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Review

Rotaviruses

Ulrich Desselberger*

Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 0QQ, UK

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ABSTRACT

Recent advances of rotavirus (RV) basic and applied research are reviewed. They consist of determination of the RV particle structure and functions of structural proteins, classification into genotypes based on whole genome analyses, description of the RV genome and gene protein assignments, description of the viral replication cycle and of functions of RV-encoded non-structural proteins as well as cellular proteins and cellular organelles involved, the present status of RV genetics and reverse genetics, molecular determinants of pathogenesis and pathophysiology, the RV-specific humoral and cell-mediated immune responses, innate immune responses and correlates of protection, laboratory diagnosis, differential diagnosis and present status of treatment, the molecular epidemiology and mechanisms of evolution of RVs, the development and universal application of RV vaccines as well as issues arising from present universal RV vaccination programs and work on alternative vaccines. The review concludes by presenting problems requiring further exploration and perspectives of future basic and translational research.

Contents

* Tel.: +44 1223 763403; fax: +44 1223 336846.
E-mail address: ud207@medschl.cam.ac.uk

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1. Introduction

Rotaviruses (RVs) were recognized as a major cause of acute gastroenteritis (AGE) in infants and young children in 1973 (Bishop et al., 1973; Flewett et al., 1973). Before that year RVs had already been discovered in diarrheic mice (Adams and Kraft, 1963), monkeys (Malherbe and Harwin, 1963) and cattle (Mebus et al., 1969) and since then in the young of many mammalian species including bats (Estes and Greenberg, 2013; He et al., 2013) and also in birds (Kindler et al., 2013; Otto et al., 2012; Trojnar et al., 2009). During the last 40 years an enormous amount of basic research on RV structure, replication, pathogenesis and immune responses has accumulated. Due to modern diagnostic techniques the molecular epidemiology of RVs has been extensively explored. Recently, two live attenuated RV vaccines have been licensed in many countries and are increasingly being applied in universal vaccination programs. In the following I will attempt to give an up-to-date review of important recent achievements in research and prevention and to formulate remaining open questions and perspectives.

2. Structure

The fully infectious RV particle (=virion) consists of 3 protein layers and is also termed triple-layered particle (TLP). By electron microscopy, TLPs resemble wheels (*lat. rota*), and this appearance has led to the name of *Rotavirus* for the genus (Flewett et al., 1974). Based on cryo-electron microscopy and image reconstruction data (reviewed by Jayaram et al., 2004), the following structure of icosahedral symmetry has been recognized: the single layered particle (SLP = core shell) is formed by 120 molecules of the viral protein 2 (VP2), arranged as 60 dimers in a $T = 1$ symmetry (Fig. 1C). Five of the dimers form a decamer around the fivefold symmetry axis, and 12 decamers make up the core protein layer which is uniform except for small pores along the fivefold axis (McClain et al., 2010). A 'fivefold hub' (density) projecting into the core interior along the fivefold axis was first thought to be contributed by the N-terminus of VP2 (McClain et al., 2010), but was more recently recognized to result from the VP1 structure (Estrozi et al., 2013). Replication enzyme complexes, consisting of VP1 and VP3, are located at the inside of the core at the axis of fivefold symmetry (Fig. 1C and D) opposite the class I channels (McClain et al., 2010; Trask et al., 2012a, 2012b; Estrozi et al., 2013) and are in intense contact with one particular dedicated genomic dsRNA segment *via* VP1 (Periz et al., 2013). The core shell encloses the viral genome of 11 segments of dsRNA as well as the viral RNA dependent RNA polymerase (RdRp), VP1 and the capping enzyme, VP3.

The genomic RNA segments have been proposed to form conical cylinders around the replication complexes (Prasad et al., 1996; Jayaram et al., 2004) (Fig. 1D) but details of the dsRNA structure within the core are just beginning to be explored. Thus, in actively transcribing double-layered particles (DLPs, see below), a decreased order of the middle (VP6) layer was found to be accompanied by increased order of the core content (Kam et al., 2014). Similar partial order of dsRNA segments was observed in core particles of bluetongue virus (Gouet et al., 1999). The 11 RNA segments (Fig. 1A) have very short completely conserved 5′ and 3′ terminal nucleotide (nt) sequences, 5′-GGC ··· ACC-3′. The untranslated regions (UTR) of the RNA segments (+ sense) are small: 9–48 nt at the 5′ end, and 17–182 nt at the 3′ end. The 5′ and 3′ ends are of partial inverted complementarity and are subject to long range interactions (LRI) (Tortorici et al., 2006; Li et al., 2010). Those at the 3′ end function as RdRp recognition signals (Tortorici et al., 2003) and also interact with NSP3 (Chizhikov and Patton, 2000; Vende et al., 2000), involved in activation of translation (see below). The RdRp structure has been solved at a resolution of 2.9 Å (Lu et al., 2008) (Fig. 2): VP1 forms a cage-like structure disrupted by 4 tunnels (leading to the catalytic site in the center of the molecule) which are proposed to allow for: (1) the entry of free nucleoside triphosphates (NTPs), (2) the entry of template ssRNA, (3) the exit of the (+) ssRNA product, and (4) the exit of (−) ssRNA (transcription) or dsRNA (replication). Tunnel 3 is positioned toward the class I channel inside of VP2 (Settembre et al., 2011; Estrozi et al., 2013), permitting release of (+) RNAs into the cytoplasm; tunnel 4 directs the newly replicated dsRNA toward the core interior. Analysis of different VP1-polyribo-oligonucleotide complexes has resulted in the finding that the 5′-UGUG-3′ sequence at the 3′ end of the RNA is specifically recognized at the template entry channel (Lu et al., 2008). In many aspects the structure of the RV RdRp is similar to the corresponding enzyme of orthoreovirus (Tao et al., 2002).

The viral core is surrounded by 260 trimers of VP6, which form the middle layer and constitute double-layered particles (DLPs) (Fig. 1C and E). The VP6 structure has also been determined (Mathieu et al., 2001): VP6 trimers make contact with both the underlying core (VP2) (Charpilienne et al., 2002) as well as VP7 and VP4 trimers on the outside.

The DLPs in turn are covered by 260 trimers of VP7 and 60 spikes of VP4 trimers (=180 molecules) to form the TLPs (Fig. 1B and C). The virions contain 132 channels along the axes of fivefold (12 channels of class I), threefold and twofold symmetry (60 class II and 60 class III channels, respectively) (Jayaram et al., 2004). The three-dimensional (3D) structure of the RV virion has recently been determined by electron cryomicroscopy (cryoEM) and single-particle tomography at about 4.3 Å resolution (Settembre et al., 2011): there is an intense interaction of the VP4 trimer with both VP6 (into which the base of VP5* is half buried) and VP7 (Fig. 3). The structure and interactions of VP4 with other structural RV proteins permit the description of a mechanism of RV entry (see below). It has previously been shown that VP4 trimers can only be observed by cryo-EM in TLPs grown in the presence of trypsin (Crawford et al., 2001). Since the stoichiometry of VP4 in RVs grown in the presence and absence of trypsin is identical, it was concluded that VP4 spikes of RV grown in the absence of trypsin are icosahedrally disordered. By single particle cryo-EM and cryo-electron tomography (cryo-ET) it has recently been demonstrated for 2 RV strains that the VP4 spike structure of RV particles grown in the absence of trypsin is indistinguishable from that of particles grown in the presence of trypsin (Rodríguez et al., 2014), suggesting that the previous lack of observation of VP4 structures in trypsin-free grown RV particles was mainly due to the imposition of icosahedral structure requirements on the previous cryo-EM data and that proteolytic cleavage of VP4 mainly achieves conformational changes enabling viral entry into cells. As an aside, the science of structural biology is closely related to talents in visual arts as noted by Stephen and Baumeister (2008).

3. Classification

Rotaviruses constitute the genus *Rotavirus*, one of the 15 genera of *Reoviridae* family which is subdivided into the sub-families of