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Characterization of GM-CSF-inhibitory factor and Uracil DNA glycosylase encoding genes from camel pseudocowpoxvirus

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ABSTRACT

The present study describes the PCR amplification of GM-CSF-inhibitory factor (GIF) and Uracil DNA glycosylase (UDG) encoding genes of pseudocowpoxvirus (PCPV) from the Indian Dromedaries (*Camelus dromedarius*) infected with contagious ecthyma using the primers based on the corresponding gene sequences of human PCPV and reindeer PCPV, respectively. The length of GIF gene of PCPV obtained from camel is 795 bp and due to the addition of one cytosine residue at position 374 and one adenine residue at position 516, the open reading frame (ORF) got altered, resulting in the production of truncated polypeptide. The ORF of UDG encoding gene of camel PCPV is 696 bp encoding a polypeptide of 26.0 kDa. Comparison of amino acid sequence homologies of GIF and UDG of camel PCPV revealed that the camel PCPV is closer to ORFV and PCPV (reference stains of both human and reindeer), respectively.

1. Introduction

Pseudocowpox virus (PCPV, previously known as Parapoxvirus bovis 2) is one of two parapoxviruses (PPVs) of cattle, along with *Bovine papular stomatitis virus* (BPSV, previously known as Parapoxvirus bovis 1). Two other virus species, namely *Orf virus* of sheep and goats (ORFV, previously known as Parapoxvirus ovis) and *Parapoxvirus of red deer in New Zealand* (NZPV), complete the genus *Parapoxvirus* within the subfamily *Chordopoxvirinae* of the family *Poxviridae* (Van Regenmortel et al., 2000). Parapoxviruses are epitheliotropic viruses identified throughout the world as causing nonsystemic, vesicular, and eruptive skin disease in domestic and wild mammals, especially ruminants (Fleming and Mercer, 2007). Individual PPV species usually display a narrow host range yet are occasionally transmitted to humans, causing localized lesions on the hands (MacNeil et al., 2010).

On the Indian subcontinent, contagious ecthyma is a major exathematous skin infection of Dromedary camels (*Camelus dromedarius*) and is caused by pseudocowpoxvirus (Nagarajan et al., 2010). This disease usually occurs during and immediately after monsoon season in Indian camels. In the camel contagious ecthyma (CCE), nodules appear on the lips followed in most cases with swelling of the face and sometimes the neck. Papules and vesicles appear later and within a few days develop into thick scabs. Lesions occur sometimes on the face, eyes and nares and healing occurs within 20–30 days in most cases (Abubakr et al., 2007).

Despite Poxviruses (PPVs) stimulating a vigorous immune response in the host, they can replicate and induce lesions. A possible explanation for this is that poxviruses, along with other large DNA viruses, express immunomodulatory virulence proteins that inhibit or mimic key effector molecules of the host immune and inflammatory response to infection (Lalani and McFadden, 1997). A common general mechanism is the production of viral proteins inhibiting early events in the host response to infection, including inflammatory cytokines, interferons, chemokines, complement function and finally apoptosis. Many of the immunomodulatory genes are orthologs of host cellular genes that have been acquired and modified by the viruses. By studying these viral immunomodulator proteins, knowledge into the mechanisms of not only virus virulence but also host protective immunity to virus infection is gained.

Several putative immunomodulating genes of parapoxviruses have been discovered: a viral ortholog of mammalian vascular endothelial growth factor (VEGF) (Lyttle et al., 1994), a viral ortholog of IL-10 (Fleming et al., 1997), and an orf virus ortholog of the vaccinia virus E3L gene, which codes for an interferon resistance protein (McInnes et al., 1998). Apart from these, ORFV has been shown to encode a protein (GM-CSF inhibitory factor- GIF) that is capable of binding and inhibiting both ovine cytokines interleukin-2 (IL-2) and granulocyte– macrophage colony-stimulating factor (GM-CSF) (McInnes et al., 2005). As GIF is a protein with unusual properties and part of a growing number of pathogen immunomodulators that will be useful not only in determining mechanisms of viral

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pathogenesis and the nature of host antipathogen immunity but also as templates for potentially therapeutic proteins or peptides (Deane et al., 2000).

Several orthopoxvirus (OPV) enzymes are being considered as targets for antiviral drugs. As the uracil DNA glycosylase (UDG) protein is highly conserved between vaccinia and variola major virus, an inhibitor could be helpful to decrease the pathogenicity to all orthopoxvirus orthologs (De Silva and Moss, 2003).

In PCPV, viral IL-10 (v IL-10) was present only in the strain F00.120R, a reindeer (*Rangifer tarandus*) isolate but absent in the reference strain VR634, a human isolate. On the other hand GIF was present in the human isolate but not in the reindeer isolate (Hautaniemi et al., 2010). We recently reported the presence of viral IL-10 in PCPV from the Dromedary camel (Nagarajan et al., 2013) but information on the presence of GIF in camel PCPV is not available. We therefore described the isolation and characterization of a GM-CSF-inhibitory factor (GIF), derived from the PCPV genome. Further, in the line of pathogenecity studies of camel PCPV, the present study is a preliminary step to understand the sequence homology of the UDG encoding gene of PCPV from the camel with other Poxviruses.

The objective of the present study is to characterize two important genes viz., GIF and UDG encoding genes of pseudocowpoxvirus from Indian Dromedary camels at sequence level and to find out their phylogenetic relationships with other poxviruses.

2. Materials and methods

2.1. Scab materials

In mid August 2011, camel calves of below 1 year of age of either sex in a camel herd in Jagthi village of Udaipur district, Rajasthan state, India were showing clinical signs of contagious ecthyma lesions around the facial region. Scab materials were collected from 10 severely affected animals and subjected to PCR.

2.2. Polymerase chain reaction

Skin scabs were collected from the suspected lesions of camel contagious ecthyma and stored at –20 °C until use. DNA was extracted from the skin scabs using GeneiUltrapure™ Mammalian Genomic DNA Purification Kit – Tissues (Bangalore GeNei Pvt. Ltd., India) according to the manufacturer's instructions. Reaction volumes for the PCR of 50 µl were used and contained 5 µl of 10× buffer with 15 mM $MgCl_2$, 10 mM of each dNTPs, 100 pmol of each oligonucleotide primer, 100 ng of DNA sample and 3U Taq DNA polymerase. GM-CSF inhibitory factor (GIF) and uracil DNA glycosylase (UDG) encoding genes of camel pseudocowpoxvirus (PCPV) obtained from India were amplified from the genomic DNA isolated from the Dromedary camel skin scabs infected with contagious ecthyma by PCR using the primers designed based on PCPV gene sequences reported in GenBank (Table 1). The reaction mixture was subjected to initial denaturation of the template at 94 °C for 5 min in a thermal cycler (Eppendorf, Germany). Cycling conditions for PCR were 35 cycles of 60 s at 94 °C, 60 s at annealing temperatures depending on the genes to be amplified (Table 1) and 60 s at 72 °C, followed by a final extension for 10 min at 72 °C. The total genomic DNA isolated from the Dromedary camels infected with camelpox was included as a negative control in the PCR.

2.3. Cloning and sequencing of PCR amplified DNA fragments

The PCR amplified products amplified from 10 individual scab materials were validated in 1.2% agarose gel. Out of 10 positive amplicons, only three amplicons corresponding to genes encoding GM-CSF inhibitory factor (GIF) and uracil DNA glycosylase (UDG) of camel pseudocowpoxvirus (PCPV) were cloned into pGEM-T Easy vector (Promega Corp., Madison, USA). The ligated mixtures for both genes were individually transformed into *Escherichia coli* DH 5α (Sambrook et al., 1989). The positive clones were confirmed by colony PCR using gene-specific primers and restriction analysis with EcoRI. Three positive clones in each gene were sequenced at the sequencing facility, Delhi University (South Campus), Delhi. Since pGEM-T easy vector was used for the cloning purpose, universal T7 and SP6 primers were used for the sequencing of recombinant clones. The primer sequences used for the sequencing were based on respective promoter sequences. Nucleotide sequences were submitted to GenBank and the accession No. was obtained (GIF gene JQ917913; UDG gene JQ728422). The determined nucleotide sequences and the deduced amino acid sequences of the GIF and UDG were analyzed with the BLAST program (NCBI) search of GenBank. Nucleotide identity, amino acid identity and comparison of the sequences with published sequences of members of *Poxviridae* available in the GenBank database were carried out using the computer software BioEdit version 7.0.9. The multiple alignment of the protein sequences was created using MUSCLE (Edgar, 2004). and a phylogenetic tree was constructed based on the nucleotide sequences in case of GIF and amino acid sequences in case of UDG, by the maximum likelihood statistical method using Mega 6 (Molecular Evolutionary genetics Analysis software with bootstrap values calculated for 1000 replicates (Tamura et al., 2013). The open reading frame (ORF) and translation of nucleotide sequences to amino acid sequences were predicted by using the computer software Generunner version 3.05 (Hastings Software Inc., Hastings, NY, USA; http://www.generunner.net).

Table 1
Primer sequences used to clone GIF and UDG encoding genes of camel PCPV.

Gene	Primer sequences (5′–3′)	Predicted size (bp)	Annealing temperature (in degree Celsius)	GenBank accession no.
GIF	Forward – 5′CGAAGGT ACCATGGCGTGTCTCAG GGTGTTCCTG 3′ Reverse – 5′ATTGGCGG CCGCGCGCACCGTCTG CATGCGGCACTT 3′	795	60	GQ329670
UDG	Forward – 5′ACTGGGAT CCATGGCGACGCCGGC GGCCGGCGCGCTGCG 3′ Reverse – 5′GTACGAA TTCTTAATTTTCACTATA AAAAGAAAATCCTCG 3′	696	57	GQ329669

3. Results

3.1. Cloning and sequence analysis of GM-CSF inhibitory factor gene

All the 10 DNA samples isolated from 10 different scab materials were showing the amplification of a fragment of around 800 bp size. Out of 10 PCR positive amplicons, only three representative of GM-CSF inhibitory factor (GIF) encoding gene of camel pseudocowpoxvirus (PCPV) were transformed into *E. coli* DH5α. The plasmids containing the gene of interest (recombinant plasmids) were confirmed by colony PCR using gene specific primers and restriction enzyme analysis with EcoRI. Three recombinant plasmids (one for each sample) extracted from individual bacterial colonies grown on LB agar containing 50 lg/ml ampicillin were chosen for sequencing. The sequence data obtained using T7 and SP6 primers for all three recombinants were identical and the consensus sequence was confirmed by the use of BLAST program.

In the present study, the length of the GIF gene of PCPV obtained from camel is 795 bp and is supposed to produce a