

Genes of variola and vaccinia viruses necessary to overcome the host protective mechanisms

Sergei N. Shchelkunov, Vladimir M. Blinov and Lev S. Sandakhchiev

Institute of Molecular Biology NPO 'Vector', Koltsovo, 633159 Novosibirsk region, Russian Federation

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Analysis of variola virus nucleotide sequence revealed proteins belonging to several families which provide the virus with the possibility of overcoming the barriers of specific and non-specific host defence against viral infection. The complement-binding proteins, lymphokine-binding proteins, and serine protease inhibitors can be assigned to this type, as can the proteins providing the orthopoxviruses with resistance to interferon. The revealed differences between the genes (proteins) of variola and vaccinia viruses under study are discussed.

Variola virus; Complement-binding protein; Serine protease inhibitor; Lymphokine receptor

1. INTRODUCTION

Poxviruses are the biggest and the most complexly organized animal viruses. They exhibit several unique characteristics among which those defeating the specific and non-specific mechanisms of host organism defence [1] are particularly prominent. Investigation of molecular genetic organization of variola virus (VAR) DNA yielded the intriguing information in this respect, this virus being the agent of the disease, smallpox, in humans.

In the present paper we have undertaken the analysis of VAR genome organization and compared it to vaccinia virus (VAC). Clusters of VAR genes determining the modulation of various stages of complex processes of non-specific and specific host organism defence against viral infection are revealed. Comparison of these genes with the analogous VAC genes has been performed.

2. MATERIALS AND METHODS

VAR strain India-1967 was isolated from a patient in India in 1967. The strain had undergone 5 passages on chorioallantoic membranes (CAM) of chick embryos and are maintained in the collection of WHO Collaborating Center on Smallpox and Related Infections (Institute for Viral Preparations, Moscow, Russian Federation). VAR culture was grown on CAM and after purification by sucrose density gradient the viral DNA was isolated by phenol deproteinization as described

for VAC [2]. We have set up a collection of plasmids and cosmids with insertions of various VAR DNA fragments which cover the entire genome of this virus [3]. Sequencing of the complete coding part of VAR genome was performed on the basis of this collection. The computer analysis of poxvirus genomic organization was carried out as described earlier [4]. VAR sequence was compared to the published VAC strain Copenhagen DNA sequence [5].

3. RESULTS AND DISCUSSION

Higher eukariotic organisms have mechanisms of specific and non-specific protection against viral infections. Viruses, in their turn, are capable of modulating the reaction of the host organism which gives them the possibility of multiplying in an infected organism over some definite period of time. In the course of evolution different viruses developed various mechanisms of overcoming the protective barriers of the host.

Inflammatory processes constitute one of the primary lines of host defence against viral infection. These processes are rapidly induced to limit the spread of a virus during the first hours and days after the infection. At that time the host may not be able to realize the efficient cellular and humoral immune responses against the virus. The more efficiently a virus succeeds in the overcoming of these primary protective barriers of the host organism, the more advantages it has for multiplication in the infected host, at least at the early stages of the infection process. It should be underlined that the inflammatory process is regulated by a number of mediators, therefore it is difficult to overcome fully such protective barrier and for this purpose a virus should synthesize proteins of different types.

At present one can single out three protein families of the poxviruses which can modulate the development of the inflammatory process in response to viral infec-

Correspondence address: S.N. Shchelkunov, Institute of Molecular Biology NPO 'Vector', Koltsovo, 633159 Novosibirsk region, Russian Federation. Fax: (7) (383) (2) 328 831.

Abbreviations: VAR, variola virus; VAC, vaccinia virus; CAM, chorioallantoic membrane; TNF, tumor necrosis factor; IL, interleukin-1; INF, interferon.

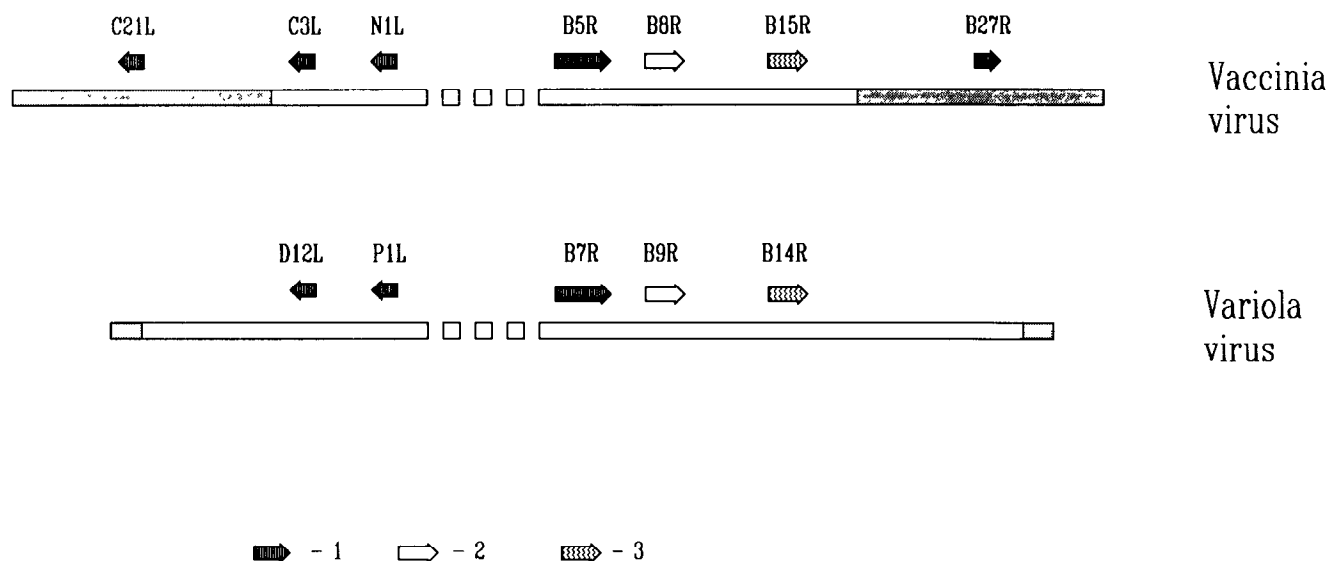


Fig. 1. Scheme of the location of the genes coding for the complement-binding proteins (1), protein-analogs of γ -interferon receptor (2) and IL-1 receptor (3) in the genome of VAC strain Copenhagen and VAR strain India-1967. Terminal inverted repeats of viral DNAs are shaded.

tion of an organism. These are the complement-binding proteins, the inhibitors of specific proteases and the analogs of lymphokine receptors [1].

The complement system is composed of more than 20 plasma proteins. The proposed antiviral action mechanisms of complement components include virus neutralization and opsonization, lysis of virus-infected cells, and amplification of inflammatory and specific immune responses [6].

The presence of genes (C21L, C3L) producing the

complement-binding proteins and inhibiting the antibody-dependent complement cascade was proved for VAC [6,7]. Gene C21L is located in the terminal inverted repeat and therefore is duplicated in the viral genome. The computer analysis [5] revealed two additional VAC genes, the products of which belong to the family of complement-binding proteins. Our analysis reveals that VAR contains the same number of the genes belonging to this family, excluding the duplicated C21L/B27R (Fig. 1). These data indicate, as we as-

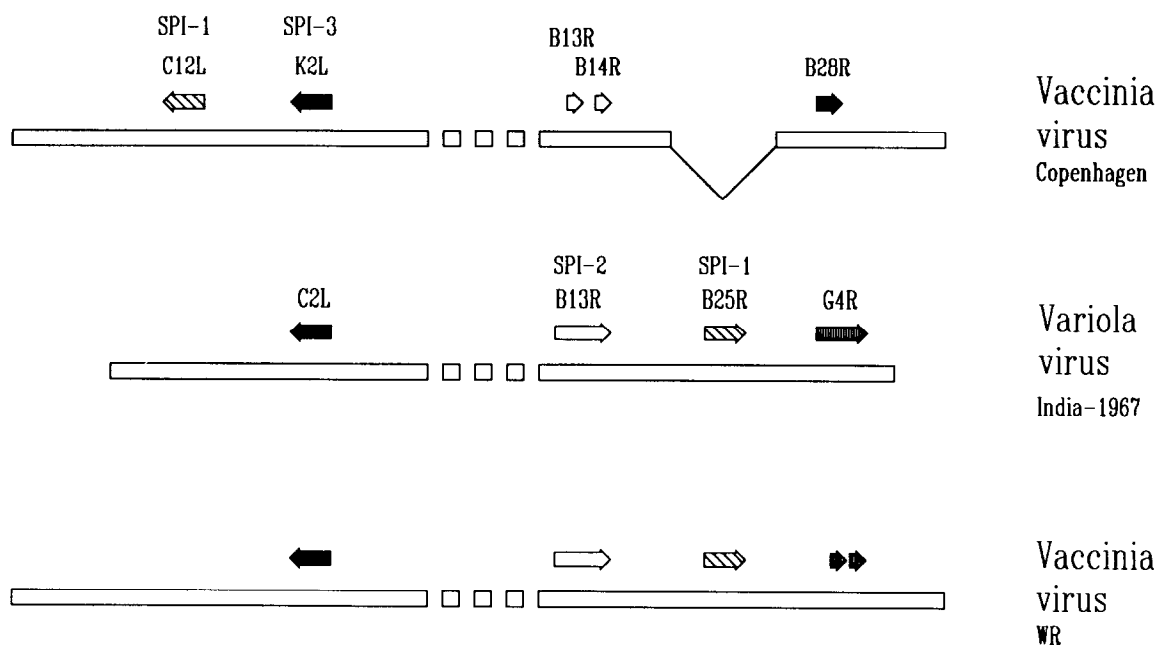


Fig. 2. Scheme of the location of the ORFs coding for the proteins of serine protease inhibitor family (SPI) and the protein-analog of TNF receptor. See text for details.

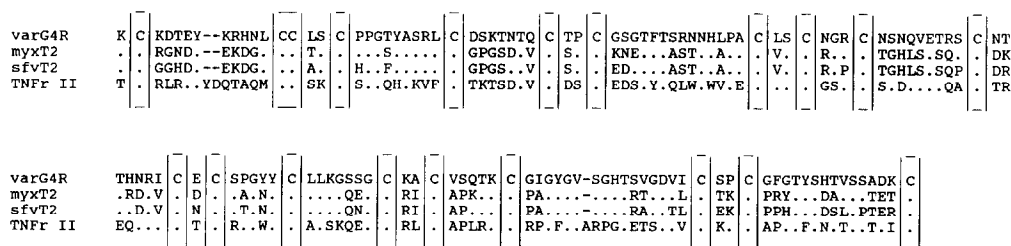


Fig. 3. Comparison of the homologous regions of human TNF receptor protein (TNFr II), G4R protein of VAR (varG4R), T2 protein of Shope fibroma virus (sfvT2) and the analogous protein of myxoma virus (myxT2) [11]. The conservative cysteine residues are enclosed in the rectangles. Dots indicate the amino acid residues identical to those of varG4R. Gaps have been introduced to maximize homology.

sumed, that VAR has more 'radical' modulators of the host protective mechanisms provided by the proteins of another type, and hence, there is no need in C21L analogous gene for VAR.

The other class of the proteins which play the important role in regulation processes of complement activation and inflammatory reactions are the proteins of serine protease inhibitors superfamily. In orthopoxviruses the first gene of this family was revealed in cowpox virus [8]. Originally it was designated as 38K gene, then as SPI-2. Smith et al. [9] found out that VAC encoded a family of genes with homology to serine protease inhibitors. Recently it was discovered [10] that SPI-2 protein inhibits the processing of pro-interleukin-1 β to mature form by the interleukin-1 β (IL-1 β) converting enzyme. But it is known that IL-1 α and IL-1 β together with the tumor necrosis factor- α (TNF- α) are the main mediators among the cytokins involved in the inflammatory processes. Functions of the other representatives of SPI family have not been clarified so far.

It is noteworthy that the SPI gene location in VAR genome corresponds to that of VAC strain WR but differs from the location in VAC strain Copenhagen (Fig. 2). Strain Copenhagen gene SPI-2 is damaged, which can result in the decrease of viral pathogenicity. The SPI-1 gene is transferred from the right end to the left end of the genome. On the whole we can see a similarity between several SPI family genes in VAR and VAC.

Drastic differences between these viruses were re-

vealed while analyzing the sequences in the right terminal region of the genome. In this region in VAR we have detected the G4R gene (Fig. 2) the protein product of which has high homology with the TNF receptor (Fig. 3). This early viral-secreted protein is likely to bind to the endogenous TNF [11]. Thus, in VAR the protein SPI-2 inhibits the formation of the mature form of IL-1 β , and the G4R gene product binds to TNF and inhibits the action of TNF. Activity suppression of the discussed main mediators of inflammatory process results in radical inhibition of the host primary protective barrier. G4R-analogous genes in VAC are inactivated.

VAR and VAC also contain the gene (B14R and B15R, respectively) which produces the secreted IL-1 binding protein (Fig. 1) [12]. The other secreted protein, namely, B9R and B8R for VAR and VAC, respectively, (Fig. 1) has reliable homology with the γ -interferon receptor (Fig. 4).

It is known that TNF, IL-1 and γ -IFN are important regulators of cellular immunity. Antagonistic proteins for these regulating proteins are able to modulate their activity. It should be noted that VAC genes K3L and

Table I
Comparison of variola (VAR) and vaccinia (VAC) virus proteins

Open reading frame		Length of protein ^a		Homology of proteins ^b
Var	VAC	VAR	VAC	
D12L	C3L	263	263	95
P1L	N1L	117	117	93
C2L	K2L	373	369	94
C3L	K3L	88	88	81
E3L	E3L	190	190	96
B7R	B5R	317	317	93
B9R	B8R	267	272	89
B13R	B13R	345	117	87
	B14R		222	90
B14R	B15R	149	149	96
B25R	C12L	372	353	89
G4R	B28R	349	123	62

^aLength of the proteins is presented as the number of amino acid residues.

^bThe homology percentage of the compared amino acid sequences is presented.

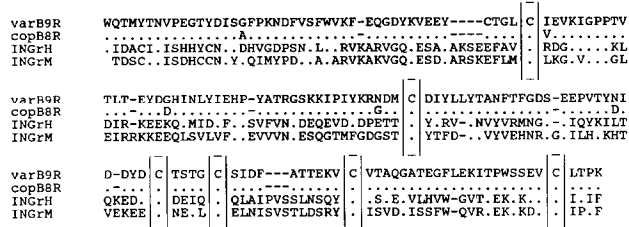


Fig. 4. Comparison of the homologous regions of human (H) and mouse (M) γ -interferon receptors (INGr) [13,14], B9R protein of VAR strain India-1967 (var) and B8R of VAC strain Copenhagen (cop). The designations are as in Fig. 3.

E3L encode the functions contributing to the interferon resistant phenotype of VAC [15]. We revealed the analogous genes C3L and E3L for VAR (Table I).

Thus, we can conclude that orthopoxviruses, and VAR in particular, have a complicated multi-factor system of modulation of non-specific and specific host organism protective reactions against these viruses. The proteins discussed have certain differences in their amino acid sequence (Table I). Hence, it is necessary to obtain the individual proteins and study their characteristics in an effort to clarify their functional distinctions.

The sequence data from this article have been deposited with the EMBL Data Library under accession number X69198.

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