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Minireview

An insight into the PB1F2 protein and its multifunctional role in enhancing the pathogenicity of the influenza A viruses

Alok K. Chakrabarti *, Gunisha Pasricha

Microbial Containment Complex, National Institute of Virology, Sus Road, Pashan, Pune-411021, India

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ABSTRACT

PB1F2 is the 11th protein of the influenza A virus. The protein has variable sizes with truncations either at the C- or N-terminal ends. The most recent example being the 2009 pandemic H1N1 virus which codes for only 11 amino-acids of the C-terminus. A review of the reports since the discovery of PB1F2 in 2001 suggests a multifunctional role for this protein that includes a proapoptotic function in immune cells and an ability to cause increased pathogenesis in animal models by dysregulating cytokines and inducing inflammation. It has also been suggested that PB1F2 regulates polymerase activity via co-localization with PB1 and causes enhanced secondary bacterial pneumonia. This review primarily focuses on understanding the proapoptotic ability of PB1F2, its sub-cellular localization and the mechanism through which it brings about apoptosis. We believe there is much more to learn about PB1F2, as many of its proposed functions are strain, host or cell-line specific.

Introduction

Influenza is an important and pathogenic viral disease infecting warm-blooded animals. This virus continuously evolves, thus causing the emergence of newer epidemics and pandemics in human swine, horses, dogs, cats, and other mammals. Aquatic birds are the natural reservoir for all the subtypes of influenza A virus and are most likely the definitive source of human pandemic influenza strains (Webster et al., 1992). Since 1500, there have been 14 or more influenza pandemics. In the past 133 years, during the so-called microbial era from 1876 to the present, there have been six pandemics. Those pandemics include the flu pandemics of 1889, 1918, 1957, 1968, and 1977, and the recent 2009 pandemic (Taubenberger et al., 2010). Compared to the pandemic influenza A strains, H5N1 viruses are more pathogenic and cause higher mortality, although they are not transmitted from human to human. Widespread and ongoing epizootic outbreaks of H5N1 viruses in Asia have increased the concern that this subtype may achieve human-to-human transmission and establish interspecies spread. Many transmission events involving H5N1 viruses to humans have been reported since 1997 (Lipatov et al., 2004). From 2003 until June 2012, six hundred and six cases of H5N1 human infection have been reported, of which 357 were fatal. To date, H5N1 has been reported in 15 countries. According to data available from up to June 2012, most cases of human infections with H5N1 virus have been reported in Indonesia and Egypt (WHO data, 2012).

Continuing evolution is most prominent in the surface glycoproteins of influenza viruses, but it also occurs in each of the eight gene segments of the virus. Among the eight segments, PB1 is of particular interest, as the PB1 gene is the only other segment that was exchanged in the pandemic viruses of 1957 and 1968 (Kawaoka et al., 1989). In addition, a novel PB1 gene was found in the 1998 swine reassortant viruses, implicating the gene's role in the pathogenesis of influenza (Karasin et al., 2000). Recent reconstruction of the 1918 virus has also confirmed that the viral polymerase is required for the pathogenicity of the recombinant 1918 virus in mice, and if it is replaced with the recent H1N1 polymerase genomic segment, the virus is attenuated in mice (Tumpey et al., 2005). Selection of the PB1 gene in the previous pandemic strains and its ability to enhance the pathogenicity and virulence of the influenza A viruses warrant further study of PB1 (Kawaoka et al., 1989; Zamarin et al., 2006; Chen et al., 2001). PB1 is encoded by segment 2 of the influenza viral genome and is a core component of the viral polymerase. The single mRNA transcribed from segment 2 encodes three proteins, PB1, PB1F2 and N40. PB1F2 is the second protein encoded by the +1 alternate open reading frame within the PB1 gene. The translation of the protein starts from the 4th initiation codon that is from nucleotide position 120 surpassing three other initiation codons of the PB1 gene (Chen et al., 2001). The fifth initiation codon of segment 2 is used to initiate translation of a third protein product

* Corresponding author.
E-mail address: aloke8@yahoo.com (A.K. Chakrabarti).

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of the PB1 gene, called PB1-N40. AUG5 of N40 is in frame with AUG1 of the PB1, and therefore, N40 is just a truncated form of PB1 that lacks the first 39 amino acids of the longer protein. N40 lacks the N-terminal region that is essential for the interaction of PB1 with PA (Wise et al., 2009).There is much to learn about the three translation products of PB1 mRNA, and it is believed that the three proteins are dependent on each other for their functions. Given the last outbreak scenario, wherein the emergence of the 2009 H1N1 influenza virus took public-health authorities by surprise, it is essential to gather in-depth knowledge about each of the viral proteins of the influenza A virus. There is a dearth of knowledge about and treatments for influenza. Thus, researchers need to develop effective vaccines and therapies, in addition to predicting newly emerging pandemic influenza viruses.

PB1F2 is a non-structural protein of the influenza A virus that was identified and characterized more than a decade ago by Chen et al. (2001). It was discovered in the A/PuertoRico/8/34 (H1N1) strain while screening for a antigenic peptides recognized by $CD8^+$ T lymphocytes (Chen et al., 2001). The PB1 gene has inefficient initiation of translation, as it does not possess a consensus Kozak sequence in that it does not have a purine nucleotide in the −3 position of the ORF. The fourth initiation codon is surrounded by an exact Kozak sequence and allows for the synthesis of PB1F2 (Košík et al., 2011). PB1F2 is absent in influenza B viruses. PB1F2 is exclusively expressed in infected cells with maximum expression at 5 h post-infection and is possibly not incorporated into the virion (Krumbholz et al., 2011). Many of the influenza A virus strains encode a full-length PB1F2 protein with 90 or 87 amino acids and a molecular weight of 10.5 kDa.

Structure of PB1F2

Chen et al. described that the novel PB1F2 has a propensity to form an amphipathic helix extending from the 69th amino acid (Leucine) to the 83rd amino acid (Phenyalanine) of the protein (Chen et al., 2001). Detailed study of the protein revealed that PB1F2 consists of two independent structural domains consisting of two close short helices at the N-terminus and an extended helix at the C-terminus. Both helical domains are connected by a flexible and unstructured hinge region (Bruns et al., 2007). The PB1F2 molecule has an intrinsic strong propensity to form oligomeric structures, a characteristic that supports the recent observation that the molecule can form membrane pores in planar lipid bilayers (Bruns et al., 2007; Henklein et al., 2005). The major oligomerization domain is located in the C-terminal helix (Bruns et al., 2007). In another study, PB1F2 was determined to belong to a group of intrinsically disordered proteins that can switch their conformation from a random to α-helical or β-sheet secondary structure depending on the environment. PB1F2 has also been reported to permeabilize cellular membranes; however, this ability is dependent on the amino acid sequence of the influenza A virus strain. The PB1F2 protein has also been shown to oligomerize and form amyloid fibers in infected cells. Amyloid fiber formation in the infected cells could give insight into the pathogenicity of the virus and also into the relationship of the influenza virus with nervous system disorders (Chevalier et al., 2010). PB1F2 has also been reported to be a phosphoprotein, wherein its function is regulated by protein kinase C (PKC). PKC phosphorylation sites have been mapped to amino acid positions 27 and 35 of the PB1F2 protein (Mitzner et al., 2009).

Varying length of PB1F2 protein

Varying sizes of the PB1F2 protein have been reported in different subtypes of influenza A viruses. A comprehensive analysis of the varying lengths of the PB1F2 protein from 20th-century pandemics and H5N1 subtypes was previously conducted by our group (Pasricha et al., 2012). The 1918 H1N1 virus responsible for the Spanish flu harbored a complete PB1F2 protein (McAuley et al., 2007). However, since 1949, most H1N1 virus strains have an incomplete PB1F2 protein with truncation either at the N- or C-terminal end (Zell et al., 2007; Pasricha et al., 2012). The N-terminal end of the protein is preserved in human hosts, while the C-terminal end is retained in swine (Table 1). Interestingly, the recent 2009 pandemic H1N1 virus harbors only an 11 amino acid C-terminal-truncated protein, which is thought to be non-functional. This indicates that this protein is not essential for the fitness of the H1N1 strain. The H2N2 subtype responsible for the 1957 pandemic (Asian flu) infected close to 250,000 people. Analysis of the 83 available PB1F2 sequences belonging to the H2N2 subtype revealed that 98.8% of the strains harbored a full-length PB1F2 protein (Table 1) and more than half of its amino acids were conserved in the strains. The circulating strains of H2N2 were replaced by H3N2 strains in 1968, which emerged as new pandemic strain (Hong Kong flu). The majority of the strains (94.27%) isolated during this pandemic also harbored a full-length PB1F2 protein (Table 1). An analysis of the length of PB1F2 protein in H5N1 strains indicates that it is comparable to that of the H2N2 and H3N2 subtypes, wherein a complete protein is found in 96% of the strains, suggesting that PB1F2 is positively selected in these subtypes and is definitely essential for the virus.

Cellular and humoral response to the PB1F2 protein

The PB1F2 protein is recognized by the human immune system and therefore has the ability to elicit both humoral and cell-mediated immune responses. The discovery of PB1F2 was based on its ability to generate a robust CD8+T cell response specific for a well-defined peptide encoded by residues 62-70 (LSLRNPILV) of the protein (Chen et al., 2001; La Gruta et al., 2008). PB1F2-specific antibodies have been detected in the sera of

Table 1
PB1F2 variants present in IAV strains H1N1, H2N2, H3N2 and H5N1 from various hosts.

S. no.	Strains/host	No. of analyzed strains	101aa	90aa	87aa	N57aa[a]	C52aa[b]	Varied size
1	**H1N1**	**1530**	**–**	**176**	**42**	**1080**	**221**	**11**
	Human	1155	–	32	41	1073	6	3
	Swine	261	–	43	1	7	202	8
	Avian	112	–	99	–	–	13	–
	Environment	1	–	1	–	–	–	–
	Others	1	–	1	–	–	–	–
2	**H2N2**	**83**	**1**	**81**	**–**	**1**	–	–
	Human	54	1	52	–	1	–	–
	Swine	–	–	–	–	–	–	–
	Avian	22	–	22	–	–	–	–
	Environment	1	–	1	–	–	–	–
	Others	6	–	6	–	–	–	–
3	**H3N2**	**2566**	**26**	**2105**	**45**	**–**	**337**	**53**
	Human	2419	25	1979	44	–	324	47
	Swine	76	–	63	1	–	7	5
	Avian	68	1	60	–	–	6	1
	Environment	2	–	2	–	–	–	–
	Others	1	–	1	–	–	–	–
4	**H5N1**	**919**	**–**	**886**	**2**	**4**	**21**	**6**
	Human	220	–	211	–	2	4	3
	Swine	13	–	13	–	–	–	–
	Avian	666	–	643	2	1	17	3
	Environment	16	–	15	–	1	–	–
	Others	4	–	4	–	–	–	–

[a] Fifty-seven amino-acid fragment with C-terminal end truncated.
[b] Fifty-two amino-acid fragment with N-terminal truncation.