

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

Received 8 December 1992; revised version received 6 January 1993

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.

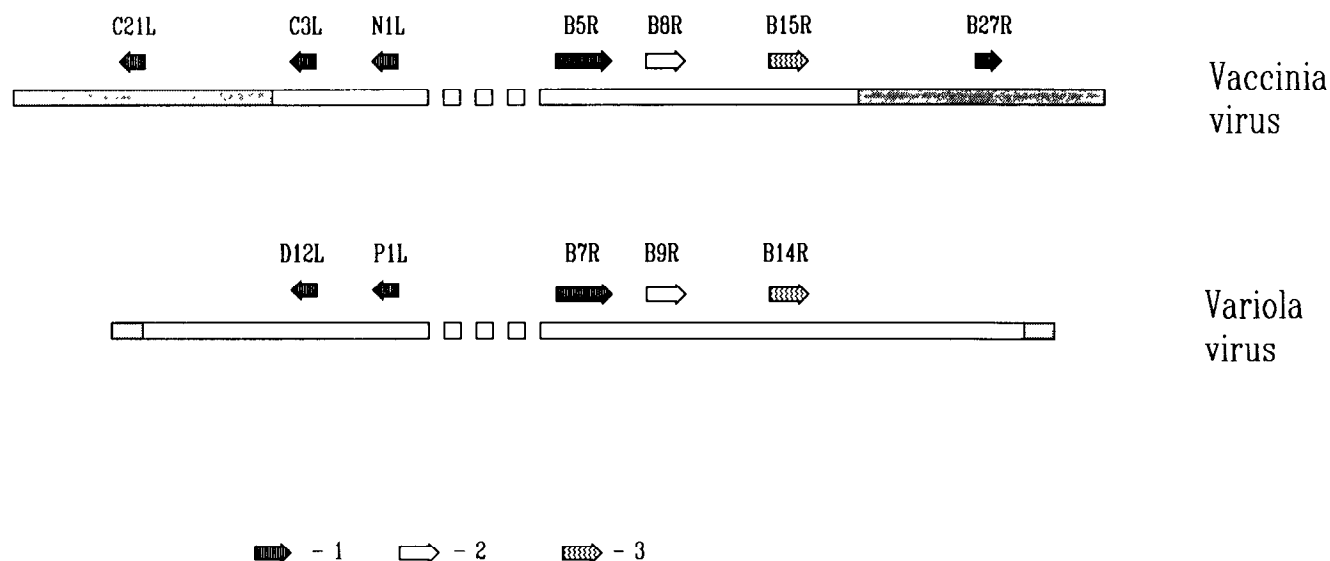


Fig. 1. Scheme of the location of the genes coding for the complement-binding proteins (1), protein-analogs of γ -interferon receptor (2) and IL-1 receptor (3) in the genome of VAC strain Copenhagen and VAR strain India-1967. Terminal inverted repeats of viral DNAs are shaded.

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-

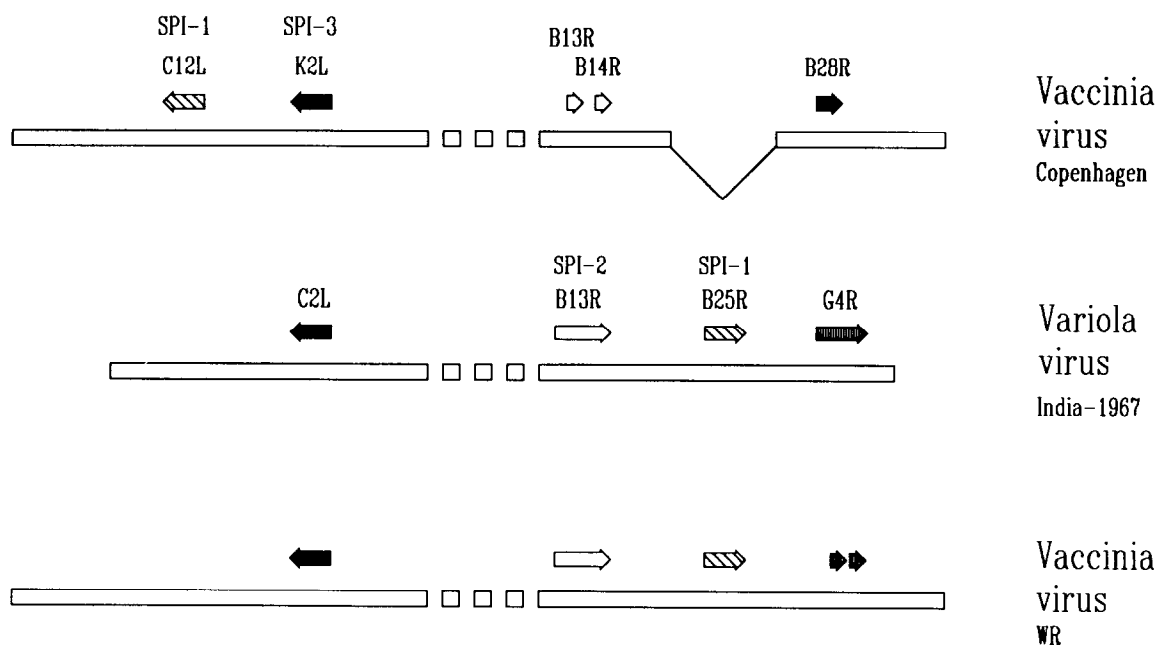
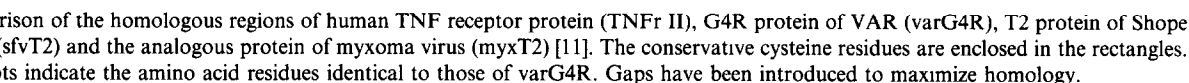


Fig. 2. Scheme of the location of the ORFs coding for the proteins of serine protease inhibitor family (SPI) and the protein-analog of TNF receptor. See text for details.



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.

REFERENCES

- [1] Buller, M.L. and Palumbo, G.J. (1991) *Microbiol. Rev.* 55, 80–122.
- [2] Shchelkunov, S.N., Stavitskii, S.B., Batenko, L.I., Gashnikov, P.V., Shchelkunova, G.A., Kostyrev, O.A. and Sandakhchiev, L.S. (1991) *Biomed. Sci.* 2, 493–497.
- [3] Shchelkunov, S.N., Marennikova, S.S., Totmenin, A.V., Blinov, V.M., Chizhikov, V.Y., Gutorov, V.V., Safronov, P.F., Pozdnyakov, S.G., Shelukhina, E.M., Gashnikov, P.V., Angzaparidze, O.G. and Sandakhchiev, L.S. (1991). *Dokl. Acad. Nauk SSSR* (in Russian) 321, 402–406.
- [4] Shchelkunov, S.N., Blinov, V.M. and Sandakhchiev, L.S. (1993) *FEBS Lett.*
- [5] Goebel, S.J., Johnson, G.P., Perkus, M.E., Davis, S.W., Winslow, J.P. and Paoletti, E. (1990) *Virology* 179, 247–266.
- [6] Kotwal, G.J., Isaacs, S.N., McKenzie, R., Frank, M.M. and Moss, B. (1990) *Science* 250, 827–830.
- [7] Isaacs, S.N., Kotwal, G.J. and Moss, B. (1992) *Proc. Natl. Acad. Sci. USA* 89, 628–632.
- [8] Pickup, D.J., Ink, B.S., Hu, W., Ray, C.A. and Joklik, W.K. (1986) *Proc. Natl. Acad. Sci. USA* 83, 7698–7702.
- [9] Smith, G.L., Howard, S.T. and Chan, Y.S. (1989) *J. Gen. Virol.* 70, 2333–2343.
- [10] Ray, S.A., Black, R.A., Kronheim, S.R., Greenstreet, T.A., Sleath, P.R., Salvesen, G.S. and Pickup, D.J. (1992) *Cell* 69, 597–604.
- [11] Upton, C., Macen, J.L., Schreiber, M. and McFadden, G. (1991) *Virology* 184, 370–382.
- [12] Smith, G.L. and Chan, Y.S. (1991) *J. Gen. Virol.* 72, 511–518.
- [13] Aguet, M., Dembic, Z. and Merlin, G. (1988) *Cell* 55, 273–280.
- [14] Cofano, F., Moore, S.K., Tanaka, S., Yuhki, N., Landolfo, S. and Appella, E. (1990) *J. Biol. Chem.* 265, 4064–4071.
- [15] Beattie-McGroth, E., Tartaglia, J., Chang, H.-W., Jacobs, B.L. and Paoletti, E. (1991) *Virology* 183, 419–422.