection of rotavirus group A in LAT using stool specimens.

2. Materials and methods

2.1. Chemicals

All reagents used were analytical-reagent grade and purchased from Sigma–Aldrich, USA.

2.2. pGEX2T-HisVP6 expression vector

Total RNA was extracted using RNAse mini kit (Qiagen, USA) from human rotavirus Wa strain that had been cultured previously in monkey kidney cells (MA-104). This RNA was used as template in the RT-PCR of part segment 6 using Superscript III one-step RT-PCR kit (Invitrogen, USA). The primers used were forward (position 17-42, 5′-CTTCgCCATggAggTTCTgTACTCAC-3′) and reverse (position 730-757, 5′-gTCgCgCCATCggCCgAATTAATTACTC-3′). The segment was cloned into a pOM vector (EUROSCARF, Germany) and subcloned into pGEX-NX-2T expression vector (David-Cordonnier et al., 1998) using standard methods (Sambrook and Russel, 2001). For subcloning primers forward, 5′-GCG GCC GCC ACC ATG GCG AAA CGC GCC AGA CCG TC and reverse 5′-AGG AAA AAA CGG CCG ATG TTT GCA GGG CTA GC, were used for amplification by the polymerase chain reaction (PCR). Bases underlined above, denote cleavage sites of restriction endonucleases *Nco*I and *Xma*III (*Eag*I), respectively, for direct cloning procedures. Positive bacterial clones were identified by plasmid DNA purification followed of restriction analysis. The amplified products and purified plasmids were evaluated by electrophoresis in 1% agarose gel in TAE buffer pH 8.0 (40 mM Tris–acetate, 1 mM ethylenediaminetetraacetic acid—EDTA) and visualized by ethidium bromide staining.

2.3. Nucleotide sequencing of pGEX2T-HisVP6 expression vector

Nucleotide sequencing was performed using dideoxy chain-termination method with dye-labeled terminators and T7 DNA polymerase (Applied Biosystems, USA), according to the manufacturer's instructions followed by analysis in an ABI Prism 3100 automated sequence apparatus (Applied Biosystems, USA). Primers annealing with pGEX2T nucleotide sequence, forward and reverse (GE Healthcare, Germany) corresponding to the nucleotide region flanking the restriction endonucleases sites *Nco*I and *Xma*III (*Eag*I) of pGEX2T-HisVP6 expression vector were used. In order to verify the integrity of part of the sequence 6 cloned, additional specific primers were used.