PB1-F2 GENE IN INFLUENZA A VIRUSES OF DIFFERENT HEMAGGLUTININ SUBTYPE

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Summary. – The second ORF frame (+1) of PB1 polymerase gene of Influenza A virus (IAV) encodes the PB1-F2 protein. The length of PB1-F2 encoded by the A/Puerto Rico/8/34 (H1N1) (PR8) virus is 87 aa. The analysis of nucleotide sequences of PB1 gene of 626 IAV isolates available in GenBank and Influenza Sequence Database revealed that this gene has mostly the capacity to encode a putative protein of 90 aa. The predicted extra three amino acids in the 90-aa PB1-F2 are to a great extent conservative. Some IAV isolates, particularly human, avian and swine with hemagglutinin (HA) of H1 subtype can potentially encode a C-terminally truncated PB1-F2 of various lengths. The C-terminally truncated PB1-F2 in H1 isolates is lacking the region responsible for mitochondrial targeting and apoptosis. About 50% of avian isolates of H9 subtype possess an ORF for truncated PB1-F2. Eighteen aa, 10 at the N-terminus and 8 at the C terminus are strictly conservative in all 148 human isolates.

Key words: Influenza A virus; PB1-F2 protein; hemaglutinin subtype; truncation

Introduction

During a systematic search for peptides recognized by $CD8^+$T lymphocytes encoded by alternative ORFs in positive-sense ssRNA of PR8 influenza virus a new protein of 87 aa was discovered (Chen *et al*., 2001). As this protein is encoded by the second ORF (+1) of PB1 polymerase gene, it has been called PB1-F2. The novel protein is encoded by nt 120–381 in the PB1 genomic segment. PB1-F2 ORF is present in most IAV isolates.

Several unusual features have been described for PR8 PB1-F2, notably significant mitochondrial localization (Chen *et al*., 2001). Interestingly, PB1 itself is not transported to mitochondria. As the mitochondrial apoptotic pathways apparently plays an important role in the pathogenesis of influenza viruses, it is anticipated that PB1-F2 is involved in this process. Several experiments suggest that it affects preferentially apoptosis of immune cells responding to influenza virus infection. Recent studies using a mouse model have also indicated the role of PB1-F2 in the pathogenicity and lethality of viruses (Zamarin *et al*., 2006).

In IAV infected cells, PB1-F2 that localizes to mitochondria is also present in the nucleus, nuclear membrane and cytoplasm of a significant fraction of cells. The localization of PB1-F2 to mitochondria is its intrinsic property, whereas its nuclear/cytoplasmic localization requires expression of PB1-F2 in the context of IAV infection. A region near the C-terminus of PB1-F2 was identified (aa 46–75) that is necessary and sufficient for its mitochondrial membrane localization (Gibbs *et al*., 2003; Yamada *et al*., 2004). This mitochondrial targeting sequence (MTS) is predicted to form a positively charged amphipathic helix. The C-terminal domain of PB1-F2 is responsible for its interaction with adenine nucleotide translocator (ANT3) in inner mitochondrial membrane, while both C- and N-terminal domains interact with the voltage-dependent anion channel 1 (VDAC1) in outer mitochondrial membrane.

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Abbreviations: ANT3 = adenine nucleotide translocator; PR8 = A/Puerto Rico/8/34 (H1N1); HA = hemagglutinin; IAV = Influenza A virus; MTS = mitochondrial targeting sequence; VDAC1 = voltage-dependent anion channel 1

The N-terminal portion of PB1-F2 might determine the cell type specificity of the proapoptotic function of the C-terminal MTS (Zamarin *et al*., 2005).

Materials and Methods

Sequence analysis and multiple alignment of PB1-F2 sequences accessible in GenBank and Influenza Sequence Database were made using the BLAST Program Version 2.0 that allowed gaps in aligning sequences (Altschul *et al*., 1997).

Results and Discussion

Here we analyzed the nucleotide and deduced amino acid sequences of the PB1-F2 gene of 626 IAV isolates (148 human, 403 avian and 75 swine) (Table 1). The sequences from human IAV isolates included the subtypes H1 (48), H2 (24), H3 (51), H5 (22), and H9 (3). The length of PB1-F2 protein encoded by PR8 PB1 gene is 87 aa (corresponding to nt 120–381). The analysis of nucleotide sequences of different IAV strains revealed that the PB1-F2 gene has mostly the capacity to encode a 90-aa protein that is longer by 3 amino acids than the PB1-F2 of PR8 at the C-terminus. The predicted extra three amino acids in the 90-aa PB1-F2 protein are to a certain extent conservative. Human IAV isolates of H1 subtype frequently contain an ORF for C-terminally truncated PB1-F2 protein. Interestingly, there is a growing number of C-terminally truncated ORFs for PB1-F2 protein in more recent isolates. The human H1 isolates from 1918–1988 have an ORF for full-length PB1-F2 protein. The later human H1 isolates from 1988–1998 have an ORF either for full-length or for C-terminally truncated PB1-F2 protein. All human H1 isolates obtained after 1998 have an ORF for truncated PB1-F2 protein only. A truncated ORF for PB1-F2 protein in human H1 isolates might encode a 57-aa protein. We found only one exception (A/Wisconsin/3523/88), an ORF encoding a PB1-F2 protein of 11 aa. Accordingly, the C-terminally truncated PB1-F2 protein in H1 isolates is lacking the region responsible for mitochondrial targeting and apoptosis. Interestingly, the H1N1 viruses are also known to cause disease less frequently than H3N2 viruses.

Table 1. Incidence of IAV isolates of human, avian and swine origin with truncated PB1-F2

Host	Subtype	No. of analyzed isolates	No. (%) of isolates with truncated PB1-F2
Human	H1	48	32 (67)
	H2	24	0
	H3	51	0
	H5	22	0
	H9	3	0
	Total	148	32 (22)
Avian	H1	3	1 (33)
	H2	12	0
	H3	37	2 (5)
	H4	19	0
	H5	156	1 (1)
	H6	29	0
	H7	18	0
	H8	1	0
	H9	101	48 (48)
	H10	11	0
	H11	5	0
	H12	5	0
	H13	1	0
	H14	1	0
	H15	3	0
	H16	1	0
	Total	403	52 (13)
Swine	H1	34	16 (47)
	H2	34	1 (3)
	H3	1	0
	H5	2	0
	H9	4	0
	Total	75	17 (23)
Total		626	101 (16)

Chen *et al*. (2004) have also analyzed the PB1 gene of 42 IAV isolates from Taiwan, including 24 H1N1 and 18 H3N2 isolates. Most of the H1N1 isolates contained a shorter putative 57-aa PB1-F2 protein, encountering a premature stop codon at a position ranging from 290 to 292. However, all of the Taiwanese H3N2 isolates from 1995–2001 contained a full-length ORF, except for A/Taiwan/1748/97, which might encode a C-terminally truncated PB1-F2 of 79-aa. Zamarin *et al.* (2005) analyzing sequences of human PB1 genes also found that the full-length PB1-F2 protein was encoded by H3N2 viruses, whereas the human and swine H1N1 isolates encoded only a C-terminally truncated PB1-F2 protein lacking the region responsible for mitochondrial targeting. The two human IAV isolates that might encode a full-length PB1-F2 protein (A/Kiev/59/79 and A/Wisconsin/10/98) apparently represent natural reassortants between H3N2 and H1N1 viruses with the PB1 gene from H3N2 virus. Similarly to human isolates, swine H1 isolates frequently posses an ORF for C-terminally truncated PB1-F2. Interestingly, the swine isolates from 1931 up to 2000 have an ORF for C-terminally truncated PB1-F2. In these isolates, an ORF for a PB1-F2 protein of 11 aa is prevalent, however, also ORFs for 25-, 34-, 57- and 63-aa PB1-F2 protein are rarely found. Unexpectedly, swine H1 isolates obtained after 2000 have an ORF for full-length PB1-F2 protein, suggesting a change in PB1 gene by reassortment.

In avian H1 isolates, an ORF for PB1-F2 protein of 79 aa is prevalent, nevertheless, rarely they contain ORFs for 11-, 43- and 57-aa PB1-F2 protein. About 50% of avian H9 isolates possess an ORF for truncated PB1-F2 protein. There is no change in the proportion of full-length to C-terminally truncated PB1-F2 during whole period of isolation of avian H9 viruses. As the number of human and swine H9 isolates analyzed here is very low, no conclusion can be drawn about the frequency of truncated ORFs for PB1-F2 protein in these isolates. Almost all the avian isolates of other than H1 or H9 subtype contain an ORF for full-length PB1-F2 protein. There are only very few exceptions from this conclusion.

As PB1-F2 domain of aa 46–75 is both necessary and sufficient for mitochondrial targeting, the C-terminally truncated PB1-F2 protein of 79 aa should be transferred into inner mitochondrial membrane where it can interact with ANT3. Nevertheless, as the interaction of PB1-F2 with DAC1 in outer mitochondrial membrane requires both N- and C-terminal domains of PB1-F2 protein, the truncated PB1-F2 protein of 79 aa would be unable of full interaction with mitochondria. Although many IAV isolates might encode a putative C-terminally truncated PB1-F2 protein, no such a protein has been identified yet in IAV-infected cells. In contrast, a N-terminally truncated PB1-F2 protein has been identified in IAV-infected cells. Zamarin *et al.* (2006) have shown that, in addition to full length protein, the C-terminal region of the PB1-F2 protein can be also simulatenously expressed from the downstream second initiation codon (nt 233–235) to produce a N-terminally truncated protein. Analyzing the sequences of PB1-F2 gene of 626 IAV isolates we found this position to a great extent conservative. It is not clear whether any biological activity of PB1-F2 protein depends on expression of both protein forms and their interaction (Zamarin *et al*., 2006).

Phylogenetic trees based on the PB1-F2 gene showed clustering of isolates according to HA subtype (data not shown). This fact is apparently the result of co-evolution. Although the PB1 polymerase gene is highly conservative, the transcript encoding the PB1-F2 protein is surprisingly under the highest positive-selection pressure for non-synonymous substitutions. The PB1-F2 protein is the third most variable protein besides HA and NA in avian strains and the sixth most variable one in non-avian strains (Obenauer *et al*., 2006).

Fig. 1

Distribution of conservative amino acids in PB1-F2 protein in human IAV isolates

Table 2. Incidence of conservative amino acids in PB1-F2 protein of human IAV isolates

Subtype	No. of analyzed isolates	No. of conservative amino acids (% of total 90 amino acids)
H1	48	31 (35)
H2	24	63 (70)
H3	51	53 (59)
H5	22	62 (69)
H9	3	88 (98)
Total	148	18 (20)

In analyzing the conservativeness of putative PB1-F2 proteins in human IAV isolates we found that the number of conservative amino acids in PB1-F2 fell down from 98 for H9 isolates to 31 for H1 isolates (Table 2). Eighteen amino acids at the positions 1, 5, 7, 8, 9 12, 13, 15, 19, 20, 24, 61, 64, 72, 77, 85, 88, and 89 were conservative in all 148 analyzed isolates (Fig. 1). Ten of these conservative amino acids are localized close to the N-terminus of PB1-F2 protein, while the remaining 8 amino acids are close to the C-terminus. In IAV isolates from all hosts 5 amino acids are absolutely conservative, namely those at the positions 1, 9, 24, 61, and 89. The second AUG at the position 233 of the PB1 gene segment is not completely conservative.

It follows from the analysis described here that the PB1-F2 protein is not essential for virus survival. We believe that further studies must focus on other than the PR8-encoded PB1-F2 protein. In addition, it will also be important to find out whether C-terminally truncated proteins are really synthesized in IAV-infected cells and to explain the role of PB1-F2 protein in IAV infection.

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