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The Genome of Equine Herpesvirus Type 2 Harbors an Interleukin 10 (IL10)-Like Gene

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Abstract

A gene was identified within the DNA sequences of the *Eco*RI DNA fragment N (4.3 kbp) of the genome of equine herpesvirus type 2 (EHV-2) coding for a protein (179 amino acid residues) homologous to the cytokine synthesis inhibitory factor (CSIF; interleukin 10) of the human and mouse, and to the Epstein-Barr virus (EBV) protein BCRF1. This finding is further significant evidence that the interleukin 10 (IL-10) and/or IL-10-like gene can indeed be present in the genomes of members of the herpesviral family.

Introduction

Interleukin 10 (IL-10) is a cytokine produced by one class of mouse helper T cell clones (1-4) and inhibits the synthesis of other cytokines, e.g., gamma-interferon. Vieira et al. (5) reported recently that a significant homology exists between human and mouse IL-10 genes (5,6) and the BCRF1 gene of Epstein-Barr virus (EBV; 7,8). This observation leads to the following questions: Firstly, is the presence of an IL-10-like gene within the genome of EBV a unique structural and functional genomic feature of this virus? Secondly, is the existence of an IL-10-like gene in EBV a common structural and functional property of the family

herpesviridae? The data presented in this report emphasize unambiguously that another herpesvirus like EHV-2 can also harbor an IL-10-like gene in its genome.

Results

Equine herpesvirus type 2 (EHV-2) has been characterized as a member of the family β-Herpesviridae with a worldwide distribution. The clinical manifestation has been associated with respiratory disease and keratoconjunctivitis (10) as well as persistent infections in horses (11). EHV-2 replicates to low titers in vitro (12) and can be propagated on equine epidermal cells (ED-2) and rabbit kidney cell cultures. The genome of EHV-2 consists of a double-stranded linear DNA molecule with a molecular weight of about 184–192 kbp (13–15). In order to identify the repetitive DNA elements within the genome of EHV-2, the *Eco*RI DNA fragment N (4.3 kbp, 0.235–0.258 viral map units) of the genome of the EHV-2 strain T400/3 was characterized by DNA nucleotide sequence analysis (16–18). The analysis of the coding strategy of this particular region of the genome of EHV-2 revealed the existence of an open reading frame (ORF) located on a subfragment (*Nsi*I/*Xho*I DNA fragment, 862 bp) of the *Eco*RI DNA fragment N (Fig. 1). As shown in Fig. 2 the deduced amino acid sequence of this ORF (ATG/TGA at nucleotide positions 120 and 659, respectively) consists of 179 amino acid residues.

A comparison between the deduced amino acid sequences of this ORF and the amino acid sequences of known proteins listed in protein databanks was carried out. A high degree of identity was detected between the amino acid sequence of this ORF and the amino acid sequences of human and mouse IL-10 proteins, and

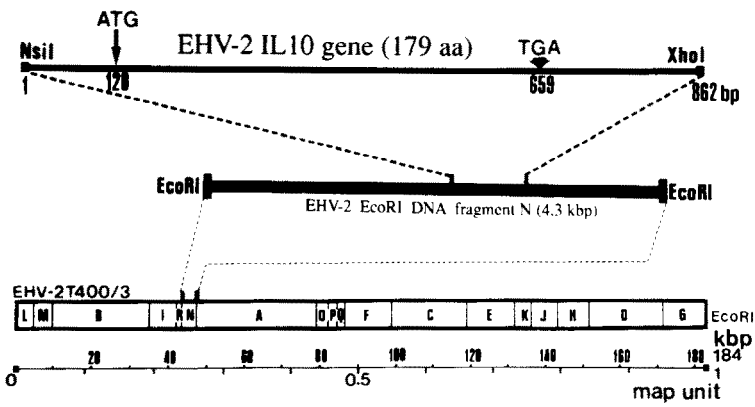

Fig. 1. Localization of the IL-10-like gene within the genome of EHV-2. The diagram of the genome of EHV-2 strain T400/3 is shown at the bottom, together with the genome coordinates and the position of the *Eco*RI recognition sites. The position of the *Eco*RI DNA fragment N is shown by magnification of the fragment as a fat line. The exact localization of the ORF encoding the EHV2-IL-10-like gene within the *Nsi*I/*Xho*I DNA fragment is shown at the top of the diagram.

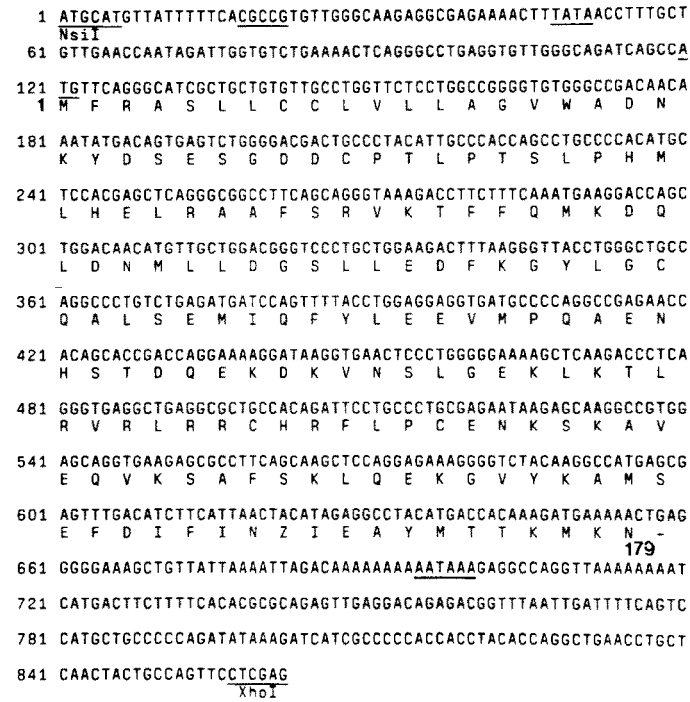

Fig. 2. DNA nucleotide sequence of the *NsiI/XhoI* (862 bp) DNA fragment harboring the EHV2-IL-10-like gene. The positions of the GC-rich region [nucleotide position (np) 18–22] and TATA-box (np 48–51) within the promoter region, the ATG start codon (np 120), and the position of the polyadenylation signal (AATAAA; np 695–700) are underlined. The deduced amino acid sequence of the EHV2-IL-10-like gene (179 amino acid residues) is shown below the corresponding DNA sequences in the one-letter code. The DNA sequence of the EHV2-IL-10-like gene has been deposited at the GenBank Data Library.

the amino acid sequences of BCRF1 protein of EBV (Fig. 3). Accordingly, this putative gene was termed the *EHV2-IL-10-like gene*. This finding indicates that indeed another member of the family herpesviridae possesses an IL-10-like gene. As shown in Table 1, the percentage of identity between EHV2-IL-10 and human-IL-10, mouse-IL-10, and EBV-IL-10 was found to be 76.4%, 68.5%, and 70.6%, respectively. The biophysical and biochemical properties of the EHV2-IL-10 protein in comparison to the human and mouse IL-10 proteins and BCRF1 protein of EBV are summarized in Table 2. As shown in Fig. 3 the N-termini of the different cellular and viral IL-10-like genes are more heterogeneous than the middle parts and the C-termini where in all four amino acid sequences at positions 74–92, 123–131, and 133–142 are 100% identical. These regions could serve as target sequences for the identification of IL-10-like genes of other animal species and viruses. The family herpesviridae, whose members are all very well adapted

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EHV-2    1 MFRASL--LCCLVLLAGVWADNKYDSESGDDCPTLPTSLPHMLHELRAAF   48
BCRFI    1 .E.RLVVT.Q.....YLAPECGG-----T.Q.DNF.----Q..RD..D..   41
HIL10    1 .HSSA.--.......T..R.SPGQGTQ.ENS.THF.GN..N..RD..D..   48
MIL10    1 .PGSA.--....L..T.MRISRGQY.REDNN.THF.VGQS...LE..T..   48
           *    +  * **+**              ++ *  +*    +** +**+**

EHV-2   49 SRVKTFFQMKDQLDNMLLDGSLLEDFKGYLGCQALSEMIQFYLEEVMPQA   98
BCRFI   42 ........T..EV..L..KE.............................   91
HIL10   49 ..............L..KE.............................   98
MIL10   49 .Q......T......I..TD..MQ.....................V.....   98
           *+****** **++**+**++**++**********************+******

EHV-2   99 ENHSTDQEKDKVNSLGEKLKTLRVRLRRCHRFLPCENKSKAVEQVKSAFS  148
BCRFI   92 .NQDPEA-..H......N.....L.....................I.N..N  140
HIL10   99 .NQDP.I-.AH......N.....L.....................N..N  147
MIL10   99 .K.GPEI-.EHL.....M.....K.....D.N  147
           *+++++  *+++*****+*****+*********** **********+*++*+

EHV-2 149 KLQEKGVYKAMSEFDIFINYIEAYMTTKMKN  179
BCRFI 141 ......I.....................I.AR-  170
HIL10 148 ......I.....................M.IR.  178
MIL10 148 ..EDQ......N........C.....MI...S  178
          **+++*+****+********+*****  * +
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Fig. 3. Alignment of the amino acid sequence of the IL-10-like gene of EHV-2 (EHV-2, line 1) with the BCRFI protein of EBV (BCRFI; line 2), human IL-10 (HIL10, line 3), and mouse IL-10 (MIL10; line 4). Identical amino acid residues are indicated by dots; introduced gaps to facilitate the alignment are indicated by dashes. Identical amino acid residues in all four sequences are indicated with asterisks, and conserved exchanges are indicated with crosses on the bottom line. Amino acid sequences were compiled and analyzed using the PC/Gene program release 6.60 (UgenBank 70-29, EMBL 29, SWISS-Prot 20; University of Geneva, Switzerland; Intelligenetics Inc., Mountain View, CA, USA).

to their hosts (20) and establish latent infections, should particularly be examined for the presence of IL-10-like genes within their genomes. These analyses will bring further information on the role of IL-10-like genes in virus infections.

The computer analysis of EHV2-IL-10 protein revealed a hydrophobic signal peptide at the N-terminus, suggesting that IL-10 protein is a viral protein that can be excreted from the infected cells. Although the N-terminus of the viral and cellular IL-10 proteins are not homologous (Fig. 3), they resemble EHV2-IL-10 in having a hydrophobic signal peptide. It is of interest that the IL-10 proteins are markedly conserved in the leucine zipper sequence (aa 109–130; Table 2). The property of the highly conserved sequence (aa 47–99) is not yet understood. However, it is possible that this sequence might be involved in receptor binding.

Table 1. Percentage of identity detected by comparison of the predicted amino acid sequences of EHV2-IL-10, human-IL-10, mouse-IL-10, and Epstein-Barr virus open reading frame BCRF1 (EBV-IL-10)

	EHV-2	Human	Mouse	EBV
EHV-2	100	76.4	68.5	70.6
Human	76.4	100	71.9	80
Mouse	68.5	71.9	100	63.5
EBV	70.6	80	63.5	100

Table 2. Comparison of the biophysical and biochemical properties of the predicted amino acid sequences of EHV2-IL-10, human-IL-10, mouse-IL-10, and Epstein-Barr virus open reading frame BCRFI (EBV-IL-10)

	Amino acid position			
Property	EHV-2	Human	Mouse	EBV
Amino acid residues	179	178	178	170
Molecular weight (kD)	20.6	20.5	20.6	19.9
Isoelectric point	6.48	8.02	8.18	5.68
Potential sites for N-glycosylation	100	134	29	127
	135		134	
Tyrosine kinase phosphorylation site	156	155	155	148
Potential sites for protein kinase C phosphorylation	119	118	118	111
	174	173		166
Potential sites for casein kinase II phosphorylation	26	24	24	50
	69	69	111	62
	103	111		104
	112	159		152
	160			
Potential sites for N-myristoylation	154	35	15	23
		153	153	146
Tyrosine sulfatation site	22			
Position of cysteine residue	8	8	8	11
	9	9	9	21
	30	24	30	27
	80	30	80	73
	127	80	126	119
	133	126	132	125
		132	167	
Leucine zipper			109–130	

The analysis was carried out according to Bairoch (19).

The cysteine residues are conserved in the sequence of the four IL-10 proteins. Table 2 provides information on potential sites for N-glycosylation, phosphorylation, N-myristoylation, and sulfatation, which are the same in all the IL-10 proteins.

Discussion

The possible role, if any, of EHV2-IL-10-like gene product is not known. Yet it was reported that IL-10 cytokine can affect the cellular immune response by a) inhibiting cytokine production by gamma-INF and LPS activated macrophages (21) and b) strongly reducing the antigen-presenting capacity of monocytes by downregulating the expression of the class II major histocompatibility complex (22). If EHV-2 expresses its IL-10-like gene during infection in horses, then this viral cytokine might be part of the viral mechanism to reduce the host immune

response to the virus. Further studies are needed to elucidate the role of the IL-10 in EHV-2 pathogenesis in horses.

Acknowledgments

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