Available online at www.sciencedirect.com

SCIENCE DIRECT

Gene 355 (2005) 40 – 47

GENE
SECTION
EVOLUTIONARY GENOMICS

www.elsevier.com/locate/gene

Evolutionary history of orthopoxvirus proteins similar to human complement regulators

Emily Ciulla[a,1,4], Andrea Emery[a,2,4], Dina Konz[a,3,4], Julia Krushkal[b,*]

[a]*Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA, USA*
[b]*Department of Preventive Medicine and Center of Genomics and Bioinformatics, University of Tennessee Health Science Center, Memphis, TN 38163, USA*

Received 10 September 2004; received in revised form 28 March 2005; accepted 10 May 2005
Available online 14 July 2005
Received by W. Makalowski

Abstract

Orthopoxviruses include many important pathogens such as variola major virus, camelpox, buffalopox, monkeypox, cowpox, and variola minor viruses. This group of viruses also includes vaccinia virus, which is extensively used in human vaccine development. Genomes of orthopoxviruses encode proteins with sequences similar to human regulators of complement activation (RCA) that contain tandem short consensus repeats (SCRs). We employed phylogenetic tree analysis to evaluate the structural relationships among SCRs of orthopoxvirus RCA-like proteins and those of human complement regulators. The human complement RCA proteins analyzed were factor H (FH), C4 binding protein alpha chain, membrane cofactor protein (MCP), decay accelerating factor (DAF), and complement receptors type 1 (CR1) and 2 (CR2). Sequences of key poxvirus regulators of complement activation, vaccinia virus complement control protein (VCP), smallpox inhibitor of complement enzymes (SPICE), and cowpox inflammation modulatory protein (IMP) were similar to SCRs 1 through 5 of C4 binding protein, alpha chain, and they were also clustered with other homologous repeats of MCP, DAF, CR1, CR2, and FH. Phylogenetic clustering of RCA sequences suggested that poxvirus complement regulators VCP, SPICE, and IMP arose from a single ancestral sequence that shared similarity with all human regulators of complement activation. Any changes in poxvirus complement regulators leading to the enhancement of their ability to regulate complement activation likely resulted from new mutations in the viral lineages.

Keywords: Regulator of complement activation; Poxvirus; Vaccinia complement control protein; Smallpox inhibitor of complement enzymes; Inflammation modulatory protein

Abbreviations: B5R, vaccinia virus B5R protein; C3, complement component C3; C3a, C3a cleavage fragment; C3b, C3b cleavage fragment; C3d, C3d cleavage fragment; C3dg, C3dg cleavage fragment; C3L, vaccinia virus complement control protein (product of the *C3L* gene of vaccinia virus, strain *WR*); C4, complement component C4; C4b, C4b cleavage fragment; C4bp, C4 binding protein; C4bpα, C4 binding protein, α chain; C4bpβ, C4 binding protein, β chain; C5, complement component C5; CR1, complement receptor type 1; CR2, complement receptor type 2; DAF, decay accelerating factor; D12L, smallpox inhibitor of complement enzymes (product of the *D12L* gene of the variola major virus, strain *India-1967*); D17L, inflammation modulatory protein (product of the *D17L* gene of the cowpox virus, strain *GRI-90*); D15L, smallpox inhibitor of complement enzymes (product of *D15L* gene of the variola major virus, strain *Bangladesh-1975*); FH, factor H; iC3b, iC3b cleavage fragment; IgG1, immunoglobulin G subclass 1; IMP, inflammation modulatory protein; MCP, membrane cofactor protein; *ps-hr*, plaque size-host range gene; RCA, regulators of complement activation; SCR, short consensus repeat; SPICE, smallpox inhibitor of complement enzymes; TBR, tree bisection-reconnection; VCP, vaccinia virus complement control protein.

* Corresponding author. Tel.: +1 901 448 1361; fax: +1 901 448 7041.
E-mail address: jkrushka@utmem.edu (J. Krushkal).
[1] Present address: Averion, Inc., Framingham, MA.
[2] Present address: Harvard Medical School, New England Primate Research Center, Southboro, MA.
[3] Present address: ViaCell, Inc., Worcester, MA.
[4] E. C., A. E., and D. K. contributed equally to this work.

0378-1119/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2005.05.008

1. Introduction

The human complement system plays a highly important role in host defense against pathogens, in regulation of humoral immune response, and in inflammation Müller-Eberhard (1985). The early stages of complement activation may be initiated via three different pathways: the classical pathway, the alternative pathway, and the mannan-binding lectin pathway. These pathways lead to the assembly of distinct multi-component protein enzymatic complexes, C3 convertases. The C3 convertases are serine proteases that proteolytically cleave complement component C3 into two fragments, C3a and C3b. After the C3 cleavage, the three initiating pathways converge on a common series of steps that lead to the formation of the C5 convertase, assembly of the terminal complement components in the membranes of pathogens, cell lysis, and recruitment and activation of phagocytes (Müller-Eberhard, 1985; Pier et al., 2004).

To avoid injury to host tissue and to prevent uncontrolled activation, the activation of complement is tightly regulated by several mechanisms (Hourcade et al., 2000). Some of these mechanisms regulate the formation and stability of C3 convertases (Hourcade et al., 2002), and they involve a group of soluble and cell membrane-anchored regulatory proteins, referred to as regulators of complement activation (RCA). The RCA proteins consist of partially homologous tandem short consensus repeats (SCRs) of 60–70 amino acids (Stehle and Larvie, 2003). The two important human soluble plasma complement regulators are C4 binding protein (C4bp) and factor H (FH) that modulate the C3 activation in the classical and the alternative pathways, respectively (Gigli et al., 1979; Pangburn and Müller-Eberhard, 1985). Structurally, C4bp contains 8 disulfide linked chains. Seven of these chains are identical (C4 binding protein α chain, or C4bpα), and they regulate complement activation. Each C4bpα chain consists of 8 short consensus repeats. The non-identical β chain of C4 binding protein (C4bpβ) participates in coagulation (Blom, 2002). The single polypeptide chain of human FH consists of 20 SCRs (Zipfel et al., 2002).

Human membrane-anchored RCA proteins that regulate complement activation to prevent uncontrolled C3 activation include decay accelerating factor (DAF), membrane cofactor protein (MCP) and complement receptor type 1 (CR1). Both DAF and MCP contain 4 SCRs, while CR1 protein has different allelic variants, ranging from 23 to 44 SCRs (Hourcade et al., 2000). Recent analysis of genomic DNA of the human *CR1* gene suggested evidence for additional SCRs that are not expressed (McLure et al., 2004a,b).

In addition to regulation of complement activation, some RCA proteins perform other important biological functions in immune regulation. For example, MCP participates in modulation of T-lymphocyte-mediated immune responses, while CR1 serves as a cofactor for factor I mediated proteolysis of iC3b to generate C3dg, which may affect IgG1 responses and elicit memory cells (Hourcade et al., 2000; Kemper et al., 2001; Marie et al., 2002). Another RCA-related protein, complement receptor type 2 (CR2), participates in B-lymphocyte activation, and it is also expressed on other cells of the immune system. It interacts with cleavage fragments iC3b, C3dg, and C3d (Ross et al., 1973; Stehle and Larvie, 2003). CR2 protein has allelic variants that consist of 15 and 16 SCRs (Fujisaku et al., 1989), and additional non-expressed SCR duplications have been identified in the *CR2* gene (McLure et al., 2004a,b). Additional SCR-containing proteins with less established function or function different from complement regulation are also known (Krushkal et al., 2000; Zipfel et al., 2002).

Viral group *Orthopoxviridae* includes variola major virus, a highly virulent agent that causes smallpox. Many other orthopoxviruses also pose a serious health threat, e.g. camelpox, buffalopox, monkeypox, cowpox, and variola minor viruses (Georges and Georges-Courbot, 1999; McFadden, 2005). Another orthopoxvirus, vaccinia virus, is extensively used in human vaccine development and in biomedical research. The genomes of orthopoxviruses encode virulence-related proteins that can regulate complement activation. These proteins consist of four SCRs and are similar in sequence to human regulators of complement activation (Kotwal et al., 1998a,b; Kotwal, 2000; Lee et al., 2003). It has been suggested that viral complement regulators originated from a host genome as a result of horizontal gene transfer (Kirkitadze et al., 1999; Uvarova and Shchelkunov, 2001). In vaccinia virus, an important virulence factor that regulates human complement activation is vaccinia virus complement control protein (VCP) (Kotwal and Moss, 1988; Kotwal, 2000). VCP is a major secretory protein of cells infected with vaccinia virus, and it also exists in a membrane-bound form (Rosengard et al., 2002). VCP inhibits both the alternative and the classical pathways, it can bind both C3b and C4b, and it blocks complement-mediated antibody-induced virus neutralization. It also binds to heparin and heparan sulfate proteoglycans, blocking chemotactic signals (Kotwal et al., 1990; Sahu et al., 1998; Smith et al., 2000, 2003).

Other orthopoxviruses express VCP homologs that are important for immune modulation and virulence. The VCP homolog in variola major virus, the most virulent orthopoxvirus, is termed the smallpox inhibitor of complement enzymes (SPICE) (Kotwal, 2000; Lee et al., 2003). SPICE differs from VCP at 11 amino acid sites (Rosengard et al., 2002). It is highly efficient in inactivating both the classical and the alternative pathways of the complement system, and it shows strong preference to human complement inactivation as opposed to other mammalian species (Rosengard et al., 2002). The VCP homolog in cowpox virus is the inflammation modulatory protein (IMP), which downregulates complement and allows the infected tissue to evade inflammation (Kotwal et al., 1998a,b).

The relationship of VCP, SPICE, and IMP to human complement regulators has not been fully elucidated. A