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In silico study of potential autoimmune threats from rotavirus infection

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ABSTRACT

Rotavirus, the major cause of infantile nonbacterial diarrhea, was found to be associated with development of diabetes-associated auto-antibodies. In our study we tried to find out further potential autoimmune threats of this virus using bioinformatics approach.
We took rotaviral proteins to study similarity with *Homo sapiens* proteome and found most conserved structural protein VP6 matches at two regions with ryanodine receptor, an autoimmune target associated with myasthenia gravis. Myasthenia gravis, a chronic neurodegenerative autoimmune disorder with no typical known reason, is characterized by fluctuating muscle weakness which is typically enhanced during muscular effort. Affected patients generate auto antibodies against mainly acetyl choline receptor and sarcoplasmic reticulum calcium-release channel protein ryanodine receptor. Further, we observed that two regions which matched with ryanodine receptor remain conserved in all circulating rotaviral strains and showed significant antigenecity with respect to myasthenia gravis associated HLA haplotypes. Overall, our study detected rotaviral VP6 as a potential threat for myasthenia gravis and enlighten an area of virus associated autoimmune research.

1. Introduction

Autoimmune disease is a state of over reactive immune system. It is triggered due to the deterioration of immunologic tolerance to auto-reactive immune cells resulting in attack on self molecules. Myasthenia gravis, a chronic neuromuscular disorder, can lead to various degrees of neurologic dysfunction characterized by fluctuating, fatigable weakness of muscles under voluntary control (Thanvi and Lo, 2004). Although the basis of the disorder is not known, the involvement of immune responses in its pathogenesis is well established. Normally myasthenia gravis (MG) patients are found to produce antibodies against acetylcholine receptor (AChR), but some have antibodies against skeletal muscle antigens in addition (Meriggioli and Sanders, 2012). One major antigen for non-AchR antibodies in MG are the Ca^{2+} release channel of the sarcoplasmic reticulum, the ryanodine receptor (RyR) (Skeie et al., 2006). RyR antibodies are developed by mainly thymoma MG patients and a few late-onset MG patients and correlate with severe MG disease (Shelton et al., 2001). MG and other multiple human autoimmune diseases are also induced through various mechanisms such as molecular mimicry, epitope spreading, direct bystander activation, and release of cryptic epitopes (Olson et al., 2001).

Molecular mimicry is defined as sharing of similar linear amino acids stretch or conformational fit between two different protein molecules of two different origins. During microbial infection, if the organism shares cross-reactive epitopes with the host, then the immune response to the infecting agent will also recognize and attack host protein, which become worsen with second, third, or repeated infection(s) that potentiate the autoimmune assault (Oldstone, 2005).

Among the potential micro-organisms particularly viruses have been proposed as a cause of several autoimmune diseases in humans (von Herrath et al., 2003). For example, human T-lymphotropic virus type 1 (HTLV1) has been implicated in various autoimmune arthropathies (Nishioka et al., 1993); herpes simplex virus type 1 (HSV-1) are linked with keratitis (Zhao et al., 1998); hepatitis C virus has been proposed as a initiator of various rheumatoid diseases (Romero Portales et al., 1997; McMurray and Elbourne, 1997) and Epstein–Barr virus has been associated with systemic lupus erythematosus (Parks et al., 2005). Coxsackieviruses and rubella virus have been proposed as causative agents of Type 1 diabetes (Atkinson et al., 1994; Yoon et al., 1989) and many viruses have been proposed as the 'cause' of multiple sclerosis (Monteyne et al., 1998). Myasthenia gravis, our subject of study, also reported to have viral connection. Molecular mimicry of

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Abbreviations

MG	Myasthenia gravis
AChR	Acetylcholine receptor
RyR	Ryanodine receptor
IA2	Inusulinoma antigen 2
GAD 65	Glutamic acid decarboxylase
IEDB	Immune Epitope Database and Analysis Resource
MHC	Major histocompatibility complex
ANN	Artificial neural network
SMM	Stabilized matrix method

herpes simplex virus (HSV) type 1 glycoprotein (Gp) D (amino acid residues 286–293) with a chain of human (Hu) AChR (amino acid residues 160–167) was suggested as a probable cause of myasthenia gravis initiation (Schwimmbeck et al., 1989)

Rotavirus, a non-enveloped double stranded RNA virus belonging to the family reoviridae, is the major cause of severe gastroenteritis in children under age of 5 years (Gray et al., 2008; Kawai et al., 2012; Bhowmick et al., 2012) and causes two million hospitalizations and four hundred fifty thousand deaths per year worldwide (Kawai et al., 2012; Bhowmick et al., 2012). Although recent reports confirmed rotavirus infection may accelerate the progression of genetically-predisposed children to type 1 diabetes (Pane et al., 2014) but direct involvement of rotavirus with autoimmune diseases such as type 1 diabetes and celiac disease is contradictory (Honeyman et al., 2000; Blomqvist et al., 2002; Stene et al., 2006). In our study we have explored potential autoimmune threats to humans from rotavirus by analyzing presence of molecular mimicry in viral proteins with host proteome. In previous reports rotaviral structural protein VP7 was found to have molecular mimicry with IA2 and GAD 65, involved with type 1 diabetes. This study initiated with search of sequence similarity of viral proteins with human proteome and found no significant matches with host proteins other than in VP6 protein sequence. VP6 is a structural protein and form the second outer layer of the virus. It was found to possess two immunogenetically active epitope regions which share high similarity with ryanodine receptor 2, a target in myasthenia gravis. Other than relatively non-variability of VP6 protein sequences it is the most immunogenic among all the structural protein which substantiates the potentiality of our finding (Tang et al., 1997; Esteban et al., 2013).

2. Methods

2.1. Sequence acquisition

We have accessed NCBI protein data bank and retrieved sequences of rotavirus A VP6 and ryanodine receptor 2 of *H. sapiens*. We downloaded 16 complete VP6 protein sequences of four most circulating rotavirus subtypes G1P8, G2P4, G3P8, and G4P8 isolated from different countries. There are only 3 protein sequences of ryanodine receptor 2 of *Homo sapiens* that are present in NCBI data bank and we downloaded them for analysis (accessed on 18.07.2013).

2.2. Protein blast

We used Blastp server to determine similarities of rotavirus VP6 protein in human proteome. Only matches with most high score (ryanodine receptor) were taken for further analysis.

2.3. Sequence alignment

To determine conserved regions within a set of protein sequences and to determine matching regions between different families of protein sequences, CLUSTAL-X (http://www.clustal.org/) (Larkin et al., 2007 Wang et al., 2013) and CLUSTAL-OMEGA (http://www.clustal.org/) (Sievers et al., 2011) software available on the web were used.

2.4. 3D structure visualization

To visualize the protein structural residues we used the crystallographic structure from PDB database and the molecular visualization tool PyMOL (www.pymol.org) (Steinkellner et al., 2009).

2.5. cell epitope prediction

To determine the antigenecity of our identified region of VP6 we used two epitope-prediction tools, viz., the IEDB (http://www.iedb.org/) (Immune Epitope Database and Analysis Resource) (Vita et al., 2010; Shehzadi et al., 2012; Kim et al., 2012) and ABCpred (http://www.imtech.res.in/raghava/abcpred/) servers (Saha and Raghava, 2006; Sun et al., 2012; Maksimov et al., 2012), which have given good results in several applications.

2.6. cell epitope prediction

The IEDB server was also used for predicting T-cell epitope and MHC (major histocompatibility complex) binding predictions (Vita et al., 2010; Shehzadi et al., 2012; Kim et al., 2012). These tools predict IC50 values for peptides binding to specific MHC molecules of specific alleles. A lower number indicates higher affinity. Most known epitopes have high or intermediate affinity. MG has specific HLA haplotypes, HLA-DRB1*0301 and HLA-B*8:01. For detecting peptides binding with HLA-DRB1*03:01 allele NN-align (netMHCII-2.2), SMM-align (netMHCII-1.1) method and for detecting peptides binding with HLA-B*08:01 allel artificial neural network (ANN), stabilized matrix method (SMM) was used.

3. Results

3.1. VP6 protein shows significant similarity with human ryanodine receptor 2

To find out any potential match of rotaviral protein VP6 in human proteome, we downloaded VP6 protein sequence of rotavirus A (accession: ACL93331) and using NCBI blastp suite (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) we found that it has significant similarity with ryanodine receptor protein (data not shown). Then to further confirm the similarity between VP6 and ryanodine receptor we downloaded ryanodine receptor protein sequences from gene data bank (accession: NP_001026) and aligned it with VP6 protein sequence using CLUSTAL-OMEGA (http://www.genome.jp/tools/clustalomega/). Result revealed similarity at different regions throughout the ryanodine receptor sequence. Among them 2 regions (region A: 261–268, region B: 374–381) showed similarity along with a stretch of 8 amino acid (Fig. 1A). Region A showed 75% similarity and 62.5% identity with 800–807 amino acid region and region B showed 87.5% similarity and 62.5% identity with 904–911 amino acid region of ryanodine receptor (Fig. 1A). These two specific regions of VP6 were represented in 3D space filling model where the two regions were colored (red: region A, yellow: region B) (Fig. 2).