Virus Research 247 (2018) 40–46

Contents lists available at ScienceDirect

Virus Research

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

Multiple proteins differing between laboratory stocks of mammalian orthoreoviruses affect both virus sensitivity to interferon and induction of interferon production during infection

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ARTICLE INFO

Keywords:
Reovirus
Interferon
Reverse genetics

ABSTRACT

In the course of previous works, it was observed that the virus laboratory stock (T3D^S) differs in sequence from the virus encoded by the ten plasmids currently in use in many laboratories (T3D^K), and derived from a different original virus stock. Seven proteins are affected by these sequence differences. In the present study, replication of T3D^K was shown to be more sensitive to the antiviral effect of interferon. Infection by the T3D^K virus was also shown to induce the production of higher amount of β and α-interferons compared to T3D^S. Two proteins, the μ2 and λ2 proteins, were found to be responsible for increased sensitivity to interferon while both μ2 and λ1 are responsible for increased interferon secretion. Altogether this supports the idea that multiple reovirus proteins are involved in the control of induction of interferon and virus sensitivity to the interferon-induced response. While interrelated, interferon induction and sensitivity can be separated by defined gene combinations. While both μ2 and λ2 were previously suspected of a role in the control of the interferon response, other proteins are also likely involved, as first shown here for λ1. This also further stresses that due caution should be exerted when comparing different virus isolates with different genetic background.

1. Introduction

In the last few years, it has been observed that so-called wild-type reovirus can slightly vary in sequence from one laboratory stock to the other, even if they are all referred to as type 3 Dearing strain. This could result from random genetic drift or to different experimental conditions for virus growth. Isolation of single plaques, sometimes used as a routine procedure to avoid accumulation of non-infectious viral mutants upon virus propagation has also likely contributed to this situation. Variations between sequences of laboratory stocks are observed when comparing the few cases where complete sequences were reported from a given stock (Kobayashi et al., 2007; van den Wollenberg et al., 2012; Chakrabarty et al., 2015; Sandekian and Lemay, 2015a). Also, differences in phenotypic properties between virus stocks were observed in few cases (Yin et al., 2004; Coffey et al., 2006; Nygaard et al., 2013; Berard et al., 2015). These variations have led to the denomination of viral subtypes such as T3D^C, T3D^F, T3D^H, T3D^N, T3D^K, T3D^S and T3D^W (Sandekian and Lemay, 2015a).

Previous work has shown that the viral stock encoded by the plasmids used in reverse genetics (herein referred to as T3D-Kobayashi, T3D^K) do differ from the laboratory virus stock in 7 out of 11 proteins (Sandekian and Lemay, 2015a). In the course of this previous work the laboratory wild-type virus stock (herein referred to as T3D-Sandekian, T3D^S) was reconstructed by site-directed mutagenesis and reverse genetics, in order to allow comparisons with viral mutants derived from this laboratory stock. Although this aspect was not specifically mentioned in the previous publication, it was noticed that the reverse genetics virus (T3D^K) is significantly more sensitive to interferon than T3D^S.

A plethora of viruses is presently considered as possible oncolytic viruses for cancer treatment as recently reviewed by many authors (Ilkow et al., 2014; Miest and Cattaneo, 2014; Pikor et al., 2015; Turnbull et al., 2015). Among these, reovirus is one of the most advanced in clinical settings, being currently in phase III. It presents the advantage of exhibiting a natural tropism for cancer cells while being essentially nonpathogenic in adult humans (Kelly et al., 2009; Harrington et al., 2010; Black and Morris, 2012; Clements et al., 2014; Chakrabarty et al., 2015). The interferon response often plays a role in determining the ability of a virus to discriminate between cancer cells, frequently exhibiting a reduced interferon response, and normal cells (Randall and Goodbourn, 2008; Naik and Russell, 2009; Kaufman et al., 2015). However, examples abound where interferon can still contribute

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https://doi.org/10.1016/j.virusres.2018.01.009
Received 13 September 2017; Received in revised form 20 January 2018; Accepted 21 January 2018
Available online 31 January 2018
0168-1702/

to limiting oncolytic activity, as recently reviewed (Vaha-Koskela and Hinkkanen, 2014; Ebrahimi et al., 2017). The original model of reovirus oncolytic activity postulated that a decreased in the interferon-induced protein kinase PKR was responsible for the increased ability of Ras-transformed cells to allow reovirus replication resulting in cell lysis (Strong et al., 1998). Further work indicated that the defective interferon secretion of Ras-transformed cells favors cell-to-cell viral propagation in these cells compared to normal cells (Shmulevitz et al., 2010). It thus appears to be essential to gain a further understanding of the viral determinants that control induction of the interferon response and the sensitivity of different viral isolates to this response. This could possibly lead to better optimization of viral strains toward oncolytic activity, as many investigators believe to be possible, and as recently reviewed (Mohamed et al., 2015; Kemp et al., 2016). This is especially envisaged since the advent of plasmid-based reverse genetics to manipulate the viral genome (Lemay, 2011; van den Hengel et al., 2013; Stuart et al., 2017). In the present study, reverse genetics was thus used to introduce each of the $T3D^K$ gene in the $T3D^S$ genetic background, either separately or in different combinations, in order to determine which protein(s) is responsible for this difference in interferon response.

While no single protein of $T3D^K$ in the $T3D^S$ background was sufficient to reconstitute the full phenotype of sensitivity observed in $T3D^K$, a combination of μ2 and λ2 was shown to be both necessary and sufficient. Also, as somewhat expected from previous studies by others (Zurney et al., 2009; Irvin et al., 2012), the μ2 protein was shown to be partly responsible for higher levels of interferon induction upon $T3D^K$ infection compared to $T3D^S$. However, an unexpected finding is that λ1 is also responsible for the full level of induction observed. At least three virus proteins thus appear to be involved in the interferon response in the context of reovirus infection.

2. Materials and methods

2.1. Cell lines and viruses

L929 mouse fibroblasts were originally obtained from the American type culture collection (ATCC® CCL-1™). The baby hamster kidney cell line (BHK) stably expressing the T7 RNA polymerase (BSR-T7 cells) has been described (Buchholz et al., 1999) and was a generous gift from the laboratory of Dr. John Hiscott (Lady Davis Research Institute, Montréal, Canada). Both cell lines were grown in minimal Eagle medium (MEM) with 5% fetal bovine serum.

Wild-type laboratory stock of reovirus type 3 ($T3D^S$) was previously described (Sandekian and Lemay, 2015a,b) and was rescued by reverse genetics following introduction of the appropriate mutations in the plasmids encoding the wild-type virus from Dr. Terry Dermody's laboratory ($T3D^K$). Other viruses, harboring various combinations of genes from $T3D^K$ in the $T3D^S$ background were obtained by reverse genetics, as described below.

All virus stocks were routinely grown on L929 cells and virus titers determined by $TCID_{50}$, as described (Danis and Lemay, 1993). In addition, since some assays used in the course of the work rely on similar cell-killing and lysis ability of the different viruses, serial binary dilutions of each virus was used to infect L929 cells in single wells of a 96-well plates. Cells were incubated for 4–5 days before being fixed and remaining cells stained with methylene blue, as previously described (Sandekian et al., 2013; Sandekian and Lemay, 2015a).

2.2. Reovirus reverse genetics

The plasmids corresponding to the 10 genes of reovirus serotype 3 Dearing, $T3D^K$, under the transcriptional control of the T7 promoter were originally obtained from the laboratory of Dr. Terence Dermody (Vanderbilt University, Nashville, Tennessee) (Kobayashi et al., 2007). The recovery of the wild-type laboratory stock $T3D^S$ was achieved by first submitting each plasmid to site-directed mutagenesis for each of the gene segment differing between $T3D^S$ and $T3D^K$. Plasmids were then used to recover infectious virus by the improved reverse genetics approach using transfection in BHK cells expressing the T7 RNA polymerase (Kobayashi et al., 2010). Rescued viruses were propagated as described before (Brochu-Lafontaine and Lemay, 2012; Sandekian and Lemay, 2015a,b).

2.3. Determination of interferon sensitivity

Mouse type I β-interferon was obtained from PBL interferon source. Two different approaches were used to determine interferon sensitivity on mouse L929 cells. Decreased virus titer at near saturating concentration, 200 international units (IU)/ml, was measured by $TCID_{50}$, as previously used. Similarly, interferon sensitivity to varying dilutions of interferon on L929 cells was determined, also as before (Sandekian and Lemay, 2015a). Briefly, binary dilutions of interferon were prepared from 500 IU/ml in single wells of a 96-well plates seeded with L929 cells. Cells were infected with the different viruses at a MOI of 0.05, incubated for 4–5 days before being fixed and stained with methylene blue. Remaining cell-retained stain was solubilized and quantitated, essentially as described before (Sandekian et al., 2013), using a Bio-TEK microplate reader Elx800. Relative cell destruction was determined by comparison with mock-infected cells.

2.4. Determination of interferon induction

Enzyme-linked immunosorbent assay (ELISA) was performed on different dilutions of mock-infected or infected cell culture medium to determine the concentration of either β-interferon or all subtypes of α-interferon (Verikine mouse interferon beta and alpha ELISA kit, PBL Assay Science). Results from mock-infected cells were always below detection level. Values were obtained using the microplate reader (BioTEK Elx800).

3. Results

3.1. Generation and characterization of $T3D^S/T3D^K$ monoreassortants

The differences in sequence between the wild-type laboratory virus stock of reovirus serotype 3 Dearing $T3D^S$ (originally obtained from ATCC) and that of the serotype 3 Dearing recovered using the plasmid-based reverse genetics system, $T3D^K$, were previously reported (Sandekian and Lemay, 2015a); for the sake of simplicity this information is repeated herein (Table 1). A total of 24 nucleotide differences, 21 transitions and 3 transversions, were observed. These resulted in 12 amino acids changes in 7 different viral proteins.

In a first set of experiments, each of these seven $T3D^K$ genes were separately used to replace their homologous gene in the $T3D^S$ background. The seven monoreassortant viruses were recovered and propagated with similar final titers. In parallel, in order to avoid discrepancies due to possible differences in cell-killing ability at the same multiplicity of infection, cells were infected with serial binary dilutions of the different viruses, as described in Materials and methods (Section 2.1). All viruses had similar replication phenotype, as measured by this approach (data not shown).

The infectious titers of the different viruses were then compared in the absence or presence of interferon at 200 IU/ml (Fig 1). Each virus exhibited resistance to interferon treatment similar to that of $T3D^S$. Only viruses harboring the M1 gene (encoding the μ2 protein) and possibly the L2 gene (encoding the λ2 protein) were slightly more sensitive to interferon but far from the high sensitivity observed for $T3D^K$. The different viruses were also examined at various interferon concentrations, as described in Materials and methods (Section 2.3). Again, no single gene can reconstitute the full $T3D^K$ sensitivity phenotype and only M1 had an effect in this assay (data not shown).