Journal of Structural Biology 175 (2011) 127–134

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Review

How vaccinia virus has evolved to subvert the host immune response

Mohammad W. Bahar [a,1], Stephen C. Graham [a,2,1], Ron A.-J. Chen [b,3], Samantha Cooray [b,4], Geoffrey L. Smith [b], David I. Stuart [a,c,*], Jonathan M. Grimes [a,c,*]

[a] Division of Structural Biology and Oxford Protein Production Facility, Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, United Kingdom
[b] Department of Virology, Faculty of Medicine, Imperial College London, St. Mary's Campus, Norfolk Place, London W2 1PG, United Kingdom
[c] Science Division, Diamond Light Source Ltd., Diamond House, Harwell Science and Innovation Campus, Didcot, Oxfordshire 0X11 0DE, United Kingdom

ARTICLE INFO

Article history:
Received 2 February 2011
Received in revised form 10 March 2011
Accepted 14 March 2011
Available online 17 March 2011

Keywords:
Structural virology
Innate immunity
Cell signalling
X-ray crystallography
Surface receptors

ABSTRACT

Viruses are obligate intracellular parasites and are some of the most rapidly evolving and diverse pathogens encountered by the host immune system. Large complicated viruses, such as poxviruses, have evolved a plethora of proteins to disrupt host immune signalling in their battle against immune surveillance. Recent X-ray crystallographic analysis of these viral immunomodulators has helped form an emerging picture of the molecular details of virus-host interactions. In this review we consider some of these immune evasion strategies as they apply to poxviruses, from a structural perspective, with specific examples from the European SPINE2-Complexes initiative. Structures of poxvirus immunomodulators reveal the capacity of viruses to mimic and compete against the host immune system, using a diverse range of structural folds that are unique or acquired from their hosts with both enhanced and unexpectedly divergent functions.

1. Introduction

Vaccinia virus (VACV), the smallpox vaccine, is the prototype member of the *Orthopoxvirus* genus of the *Poxviridae*: a family of large, complex dsDNA viruses that replicate in the cytoplasm of host cells and form virions with a unique morphology (Condit et al., 2006; Moss, 2007). The VACV genome reflects the complexity of poxviruses in both gene composition and structure. Its linear dsDNA genome ranges from 185–200 kbp in size, with a capacity to encode around 200 distinct proteins (Lefkowitz et al., 2006; Moss, 2007). The highly conserved central portion of most poxvirus genomes contains essential genes involved in key functions such as transcription, DNA replication and virion assembly. In contrast, genes that cluster at the ends of the genome are usually species- or host-specific and encode virulence factors that modulate the host immune system (Gubser et al., 2004; Jackson et al., 2005). Analysis of poxvirus genomes has shed new light on poxvirus phylogeny and evolution (Lefkowitz et al., 2006) showing that poxvirus proteins are generally more similar to eukaryotic proteins than bacterial, suggesting that gene acquisition by horizontal gene transfer from their eukaryotic hosts has been a slow but ongoing process that has contributed to the evolution of poxviruses (Esposito et al., 2006; Lefkowitz et al., 2006). Many of these host-derived genes facilitate poxvirus immune evasion. VACV immunomodulators function both outside and inside infected host cells. Proteins that are secreted from infected cells are directed toward binding and disrupting the function of complement, interferons (IFNs), cytokines and chemokines (Alcami, 2003; Alcami and Koszinowski, 2000; McFadden and Murphy, 2000; Perdiguero and Esteban, 2009), as well as semaphorin signalling (Seet et al., 2003). Conversely, intracellular immunomodulators modulate apoptosis, the antiviral effects of IFNs, innate immune signalling and host gene transcription (Haga and Bowie, 2005; Perdiguero and Esteban, 2009; Seet et al., 2003; Taylor and Barry, 2006).

Abbreviations: Bcl-2, B-cell lymphoma-2; CPXV, Cowpox virus; dsDNA, double-stranded DNA; ECTV, ectromelia virus; GAGs, glycosaminoglycans; GPCRs, G-protein coupled receptors; IFN, interferon; IG, immunoglobulin; PDB, protein data bank; RPXV, rabbitpox virus; r.m.s.d., root mean square deviation; SPINE, Structural Proteomics In Europe; TLR, Toll-like receptors; TNF, tumour necrosis factor; TNFR, tumour necrosis factor receptor; VACV, vaccinia virus; vCCI, viral CC-chemokine inhibitor; eIF2α, eukaryotic translation initiation factor 2 alpha; TRAF6, TNF-receptor-associated factor 6; IRAKs, IL-1 receptor associated kinases; IKK, IκB kinase.

* Corresponding authors.
E-mail addresses: dave@strubi.ox.ac.uk (D.I. Stuart), jonathan@strubi.ox.ac.uk (J.M. Grimes).

[1] These authors contributed equally to this work.

[2] Present address: Cambridge Institute for Medical Research and Department of Clinical Biochemistry, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 0XY, United Kingdom.

[3] Present address: Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, United Kingdom.

[4] Present address: Molecular Immunology Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, United Kingdom.

1047-8477/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jsb.2011.03.010

In this review we assess the current structural knowledge on poxvirus immunomodulators (summarised in Table 1), focussing on what we have learnt from the five specific examples of extra- and intracellular immune modulators of VACV that have been solved as part of SPINE2-Complexes (shown in their functional context in Fig. 1). We highlight how VACV has evolved to use two broad classes of immunomodulators: those acquired from the host and those that appear to have no relationship to known host proteins. This work emphasises how poxviruses are able to acquire and replicate a number of structural scaffolds to carry out related but distinct immunomodulatory functions and underscores the observation that apparently unrelated sequences have often diverged from host acquired genes whilst conserving structure.

2. Extracellular immune evasion

Two structures of secreted VACV proteins that inhibit cytokines and chemokines were determined as part of the SPINE2-complexes activity and a number of structures have been determined by others, which are summarised first (Table 1). The crystal structure of the complex between ectromelia virus (ECTV) interleukin (IL)-18 binding protein and human IL-18 (Krumm et al., 2008) reveals that the viral protein has a canonical immunoglobulin (IG)-like fold and functions by blocking a putative receptor binding site on IL-18 (Krumm et al., 2008). The ECTV IFN-γ binding protein (IFN-γBP) complexed with IFN-γ also reveals a conservation of structure with the extracellular domain of the host IFN-γ receptor (Nuara et al., 2008). Furthermore, in this complex it was shown that ECTV IFN-γBP forms secreted tetramers that sequester two dimers of host IFN-γ. Oligomerisation of ECTV IFN-γBP is achieved through a helix-turn-helix motif that is similar in structure to the transcription factor TFIIA, demonstrating poxvirus acquisition of structural folds that have been adapted for additional functions by immunomodulators (Nuara et al., 2008). Lastly, recent structures of the VACV secreted protein A39 have provided mechanistic information about viral inhibition of semaphorins, a family of conserved signalling molecules that play crucial roles in the development of the nervous system and in immune regulation, through interactions at the cell surface with their cognate plexin receptors (Suzuki et al., 2008). VACV A39 is a secreted poxviral homolog of sema7A, and the crystal structure of the A39-PlexinC1 complex confirms that viral semaphorins share a conserved binding mode – adapted for higher affinity – to host semaphorin-plexin interactions (Liu et al., 2010, and see Bowden et al. in this issue). In each of these examples the relationship between the virus protein and its cellular counterpart(s) was deduced from comparison of primary amino acid sequence and prompted specific experiments to test function. However, in some other examples below, no such primary sequence similarity was evident and protein structure provided inference about possible function.

2.1. Poxvirus inhibition of TNFα – CrmE and Tanapoxvirus protein 2

Tumour necrosis factor α (TNFα) is the prototypic member of a superfamily of potent pro-inflammatory cytokines that can induce an anti-viral state and promote apoptosis in virus-infected cells (Aggarwal, 2003; Locksley et al., 2001). Poxviruses have countered the selection pressure of the TNF superfamily by evolving proteins that disrupt TNFα-induced apoptosis (Alcami, 2003; Taylor and Barry, 2006). Two distinct classes of secreted poxvirus TNFα-binding proteins have now been characterised structurally (Table 1): those that share sequence similarity to the extracellular domain of cognate host TNF receptors (TNFRs) (Cunnion, 1999; Saraiva and Alcami, 2001) and, more recently, a separate protein that resembles the mammalian MHC class I heavy chain (Brunetti et al., 2003). The structure of VACV cytokine response modifier E (CrmE), solved as part of SPINE2-Complexes, was the first crystal structure of a virus-encoded TNFR (Graham et al., 2007). CrmE shares sequence identity (30%) with the human TNFR superfamily proteins 1A (TNFRSF1A), and the structure of CrmE (Figs. 1 and 2) adopts the canonical fold of the TNFR family of proteins, comprising three cysteine-rich domains (CRDs 1–3). Each CRD contains three intra-domain disulphide bonds (Fig. 2). The structure of CrmE has shown that only one of the two ligand-binding loops present in human TNF receptors are conserved in this viral counterpart. This has provided a structural basis for understanding the higher affinities of poxvirus TNFRs for the cytokine TNFα over other cytokines such as lymphotoxin-α (Graham et al., 2007), which is supported by the recent structure of TNFR2 in complex with TNFα (Mukai et al., 2010). In contrast to VACV CrmE, the TPXV protein 2 binds TNFα to inhibit host antiviral responses yet does not share sequence similarity to host TNFRs (Brunetti et al., 2003). The recent structure of a TPXV 2-TNFα complex has revealed that the TPXV protein 2 adopts an MHC class I structural fold but lacks a peptide binding groove for antigen presentation (Yang et al., 2009). The high-affinity binding between TPXV protein 2 and TNFα (Brunetti

Table 1
Structures of poxvirus immunomodulators present in the PDB.

	Description	References	PDB ID
Extracellular immunomodulators			
A41	Chemokine inhibitor	Bahar et al. (2008)	2VGA
CPXV vCCI	Chemokine inhibitor	Carfi et al. (1999)	1CQ3
Rabbitpox virus vCCI	Chemokine inhibitor (complex)	Zhang et al. (2006)	2FIN
ECTV vCCI	Chemokine inhibitor	Arnold and Fremont (2006)	2GRK
CrmE	TNF-α inhibitor	Graham et al. (2007)	2UWI
TPXV 2	MHC-like TNF-α inhibitor	Yang et al. (2009)	3IT8
IL-18 BP	Blocks IL-18 binding to IL-18R	Krumm et al. (2008)	3F62
IFN-gamma BP	Blocks IFNγ - binding to IFN-γR	Nuara et al. (2008)	3BES
A39	Semaphorin 7A mimic	Liu et al. (2010)	3NVX
Intracellular immunomodulators			
N1	Bcl-2–like inhibitor of apoptosis and toll-like receptor signalling to NF-κB	Cooray et al. (2007); Aoyagi et al. (2007)	2UXE 2I39
B14	Bcl-2–like inhibitor of NF-κB	Graham et al. (2008)	2VVY
A52	Bcl-2–like inhibitor of NF-κB	Graham et al. (2008)	2VVW
K7	Bcl-2–like inhibitor of NF-κB and IFN-β	Oda et al. (2009)	3JRV
F1	Bcl-2–like anti-apoptotic	Kvansakul et al. (2008)	2VTY
M11	Bcl-2–like anti-apoptotic	Kvansakul et al. (2007)	2JBY
K3	Inhibits PKR mediated phosphorylation of eIF2α	Dar and Sicheri (2002)	1LUZ
VH1	Dephosphorylates STAT-1, blocks expression of IFN induced genes (ISGs)	Koksal et al. (2009)	3CM3

Structures highlighted in bold were determined as part of SPINE2-Complexes.