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Species-specific differences in the structure of orthopoxvirus complement-binding protein

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Abstract

Vaccinia virus complement-binding protein (VCP) is secreted from the cells infected with the virus and controls the complement activation reactions. This protein contains four short consensus repeats (SCR), typical of the protein family of complement activation regulators. Organization of the VCP genes/proteins of orthopoxviruses—monkeypox (MPV), variola, cowpox and vaccinia viruses—and their cellular homologues (DAF and C4BP) were studied comparatively. For this purpose, VCP genes of three MPV strains were sequenced. VCP gene sequences of other human-pathogenic orthopoxvirus species and strains determined earlier were involved in the analysis. It has been demonstrated that a premature termination of the MPV VCP open reading frame results in a truncated protein sequence carrying a deletion of the C-terminal SCR4. This is an essential distinction of MPV from other orthopoxvirus species.

Keywords: Complement-binding protein; Orthopoxvirus; Monkeypox virus

The human complement system comprises over 20 blood plasma proteins and ten cell membrane-associated proteins. Mechanisms of the complement-mediated antiviral response include virus neutralization and opsonization, lysis of virus-infected cells and increases in the nonspecific inflammatory and specific immune responses (Kotwal, 1996). Vaccinia virus complement-binding protein (VCP), the first identified soluble microbial protein with a complement control activity (Kotwal and Moss, 1988), is composed of four short consensus repeats (SCRs), each with a length of 60–70 amino acid residues, typical for the family of regulators of complement activation (RCA) (Liszewski and Atkinson, 1998). It was assumed (Kirkitadze et al., 1999) that VCP gene originated initially as a result of insertion of the entire coding sequence of a host protein from RCA family or its part into the virus genome followed by adaptation (changes) of this gene for performing the functions necessary for the virus. It was demonstrated that VCP SCR sequences formed discrete compactly folded modules joined in an end-to-end manner (Smith et al., 2000).

VCP is a unique multifunctional viral protein, exhibiting functional similarities to factor H, membrane cofactor protein, complement receptor

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1 and decay-accelerating factor (DAF) (Reid and Day, 1989). It has been demonstrated that VCP (1) binds to C3b and C4b; (2) blocks the complement cascade at multiple sites and inhibits both the classical and alternative pathways; (3) blocks the complement-mediated antibody-enhanced virus neutralization; and (4) binds to heparin-like molecules lining the surface of endothelial cells, blocking thereby the chemokine binding and, subsequently, the chemotactic signal (McKenzie et al., 1992; Kotwal, 1996; Sahu et al., 1998; Rosengard et al., 1999; Smith et al., 2000).

It has been demonstrated that destruction of VCP gene results in attenuation of vaccinia virus (Isaacs et al., 1992). Using the model of cowpox virus-infected mice, VCP was demonstrated to inhibit the development of the in vivo inflammatory response (Miller et al., 1995, 1997; Howard et al., 1998).

Since human-pathogenic orthopoxvirus species differ essentially in their virulence for both humans and sensitive animals (Marennikova and Shchelkunov, 1998), it was of great interest to compare the organizations of VCP genes/proteins as one of the major molecular virulence factors in genomes of these viruses. Vaccinia, variola and cowpox virus VCP genes have been sequenced earlier. In this work, we determined the VCP gene primary sequences of three monkeypox virus (MPV) strains. DNA samples of strains CONGO-8 (CNG-8) (Marennikova et al., 1972), 77-0666 (ZAI-77) (Breman et al., 1980) and Zaire-96-I-16 (ZAI-96) (Mukinda et al., 1997) were kindly provided by Dr J.J. Esposito. Each VCP gene was amplified using polymerase chain reaction and the oligonucleotide primers shown below calculated with the program Oligo:

5′-CCCGGATCCGTCGGTAGACGATACCGT TA-3′
5′-CCCGAATTCCACTGCCATTGTTTTTGAG C-3′

The fragments amplified were hydrolyzed with *Bam*HI and *Eco*RI restriction endonucleases, added to vector plasmid pMGC20 (Ryazankina et al., 2000) digested with *Bam*HI and *Eco*RI restriction endonucleases and ligated according to the conventional technique (Maniatis et al., 1982). Upon transforming the competent *Escherichia coli* XL-blue cells with the ligation mixture, the hybrid clones were selected on LB agar nutrient medium supplemented with 50 μg/ml ampicillin, 30 μg/ml IPTG and 100 μg/ml X-gal. Hybrid plasmids were isolated from the clones selected, subjected to restriction analysis and used to sequence the cloned DNA fragments as described earlier (Shchelkunov et al., 1993b) using [^{33}P]-labeled deoxynucleotides (Izotop, Russia). MPV sequence data from this article have been deposited with the GenBank under the accession numbers AF346404, AF346405 and AF346406. The programs Gene Constructor v1.1 and Alignment Service v4.3 (Resenchuk and Blinov, 1995) were used for computer analysis of the sequence data.

VCP amino acid sequences of the three MPV strains in question appeared identical, although the nucleotide sequences displayed sporadic distinctions in their noncoding regions (Fig. 1). The MPV strains under study were isolated from human patients in one region but in different years (CNG-8, 1970; ZAI-77, 1977; ZAI-96, 1996); this suggests that VCP is highly conservative, at least in this orthopoxvirus species, circulating in Central Africa.

Further analysis also involved the earlier determined sequences of VCP gene of variola virus strains, India-1967 (VAR-IND) (Shchelkunov et al., 1993a,c), Bangladesh-1975 (VAR-BSH) (Massung et al., 1993, 1994), Garcia-1966 (VAR-GAR) (Massung et al., 1996; Shchelkunov et al., 2000); cowpox virus strains GRI-90 (CPV-GRI) (Shchelkunov et al., 1998), Brighton (CPV-BRI) (Howard et al., 1998), vaccinia virus strains Copenhagen (VAC-COP) (Goebel et al., 1990) and Western Reserve (VAC-WR) (Kotwal and Moss, 1988).

Results of the comparative analysis of VCP sequences of different orthopoxvirus species and strains are shown in Fig. 2. The VCP amino acid sequences of the three MPV strains studied are identical; therefore, only one VCP sequence of the orthopoxvirus species in question was used for analysis. It appeared that a premature termination of the MPV VCP open reading frame resulted in

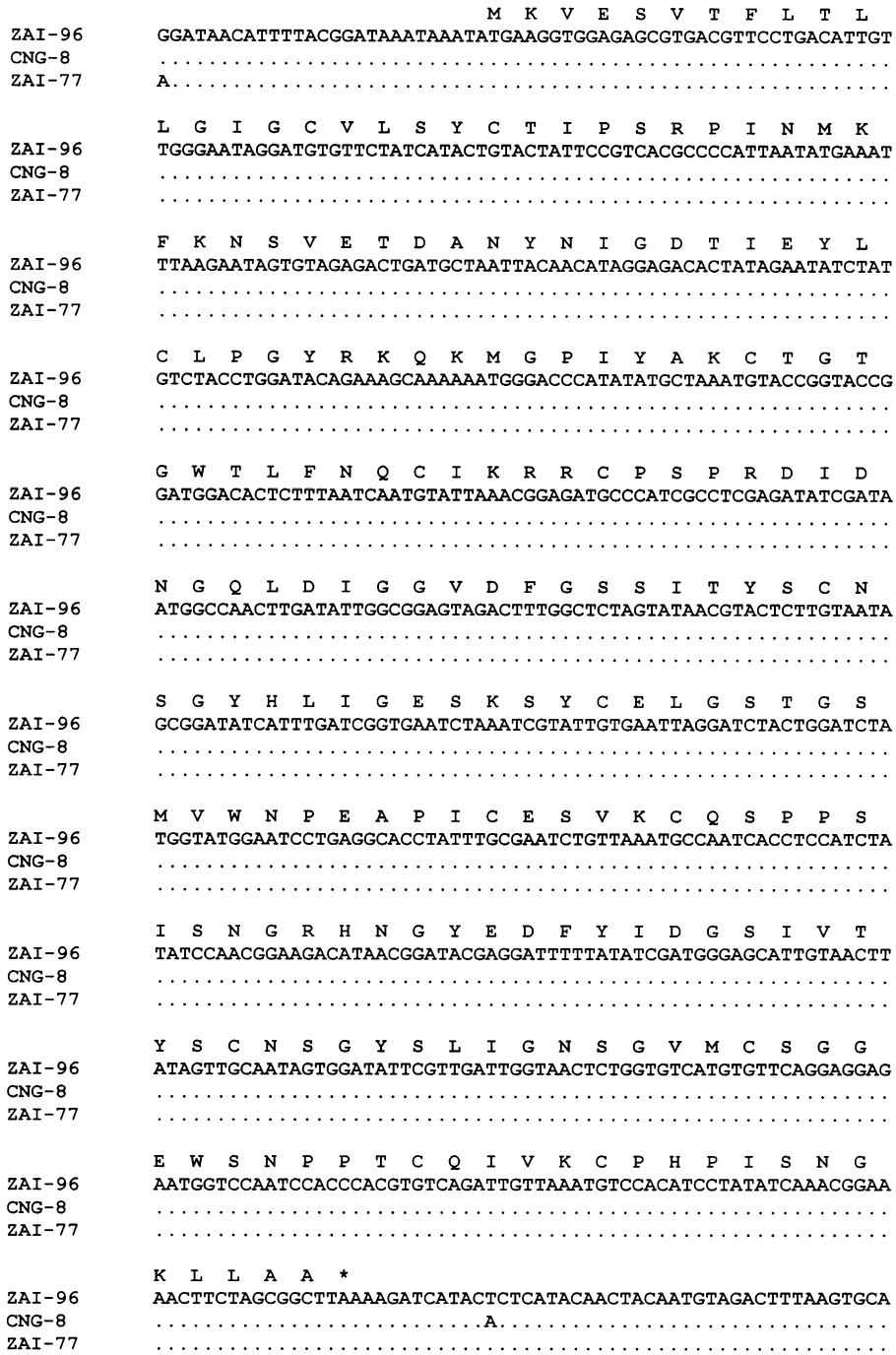

Fig. 1. VCP amino acid and nucleotide structures of monkeypox virus strains ZAI-96, CNG-8 and ZAI-77. Dots indicate the nucleotides of CNG-8 and ZAI-77 sequences coinciding with the corresponding nucleotides in ZAI-96 sequence.

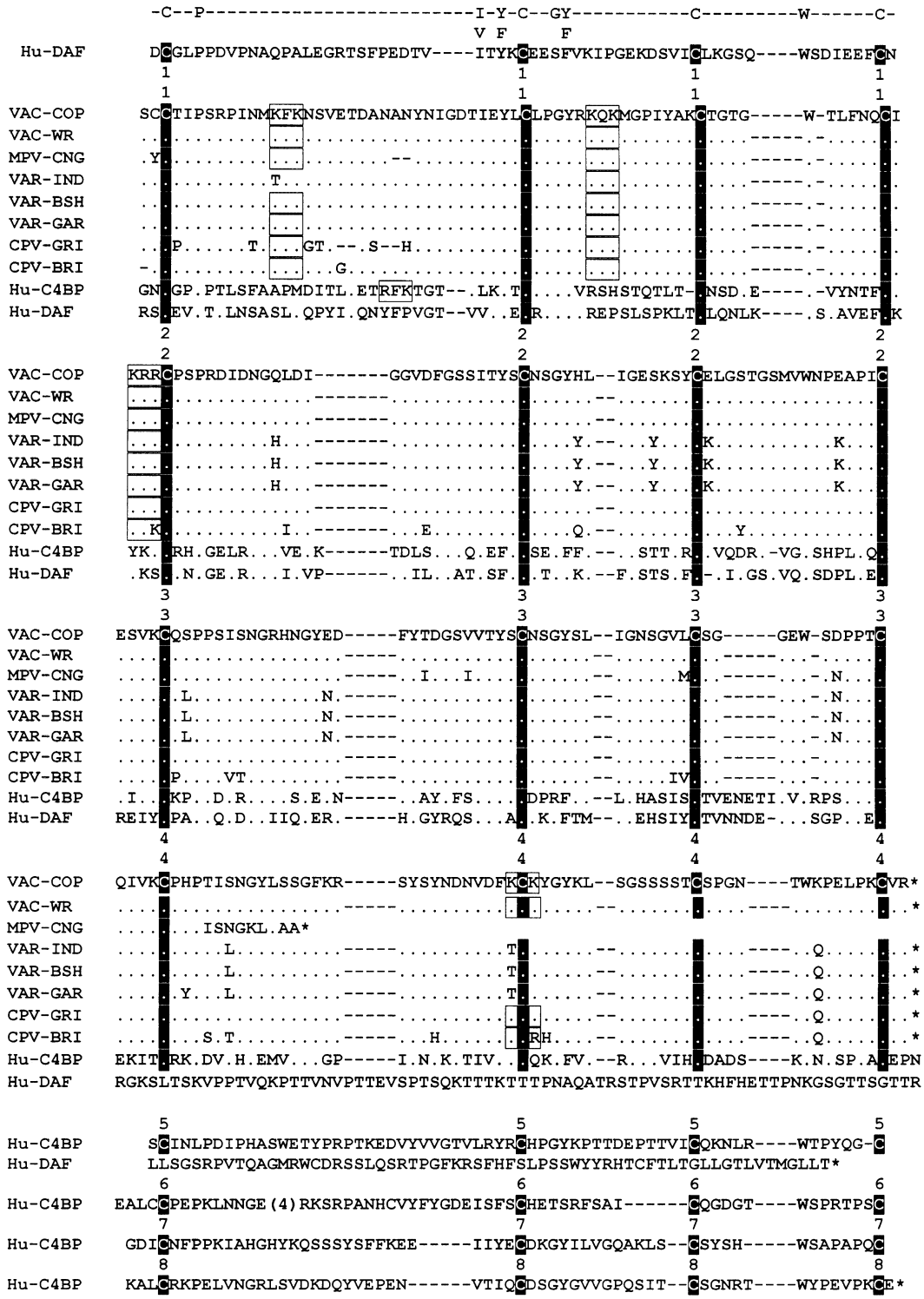

Fig. 2. (*Continued*)

a truncated protein carrying a deletion of the C-terminal SCR 4 (Fig. 2), distinguishing MPV from other human-pathogenic orthopoxvirus species studied. These data attracted attention to the homology between DAF SCRs 2–4 and VCP SCRs 1–3 (Fig. 2), which we discovered earlier (Shchelkunov et al., 1996). It was experimentally demonstrated that SCRs 2–4 of DAF were responsible for its regulatory activities (Coyne et al., 1992). Specifically, the classical C3 convertase regulatory activity pathway resides within SCRs 2–3, while alternative C3 convertase regulatory activity pathway, within SCRs 2–4 (Brodbeck et al., 1996).

Variola, cowpox and vaccinia viruses contain four SCRs. Note that VCP sequences of two vaccinia strains are identical; however, they differ from VCP sequences of variola virus strains, highly conservative within the species, by 12 amino acids. VCPs of two cowpox virus strains analyzed failed to display high intraspecies conservatism. This is likely to indicate an intraspecies heterogeneity of cowpox virus strains, which manifests itself while comparing both their biological properties and genomic structures (Marennikova and Shchelkunov, 1998).

It has been discovered that VCPs of vaccinia and cowpox viruses contain four putative heparin-binding sites (HBS 1–4; Smith et al., 2000; Fig. 2), whereas HBS 4, localized to SRC 4, in variola virus genome is mutationally changed and is completely deleted in the monkeypox virus protein. Note also that HBS 1 of the variola virus most virulent strain VAR-IND, localized to the VCP N-terminus, is mutationally altered too. These data allow us to propose that HBS 2 and 3 are most important functionally; in addition, it was shown that they are spatially close in the VCP molecule (Smith et al., 2000).

A more detailed analysis allowed us to discover the species-specific distinctions in the predicted VCP total charges, which might be important for manifesting of at least such property of VCPs as heparin binding activity (Smith et al., 2000). Total estimated VCP charges of VAC-COP and CPV-GRI are 2.55 and 2.64, respectively; VAR-IND, 5.54; and MPV, -0.3.

Summing up, the data obtained suggest that VCPs of cowpox and vaccinia viruses are most similar. VCPs of variola virus display most pronounced differences in their amino acid sequences. Monkeypox virus VCP is similar to the corresponding protein of vaccinia virus in its amino acid sequence; however, it is truncated by the deletion of the C-terminal part. It is possible that the differences in VCP structures of the viruses studied underlie the corresponding distinctions in their biological properties. This aids our capabilities in studying the fine mechanisms of function of both these proteins and the overall complement system and suggests a variety of potential therapeutics involving these unique viral proteins.

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Fig. 2. Alignment of the amino acid sequences of the proteins belonging to the family of complement control proteins. ORFs of VAC-COP, VAC-WR, MPV-CNG, VAR-IND, VAR-BSH, VAR-GAR, CPV-GRI and CPV-BRI are shown as well as cellular proteins Hu-C4BP (Aso et al., 1991) and Hu-DAF (Reid and Day, 1989). Black vertical blocks indicate conservative cysteine residues; putative heparin-binding sites are framed. Numbers of Hu-DAF SCRs are shown under Cys of these domains; SCRs numbers of other proteins, above the corresponding Cys.

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