**Review**

**Correspondence**

R. Mark L. Buller
bullerrm@slu.edu

---

### Ectromelia virus: the causative agent of mousepox

David J. Esteban\(^1\) and R. Mark L. Buller\(^2\)

\(^1\)University of Victoria, Department of Biochemistry and Microbiology, PO Box 3055 STN CSC, Victoria BC, Canada V8W 3P6

\(^2\)St Louis University Health Sciences Center, Department of Molecular Microbiology and Immunology, 1402 S. Grand Blvd, St Louis, MO 63104, USA

**Ectromelia virus** (ECTV) is an orthopoxvirus whose natural host is the mouse; it is related closely to *Variola virus*, the causative agent of smallpox, and *Monkeypox virus*, the cause of an emerging zoonosis. The recent sequencing of its genome, along with an effective animal model, makes ECTV an attractive model for the study of poxvirus pathogenesis, antiviral and vaccine testing and viral immune and inflammatory responses. This review discusses the pathogenesis of mousepox, modulation of the immune response by the virus and the cytokine and cellular components of the skin and systemic immune system that are critical to recovery from infection.

---

**Introduction**

Smallpox was once the most serious disease faced by humankind. Through international efforts, global eradication was achieved and no naturally occurring infections have been reported since 1977 (Fenner *et al*., 1988). The causative agent, *Variola virus* (VARV), is secured in repositories at the Centers for Disease Control (Atlanta, GA, USA) and Vector Institute (Koltsovo, Russia). However, the possibility of clandestine stocks held by rogue nations or terrorist groups has in recent years increased the attention given to the orthopoxviruses in general and VARV in particular. The genus *Orthopoxvirus* comprises related viruses that are classified based on genetic similarity, as well as cross-protection following vaccination and cross-neutralization by antibodies developed following infection. The genus includes *Vaccinia virus* (VACV) (the virus used as the smallpox vaccine), *Cowpox virus* (CPXV) (believed to be the virus used by Edward Jenner in the first recorded successful vaccination), *Monkeypox virus* (MPXV) (an emerging zoonosis with disease in humans similar to smallpox) and *Ectromelia virus* (ECTV).

Infectious ectromelia was identified in 1930 when the mouse was first introduced as an experimental laboratory animal (Marchal, 1930). Wild populations of mice and other rodents in Europe are suspected to be infected naturally with ECTV and the virus is transmitted easily among wild- and laboratory-mouse populations under experimental conditions (Fenner, 1981). Mice that survive the acute phase of disease develop a pustular rash, reminiscent of smallpox in humans. The genetic similarity of ECTV and VARV, the common features of the resulting disease and the convenience of the mouse as a laboratory animal have led to the use of ECTV infections in mice as a model of smallpox and exanthematous diseases in general. Furthermore, studies using ECTV have helped to elucidate critical mechanisms of viral pathogenesis and host defence, such as the importance of cytotoxic T cells (Tc cells) in recovery from viral infections, nitric oxide as a host-defence molecule (Karupiah *et al*., 1993) and elucidation of viral immune-evasion mechanisms (Alcamí, 2003). Mousepox is also a useful model for testing orthopoxvirus antivirals and vaccines (Buller *et al*., 2004).

**ECTV strains**

The original Hampstead strain of ECTV was discovered in 1930 in a laboratory-mouse colony (Marchal, 1930). Since then, other ECTV strains have been isolated from outbreaks in Europe and the USA, with differences in disease severity (Osterhaus *et al*., 1981; Osterrieder *et al*., 1994). ECTV strain Moscow (Mos) is the most virulent, isolated by V. Sololiev and first described by Andrews & Elford (1947). ECTV strain Naval (Nav) was isolated from an outbreak in a US naval research facility, which manifested as a lethal disease in BALB/c mice and a mild disease with low morbidity and mortality in CD-1 mice (Dick *et al*., 1996). The source of the agent was identified as commercial mouse serum from retired breeder mice of multiple backgrounds, including the laboratory and pet trade. Although the USA is generally believed to be mousepox-free, this suggests a possible North American reservoir of ECTV unless the virus was introduced from mouse sera obtained from non-domestic sources.

The genomes of ECTV-Nav and ECTV-Mos have been sequenced and are approximately 208 and 210 kbp, respectively (Chen *et al*., 2003) (http://www.sanger.ac.uk/Projects/Ectromelia_virus/) and show 99.5% similarity. The genomes of numerous poxviruses have been sequenced [available with bioinformatics analysis tools at www.poxvirus.org](http://www.poxvirus.org)
(Lefkowitz et al., 2005) and www.virology.ca and the family is characterized by a similar overall organization with highly conserved, essential genes in the centre (mostly involved in virus replication) and unique genes in the terminal regions (many involved in virus–host interactions) (Fig. 1). Most of the differences between ECTV-Mos and ECTV-Nav are close to, or within, the inverted terminal repeats at the extreme termini of the genome, a region containing a concatamer-resolution motif (required for DNA replication), the terminal hairpin and some repeating and non-repeating sequences (Chen et al., 2003). Major differences are seen in only three open reading frames (ORFs; Fig. 1).

Within all the orthopoxviruses, including ECTV, the central 100 kbp region is >90% similar (Gubser et al., 2004). Overall, the orthopoxviruses are remarkably well-conserved compared with other members of the subfamily Chordopoxvirinae, suggesting that they diverged only recently from their common ancestor. Phylogenetic analysis of highly conserved nucleotide sequences from the centre of the genome places ECTV in a unique branch of the orthopoxviruses, not grouping with any other virus (Gubser et al., 2004). Analysis of the terminal regions, however, shows that the right-hand end of the genome is more similar to the VACV branch than to other orthopoxviruses, perhaps suggesting that recombination between viruses occurred at some point in their evolutionary history. ORFs encoding immunomodulatory proteins from diverse ECTV isolates show high conservation (Ribas et al., 2003).

**The skin: primary site of ECTV infection**

The natural route of infection is believed to be through abrasions in the skin and virus transmission may occur directly from animal to animal or via fomites, such as contaminated bedding in a mouse-holding facility (Marchal, 1930; Fenner, 1981). Experimentally, this is mimicked most often by the footpad route of infection. Replication in the epidermis and release of viral progeny from the initial site of infection results in spread to the lymph nodes, bloodstream and other organs (Fig. 2). The steps in the progression of disease were reviewed by Buller & Palumbo (1991).

Although little is known about the specific events surrounding ECTV infection in the skin, it is possible to speculate about the environment in which the virus finds itself.
itself and the host processes that are probably critical at this stage of the viral life cycle. The skin contains a large number of specialized cell types that are capable of responding to infectious agents by first expressing, and then responding to, a wide array of signalling molecules. This induced signalling circuitry is tailored to the type of pathogen, occurs in both the epidermis and dermis and is instructive for the adaptive immune response (Williams & Kupper, 1996). The epidermis probably generates the majority of signalling molecules of the skin, as it contains most of the cells. The major epidermal cells of the mouse are keratinocytes (90 %), melanocytes (2–5 %), Langerhans cells (LCs) (2–4 %) and dendritic epidermal T cells (DETCs) (1–2 %). By weight of numbers, their responsiveness to perturbations in the epidermal environment and their ability to make a wide variety of chemokines and cytokines, keratinocytes are one of the key cells in the ‘skin immune system’ (Streilein, 1983).

Microabrasions provide direct access for ECTV to the lower layers of the epidermis and dermis, where the virus initiates infection (Fig. 3) (Roberts, 1962). These abrasions probably cause keratinocyte injury with the release of intracellular stores of interleukin (IL) 1β, proIL1β and proIL18, and the induction of a cutaneous wound-healing response with the associated production of growth factors and additional IL18 (Kämpfer et al., 1999, 2000). Thus, ECTV infection occurs in tissue that is already reactive, although the exact nature of the cytokine milieu encountered awaits direct experimental study. Keratinocytes activated by epidermal inflammatory cytokines generated in the initial stages of infection and wound healing express genes for growth factors [granulocyte–macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF)], chemokines [C-C (MCP-1) and C-X-C (IL8)], cytokines [IL1x, IL1β, IL6, IL7, IL10, IL12, IL15, IL18 and tumour necrosis factor alpha (TNF-α)] and interferons (IFN-α, β and γ) (Williams & Kupper, 1996; Uchi et al., 2000; Gröne, 2002). These cytokines act in a paracrine and autocrine manner to amplify the initial inflammatory response to include proximal keratinocytes and cells in the dermis (Murphy et al., 2000; Gröne, 2002).

Keratinocytes can be activated by environmental stimuli, including UV radiation, toxic chemicals and direct trauma, or following microbial infections through interactions with pathogen-associated molecular patterns, such as cellsurface Toll-like receptors (TLRs) (Uthaisangsook et al., 2002). TLRs, which are expressed in a wide variety of cells, are capable of recognizing conserved viral components, such as double-stranded RNA (TLR-3) or unmethylated CpG DNA (TLR-9), and are known to be activated in response to viral infections (Morrison, 2004). Through TLR-3 and TLR-4, an antiviral response is activated that results in expression of IFN and other innate antiviral genes (Doyle et al., 2002, 2003).

DETCs are a γ/δ T-cell population exhibiting restricted T-cell receptor (TCR) diversity, with important roles in wound healing and possibly defence against pathogens. In the context of wound healing, DETCs produce IFN-γ and keratinocyte growth factor (Williams & Kupper, 1996; Hayday, 2000). Melanocytes are another minor cell population in the epidermis that may contribute to the host response through phagocytosis and by secreting a limited repertoire of cytokines (IL1 and IL6) on activation.

http://vir.sgmjournals.org
Fig. 3. The cytokine and cellular network in the skin in response to infection. Infection by ECTV occurs through breaks in the skin, initiating a cascade of cytokines, chemokines and cellular activation. The cascade is further amplified by the autocrine and paracrine pro-inflammatory activities of the secreted cytokines and chemokines that activate resident and infiltrating cells, as well as vascular endothelial cells. Cytokines or chemokines in red indicate those that are predicted to be specifically inhibited by ECTV proteins. Langerhans cells in the epidermis become activated and carry viral antigen to the lymph nodes, where they can initiate an antigen-specific immune response. Circulating lymphocytes adhere to activated vascular endothelial cells and enter the infected tissue by diapedesis. Dashed black lines indicate secretion of soluble inflammatory mediators. Solid black or white lines indicate cellular movements. The dashed white box indicates additional resident cells that respond to and contribute to the cytokine cascade. See text for full details.

(Mackintosh, 2001). Mediators GM-CSF, IL1, IFN-α/β, TNF-α, IL18 and IL10/IL12 activate LCs that are constantly sampling their environment for antigens (Murphy et al., 2000). Activated LCs containing endogenous (virus infection) or exogenous (uptake of apoptotic bodies and necrotic cells, macropinocytosis and endocytosis) viral antigens downregulate E-cadherin-mediated adhesion to keratinocytes. The LCs then traverse the basement membrane and migrate through the dermal extracellular matrix to the afferent lymphatics and, from there, reach the draining lymph node (Jakob et al., 2001). As a result of LC migration, both VACV and Molluscum contagiosum virus (MOCV) skin lesions are associated with low densities of LCs (Bhawan et al., 1986; Hernando et al., 1994). In the lymph node, LCs present viral antigens to naive T and B cells or, if ECTV infection compromises their antigen-presentation function,
antigens may present by cross-priming mechanisms (Bronte et al., 1997; Engelmayr et al., 1999; Drillien et al., 2000; Norbury & Sigal, 2003).

The cytokines and chemokines produced in the epidermis are thought to pass through the basement membrane and act on dermal cells and the endothelium of the post-capillary venules (Kondo et al., 1997). The post-capillary venules are major sites of diapedesis of inflammatory cells. Epidermal IFN-γ, TNF-α, IL1 and IL18 activate dermal fibroblasts to secrete TNF-α, IL8 and IL6 and, in turn, this mix of cytokine and chemokines activates dermal macrophages, dendritic cells and mast cells associated with the microvasculature (microvascular units) (Streilein, 1983). Activated dermal macrophages secrete IL1β, IL6, IL12, IL18, TNF-α, GM-CSF, IFN-β, nitric oxide and IFN-γ (Stoy, 2001).

Modulation of the immune response by ECTV

Intradermal infection of the ear of a disease-susceptible A/NCr mouse strain results in skin lesions without the apparent development of an inflammatory response. The lesions lack infiltrating inflammatory cells in the dermis and there is no pooling of inflammatory cells in the capillaries, despite detectable viral antigen (Fig. 4). The failure to observe a host response is probably due to the intact vasculature and virus-encoded host-response modifiers (HRMs; Table 1). The primary mechanism of action of HRMs is by disrupting receptor–ligand interactions, but also occurs through inhibiting cytokine secretion or modulating post-ligation signalling. This probably results in a lower concentration of primary and secondary cytokines in the epidermis and dermis and a failure to generate optimal gradients of chemokines to direct first inflammatory cells [such as macrophages, neutrophils and possibly natural killer (NK) cells] and then immune T cells to the site of infection (Fig. 3). Thus, the main function of HRMs may be to attenuate the innate local responses to infection in the skin, as replication at the primary site of infection is probably sufficient for transmission of progeny virus to contact mice and completion of the virus life cycle. A second type of lesion is occasionally seen at later time points in infection, showing extensive necrosis and modest cellular influxes. As endothelial-cell function is compromised by viral or host toxic effects, vessel haemorrhage may lead to ‘direct access’ of host inflammatory immune cells to infected tissue.

Inhibition of inflammatory cytokines and chemokines

IL1 induces a broad range of cytokines with systemic and local effects and plays a primary role in initiating the inflammatory response to infection. The activities of IL1α and IL1β generally overlap, as they are bound by the same cellular receptors with high affinity. A secreted protein expressed in ECTV-infected cells, vIL1βR, which is similar in sequence to the extracellular domain of the type II receptor, inhibits binding of IL1 to the receptor. Unlike the receptor, vIL1βR is specific for IL1β and shows no interaction with mouse or human IL1α (Smith & Alcamí, 2000). Soluble IL1α-binding activity has not been identified in media from cells infected with ECTV or other orthopoxviruses, perhaps suggesting that, despite their functional similarities, IL1β may play a more important role in defence against infection. Furthermore, orthopoxvirus vIL1βRs do not bind IL1RA, a natural competitive inhibitor of IL1 activity, demonstrating the adaptation of the virus to the host response by avoiding interference with the endogenous antagonist (Alcamí & Smith, 1992). This binding pattern is shared by the VACV and CPXV vIL1βR homologues (Alcamí & Smith, 1992; Spriggs et al., 1992) and the molecular basis for this specificity is unidentified. There are, however, some important differences between the orthopoxvirus vIL1βRs. Although the binding patterns are similar, the total amount of IL1β-binding activity is significantly lower in medium from cells infected with
Table 1. Orthopoxvirus host-response modifier (HRM) gene families represented in ECTV

NA: Gene disrupted in ECTV-Mos. References in bold discuss ECTV genes specifically; non-bold references refer to other orthopoxviruses.

<table>
<thead>
<tr>
<th>ORF</th>
<th>Family name</th>
<th>Proposed function</th>
<th>Localization*</th>
<th>Gene conservation†</th>
<th>Orthologue</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>001/172</td>
<td>VCKBP</td>
<td>Binds CC chemokines</td>
<td>Secreted</td>
<td>15</td>
<td>VACV-Cop C23L</td>
<td>Graham et al. (1997); Smith et al. (1997); Smith &amp; Alcamí (2000)</td>
</tr>
<tr>
<td>003/170</td>
<td>TNF receptor (CrmD)</td>
<td>Binds and inhibits TNF, LT-α</td>
<td>Secreted</td>
<td>4</td>
<td>CPXV-BR-221</td>
<td>Smith et al. (1997); Loparev et al. (1998); Smith &amp; Alcamí (2000)</td>
</tr>
<tr>
<td>008</td>
<td>TNF receptor (similar to CrmB)</td>
<td>Binds and inhibits TNF</td>
<td>Secreted</td>
<td>13</td>
<td>CPXV-BR 013</td>
<td>Smith et al. (1996); Alcamí et al. (1999)</td>
</tr>
<tr>
<td>009</td>
<td>vCD30</td>
<td>CD30 homologue, binds CD30L</td>
<td>Secreted</td>
<td>4</td>
<td>CPXV-BR 015</td>
<td>Saraiva &amp; Alcamí (2001); Panus et al. (2002)</td>
</tr>
<tr>
<td>011</td>
<td>IL1-receptor antagonist</td>
<td>Peptide has antagonist activity</td>
<td>Cytoplasmic</td>
<td>16</td>
<td>VACV-Cop C10L</td>
<td>Alcamí et al. (1999)</td>
</tr>
<tr>
<td>012</td>
<td>Ubiquitin ligase</td>
<td>E3 ubiquitin ligase/apoptosis inhibition</td>
<td>Virus factories</td>
<td>12</td>
<td>CPXV-BR 023</td>
<td>Upton et al. (1994); Senkevich et al. (1994); Senkevich et al. (1995); Brick et al. (2000); Huang et al. (2004); Nerenberg et al. (2005)</td>
</tr>
<tr>
<td>013</td>
<td>IL18-binding protein</td>
<td>Blocks IL18 activity</td>
<td>Secreted</td>
<td>13</td>
<td>VARV-Bsh D7L</td>
<td>Novick et al. (1999); Born et al. (2000); Caldera et al. (2001); Symons et al. (2002a); Esteban &amp; Buller (2004); Esteban et al. (2004)</td>
</tr>
<tr>
<td>017</td>
<td>Complement binding (EMICE/VCP)</td>
<td>Complement control</td>
<td>Secreted</td>
<td>14</td>
<td>VACV-Cop C3L</td>
<td>Kotwal &amp; Moss (1988); Isaacs et al. (1992)</td>
</tr>
<tr>
<td>019</td>
<td>IκB-kinase inhibitor</td>
<td>Inhibition of TLR, TNF signalling</td>
<td>Cytoplasmic</td>
<td>16</td>
<td>VACV-Cop N1L</td>
<td>Kotwal et al. (1989); Bartlett et al. (2002); DiPerna et al. (2004)</td>
</tr>
<tr>
<td>022</td>
<td>NF-κB inhibition</td>
<td>Inhibition of IκB degradation</td>
<td>Cytoplasmic</td>
<td>16</td>
<td>VACV-Cop K1L</td>
<td>Chen et al. (1993); Shibler &amp; Jin (2004)</td>
</tr>
<tr>
<td>023</td>
<td>Serpin (SPI-3)</td>
<td>Serine protease inhibitor</td>
<td>Membrane/virion</td>
<td>16</td>
<td>VACV-Cop K2L</td>
<td>Law &amp; Smith (1992); Zhou et al. (1992); Turner &amp; Moyer (1995); Wallich et al. (2001)</td>
</tr>
<tr>
<td>025</td>
<td>Apoptosis inhibitor</td>
<td>Inhibition of apoptosis by blocking release of cytochrome c</td>
<td>Mitochondrion-associated</td>
<td>16</td>
<td>VACV-Cop F1L</td>
<td>Wasielenko et al. (2003); Stewart et al. (2005)</td>
</tr>
<tr>
<td>043</td>
<td>IFN resistance</td>
<td>Inhibits activity of PKR</td>
<td>Cytoplasmic</td>
<td>16</td>
<td>VACV-Cop E3L</td>
<td>Chang et al. (1992); Beattie et al. (1996); Langland &amp; Jacobs (2002)</td>
</tr>
<tr>
<td>083</td>
<td>Tyr/Ser phosphatase</td>
<td>Blocks IFN signalling</td>
<td>Cytoplasmic</td>
<td>16</td>
<td>VACV-Cop H1L</td>
<td>Najarro et al. (2001)</td>
</tr>
<tr>
<td>138</td>
<td>CD47-like</td>
<td>Similar to mammalian integrin-associated protein</td>
<td>Membrane</td>
<td>16</td>
<td>VACV-Cop A38L</td>
<td>Parkinson et al. (1995); Sanderson et al. (1996); Cameron et al. (2005)</td>
</tr>
<tr>
<td>139</td>
<td>Semaphorin</td>
<td>Blocks phagocytosis and cell migration</td>
<td>Secreted</td>
<td>13</td>
<td>VACV-Cop A39R</td>
<td>Comeau et al. (1998); Gardner et al. (2001); Walzer et al. (2005a, b)</td>
</tr>
<tr>
<td>140</td>
<td>Unknown</td>
<td>Attenuates inflammatory-cell influx</td>
<td>Secreted</td>
<td>16</td>
<td>VACV-Cop A41L</td>
<td>Ng et al. (2001)</td>
</tr>
<tr>
<td>ORF</td>
<td>Family name</td>
<td>Proposed function</td>
<td>Localization*</td>
<td>Gene conservation†</td>
<td>Orthologue</td>
<td>Reference(s)</td>
</tr>
<tr>
<td>-----</td>
<td>-------------</td>
<td>-------------------</td>
<td>---------------</td>
<td>-------------------</td>
<td>------------</td>
<td>--------------</td>
</tr>
<tr>
<td>143</td>
<td>Hydroxysteroid dehydrogenase</td>
<td>Attenuates inflammatory response</td>
<td>Cytoplasmic</td>
<td>16</td>
<td>VACV-Cop A44L</td>
<td>Moore &amp; Smith (1992); Šroller et al. (1998); Reading et al. (2003a)</td>
</tr>
<tr>
<td>144</td>
<td>Superoxide dismutase-like</td>
<td>Lacks catalytic activity</td>
<td>Virus factories/virion</td>
<td>16</td>
<td>VACV-Cop A45R</td>
<td>Almazán et al. (2001); Cao et al. (2002); Teoh et al. (2003)</td>
</tr>
<tr>
<td>145</td>
<td>Similar to Toll/IL1 receptor (TIR) domain</td>
<td>Inhibition of TIR domain-dependent signalling</td>
<td>Cytoplasmic</td>
<td>16</td>
<td>VACV-Cop A46R</td>
<td>Bowie et al. (2000)</td>
</tr>
<tr>
<td>158</td>
<td>IFN-γ-binding protein</td>
<td>Blocks IFN-γ interaction with receptor</td>
<td>Secreted</td>
<td>15</td>
<td>VACV-Cop B8R</td>
<td>Upton et al. (1992); Mossman et al. (1995); Smith &amp; Alcamì (2002); Bai et al. (2005); Alcamì &amp; Smith (1995)</td>
</tr>
<tr>
<td>161</td>
<td>CrmA/SPI-2 (Serpin)</td>
<td>Inhibition of apoptosis</td>
<td>Cytoplasmic</td>
<td>15</td>
<td>VACV-Cop B14R</td>
<td>Palumbo et al. (1989); Ray et al. (1992); Turner et al. (2000); Wallich et al. (2001)</td>
</tr>
<tr>
<td>163</td>
<td>IL1β-binding protein</td>
<td>Blocks IL1β interaction with receptor</td>
<td>Secreted/membrane</td>
<td>13</td>
<td>VACV-Cop B16R</td>
<td>Alcamì &amp; Smith (1992); Spriggs et al. (1992); Smith &amp; Alcamì (2000)</td>
</tr>
<tr>
<td>166</td>
<td>IFN-α/β-binding protein</td>
<td>Inhibition of IFN-α activity</td>
<td>Secreted</td>
<td>15</td>
<td>VACV-Cop B19R</td>
<td>Colamonici et al. (1995); Symons et al. (1995); Smith &amp; Alcamì (2002)</td>
</tr>
<tr>
<td>168</td>
<td>Serpin (SPI-1)</td>
<td>Serine protease inhibitor</td>
<td>Cytoplasmic</td>
<td>15</td>
<td>VACV-Cop C12L</td>
<td>Upton et al. (1990); Kettle et al. (1995); Shisler et al. (1999); Wallich et al. (2001)</td>
</tr>
<tr>
<td>NA</td>
<td>eIF-2x homologue</td>
<td>PKR inhibition</td>
<td>Cytoplasmic</td>
<td>14</td>
<td>VACV-Cop K3L</td>
<td>Davies et al. (1992); Langland &amp; Jacobs (2002)</td>
</tr>
<tr>
<td>NA</td>
<td>CrmE</td>
<td>TNFR homologue</td>
<td>Secreted/membrane</td>
<td>1</td>
<td>CPXV-GRI K3R</td>
<td>Saraiva &amp; Alcamì (2001); Reading et al. (2002)</td>
</tr>
<tr>
<td>NA</td>
<td>TLR/IL1R signalling inhibitor</td>
<td>Inhibition of NF-κB</td>
<td>Cytoplasmic</td>
<td>9</td>
<td>VACV-Cop A52R</td>
<td>Harte et al. (2003)</td>
</tr>
<tr>
<td>NA</td>
<td>CrmC</td>
<td>TNFR homologue</td>
<td>Secreted</td>
<td>8</td>
<td>CPXV-BR 191</td>
<td>Smith et al. (1996)</td>
</tr>
</tbody>
</table>

*Experimentally determined or predicted protein localization.
†No. (out of 16) fully sequenced orthopoxviruses that express an orthologue.
‡Present in ECTV-Nav (gene 174).
ECTV than that from cells infected with VACV or CPXV. This could be the result of lower expression levels or of lower-affinity binding caused by the differences in amino acid sequence; the ECTV vIL1/βR sequence is 82 and 83 % similar to the VACV and CPXV proteins, respectively, whilst the latter two are 92 % similar to each other. Alternatively, it is possible that the majority of the protein remains membrane-bound. PSORT predicts the protein to be membrane-associated, due to an uncleaved signal sequence (Chen et al., 2000).

The contribution of the ECTV vIL1/βR to pathogenicity in the mousepox model is not known and is difficult to predict, based on contrasting results using VACV by different routes of infection. Mice infected intranasally with vIL1/βR- VACV show increased morbidity and weight loss and succumb to infection 1 day earlier than wild type-infected animals, implicating IL1/β as a contributor to pathogenesis (Alcamí & Smith, 1992), and blocking IL1/β activity may help virus replication or transmission by dampening the inflammatory response and thus improving the overall health of the infected animal. In contrast, intracranial inoculation with vIL1/βR- VACV results in decreased pathogenicity compared with the wild type, suggesting that IL1/β activity may be a contributor to disease in this route of infection (Spriggs et al., 1992). Furthermore, intradermal infection shows no difference between the wild type and the deletion mutant (Tscharke et al., 2002).

IL18, a member of the IL1 family of cytokines, is expressed by keratinocytes, macrophages and other cell types in the mouse epidermis (Stoll et al., 1997; Kämpfer et al., 1999). It has potent pro-inflammatory activity through its induction of IFN-γ and other cytokines and chemokines, and is an activator of NK cells (Okamura et al., 1995; Lauwersy et al., 1999). IL18 activity is targeted by ECTV via the IL18-binding protein (BP) p13. The immune response to ECTV deficient in p13 expression shows heightened NK-cell activity and increased IFN-γ levels, demonstrating its ability to downregulate the function of IL18 (Born et al., 2000). Studies using a VACV IL18BP knockout showed similar results in an intranasal model of infection (Symons et al., 2002a; Reading & Smith, 2003). A member of the IL18BP family, p13 is a 13 kDa secreted protein with high sequence similarity to IL18BPs found in other poxviruses, as well as humans and mice, but bears no significant sequence similarity to the cellular receptor (Aizawa et al., 1999; Novick et al., 1999). A number of orthopoxviruses, as well as MOCV, have been shown to encode active IL18BPs (Xiang & Moss, 1999; Born et al., 2000; Smith et al., 2000; Calderara et al., 2001; Esteban & Buller, 2004). Mutagenesis studies of p13, MC54L and hull18BP have identified critical residues involved in ligand binding and demonstrated that the binding mode is conserved within the family (Xiang & Moss, 2001a, b; Esteban et al., 2004).

In addition to inhibition of IL1 and IL18 at the level of receptor engagement, ECTV-encoded proteins can block maturation of these cytokines prior to release or block ligand-induced signalling in the infected cell. IL1 and IL18 are expressed as inactive precursors in the cytoplasm, which are processed to the mature form by caspase 1 following appropriate stimulation (Ghayur et al., 1997; Gu et al., 1997). The serine protease inhibitor (serpin) SPI-2 has been shown to inhibit the proteolytic activity of caspases 1 and 8 in in vitro assays (Turner et al., 2000). Although it has not been demonstrated that SPI-2 can block the processing of IL18, the CPXV orthologue, CrmA, can inhibit maturation and release of both IL1/β and IL18 (Ray et al., 1992; Fujino et al., 2003). Despite the presence of IL18 mRNA and protein in keratinocytes, the primary site of poxvirus replication in the skin, expression of caspase 1 in these cells remains controversial (Stoll et al., 1997; Kämpfer et al., 1999; Companjen et al., 2000; Mee et al., 2000; Koizumi et al., 2001). Bioactive IL18 is present in the epidermis in the context of the wound-healing response and is detectable in keratinocytes in culture, but definitive demonstration of caspase 1 expression is elusive and the possibility of alternative processing mechanisms remains. Therefore, the role of SPI-2 inhibition of IL18 maturation in ECTV infection in vivo awaits clarification.

IL18R, IL1R and TLR have a common intracellular domain, the Toll-like/IL1 receptor (TIR) domain, involved in protein interactions with signalling components. The signalling cascade results in the activation of inflammatory-cytokine expression through NF-κB and interferon regulatory factor-3 (IRF3). Signalling can be blocked at multiple stages by the VACV gene products A52R, A46R, K1L and N1L. A52R blocks signalling by acting as an inhibitor of the adapter MyD88 and an inhibitor in IRAK2, TRAF6 and NF-κB activation (Bowie et al., 2000). However, this mechanism of host interference is probably not active in ECTV, because the A52R gene is fragmented (Chen et al., 2003). The A46R protein of VACV also inhibits TLR and IL1R signalling by binding four TIR adaptors involved in signalling, including MyD88, and deletion of A46R results in attenuation in an intranasal model of infection (Bowie et al., 2000; Stack et al., 2005). The orthologue in ECTV is predicted to be full-length and, therefore, A46R may be more important in blocking signalling in ECTV infections. A third VACV protein, N1L, inhibits activation of NF-κB and IRF3 (DiPerna et al., 2004) and the ECTV orthologue is 93 % similar, but its function has not been tested experimentally. Finally, VACV-K1L has recently been shown to inhibit NF-κB by preventing IkB degradation (Shisler & Jin, 2004) and ECTV encodes an orthologue that is 97 % similar.

Another important early cytokine involved in the antiviral response is TNF-α. The effects of TNF-α are mediated through two receptors, p55 and p75. Receptor-knockout mice on a genetically resistant background show severe signs of disease and mortality when infected with ECTV (Ruby et al., 1997). Virus is detectable in spleen and liver, whilst in wild-type mice, virus replication is limited to the site of infection. Both TNF-α receptors appear to play a role
in the antiviral activity of TNF-\(\alpha\); however, mice deficient in p75 or both p75 and p55 show a greater defect in control of infection. This suggests that membrane-bound TNF-\(\alpha\), the preferred ligand of receptor p75, may have an important antiviral role. Viral inhibition of TNF-\(\alpha\)-receptor engagement is mediated by the \(vTNFR\), a secreted receptor homologue. ECTV expresses soluble TNF-\(\alpha\)-binding activity, encoded by the \(CrmD\) homologue that is also present in other orthopoxviruses (Loparev et al., 1998; Smith & Alcami, 2000). Other \(vTNFR\) genes in CPXV, \(CrmC\) and \(CrmE\), are fragmented in ECTV and \(CrmB\) appears to be missing, although the product of ORF 008 is related (42\% similarity) to the CrmB family (Chen et al., 2003). Deletion of \(CrmE\) from VACV results in attenuation in an intranasal model of infection (Reading et al., 2002). Competition experiments revealed that \(vTNFR\) has broad species binding; however, its ability to bind LT-\(\alpha\) was inconsistent among published studies (Loparev et al., 1998; Smith & Alcami, 2000). \(CrmD\) shows higher sequence similarity to p75 than p55, again implicating a more important role for membrane-bound TNF-\(\alpha\) in defence against ECTV infection.

ECTV encodes another member of the TNFR superfamily, a secreted CD30 homologue. The exact role of CD30 in antiviral immunity is not clear, but it is expressed on various cell types, including B cells, T cells and macrophages. Cellular CD30L (CD153) is a membrane-bound protein that induces reverse signalling (Saraiva et al., 2002). Despite its ability to compete with cellular CD30 for ligation, \(vCD30\) does not behave as a true decoy receptor because it is also capable of inducing signalling in CD30L-expressing cells. In vivo, recombinant \(vCD30\) is capable of down-regulating a type 1 cytokine-mediated granuloma in response to mycobacteria. It has been hypothesized that CD30–CD153 signalling between \(\gamma/\delta\) T cells and activated macrophages may be a target of \(vCD30\) in CPXV infection (Panus et al., 2002).

ECTV, as well as other orthopoxviruses and chordopoxviruses, expresses a soluble inhibitor of C-C chemokines. The viral chemokine-binding protein (\(vCC\)-CKBP) is the most abundant secreted protein of ECTV (Chen et al., 2000). It is a 35 kDa protein that bears no sequence similarity to the cellular seven transmembrane-domain chemokine receptor, but is nonetheless capable of high-affinity binding. The protein is well-conserved among the orthopoxviruses; the ECTV protein has \(>85\%\) similarity to the homologue in CPXV, and all ECTV strains express this protein except Mill Hill, which has an early stop codon within the orthologue (Smith & Alcami, 2000). The role of \(vCC\)-CKBP in ECTV infections has not been analysed; however, infections by MYXV or Rabbitpox virus (RPXV) lacking \(vCC\)-CKBP present with higher leukocyte infiltration into the site of infection, consistent with its ability to block receptor–chemokine interactions (Graham et al., 1997; Lalani et al., 1999). Infection of mice intranasally with a recombinant VACV-WR expressing \(vCC\), the homologous protein from VACV-Lister, shows decreased cellular influx in the lungs (Reading et al., 2003b). It would not be surprising to find a similar role for \(vCC\)-CKBP of ECTV.

Numerous studies have supported the critical importance of IFN-\(\alpha/\beta\) and IFN-\(\gamma\) in recovery from ECTV infection (Karupiah et al., 1993a). In vitro, ECTV is resistant to the antiviral effects of IFN. The activity of IFN-\(\gamma\) can be inhibited at the level of receptor engagement by \(vIFN-\gamma R\) (Mossman et al., 1995; Smith & Alcami, 2002). All orthopoxviruses tested have been shown to encode and secrete a viral homologue of the extracellular domain of the cellular IFN-\(\gamma\) receptor, except for modified vaccinia Ankara (Blanchard et al., 1998). The ECTV \(vIFN-\gamma R\) shows only 20\% sequence similarity to the host receptor; however, it is capable of binding IFN-\(\gamma\) and inhibiting its biological activity in vitro (Smith & Alcami, 2002). Unlike the receptor, ECTV \(vIFN-\gamma R\) is non-species-specific, having the capability to bind mouse, rat and rabbit IFN-\(\gamma\) with the same relative affinity (Mossman et al., 1995). Deletion of the VACV IFN-\(\gamma R\) has no effect on virulence in an intranasal model of infection, consistent with the low affinity of VACV IFN-\(\gamma R\) for mouse IFN-\(\gamma\) (Symons et al., 2002b) and the inability of VACV IFN-\(\gamma R\) to inhibit the biological activity of mouse IFN-\(\gamma\) (Alcami & Smith, 1995). IFN-\(\alpha/\beta\) establishes an antiviral state in cells that can inhibit virus replication. IFN-\(\alpha\)–binding activity is detectable on the surface of ECTV-infected cells (Colamonici et al., 1995) and an inhibitor of mouse IFN-\(\alpha\) antiviral activity is present in supernatants (Smith & Alcami, 2002). Binding activity both in the supernatants and on the surface of infected and uninfected cells is also seen in VACV and suggests that secreted \(vIFN-\alpha/\beta R\) is capable of additional interactions with structures on the cell surface (Colamonici et al., 1995; Alcami et al., 2000). Surprisingly, the \(vIFN-\alpha/\beta R\) does not inhibit the activity of mouse IFN-\(\beta\), but can bind and block human IFN-\(\beta\) activity.

**Inhibition of apoptosis**

Induction of apoptosis is an early cellular protective response to eliminate virus-infected cells and limit virus replication. Apoptosis can be induced through a variety of pathways, including binding of ligands to death-effector domain (DED)-containing transmembrane receptors, UV light and viral infection. ECTV encodes a RING finger-domain protein, p28, which blocks UV-induced apoptosis specifically by acting upstream of caspase 3, a protease central to the execution of apoptosis in response to several stimuli (Brick et al., 2000). An intact p28 gene has been identified in the majority of species of the genus *Orthopoxvirus* and representatives of the genera *Leporipoxvirus, Yatapoxvirus* and *Capripoxvirus*, although it is disrupted or deleted in the WR, Cop, MVA and Tian-Tan strains of VACV. The prominent feature of the protein is a C-terminal RING zinc finger that is minimally defined by a configuration of eight Cys (C) and His (H) residues necessary for chelation of two zinc ions (Fremont et al., 1991). A p28-deficient ECTV is defective in replication in UV-exposed cells in culture; however, it replicates normally
In unexposed cells. In vivo studies have suggested that p28 has a significant role in the natural life cycle of ECTV. Following footpad inoculation, the LD_{50} of p28^{-} virus in A/NCr mice is at least 10^{3}-fold higher and the infectivity burden in liver and spleen is 1000-fold lower than that of wild-type virus (Senkevich et al., 1994). This attenuation of ECTV pathogenicity can be explained by a failure of the virus to replicate in macrophage-lineage cells at successive steps in the spread of virus from the skin to liver (Senkevich et al., 1995). The diminished replication of p28^{-} virus in macrophages from A/NCr mice may result from inefficient suppression of apoptosis. More recently, E3 ubiquitin-ligase activity of p28 has been demonstrated, which requires the RING domain (Huang et al., 2004; Nerenberg et al., 2005). The natural target and whether the activity is associated with the anti-apoptotic effect remain to be determined.

Other viral proteins are similarly able to block apoptotic pathways in infected or neighbouring uninfected cells. TNFs can induce proinflammatory cytokines, as well as activate a cell-death response through a caspase-mediated pathway. Already discussed in the context of blocking cytokine release, the CrmA homologue SPI-2 is also capable of blocking TNF-α-mediated apoptosis through inhibition of caspases 1 and 8 (Turner et al., 2000). TNF-α-mediated apoptosis can also be inhibited by the secreted vTNFR CrmD (Loparev et al., 1998).

The cellular immune response to ECTV

The mousepox model presents an opportunity to study the components of the immune system that are required for an efficient immunological response to a natural poxvirus infection in a well-understood animal model that can be further manipulated by targeted inactivation or expression of genes. The role of the cellular components of the immune response has been investigated by using mouse strains with differing susceptibilities or depleted of cells and signalling molecules by genetic and mAb approaches. Although the humoral response may contribute to complete recovery or protection after re-exposure, a polarized type 1 response coupled with a strong cellular response are the principal immune forces responsible for recovery from a primary infection (Chaudhri et al., 2004). Resistant strains (such as C57BL/6) show more rapid, stronger and sustained cytolytic responses in the NK cell and cytotoxic T-lymphocyte (CTL) compartments, compared with susceptible strains.

A large body of evidence also supports the vital role of the cell-mediated immune response, possibly including NK cells (Jacoby et al., 1989; Delano & Brownstein, 1995; Mahalingam et al., 2001). In the absence of CD8 T cells, 100% mortality is observed and it is likely that both the cytokine contribution from these cells (especially IFN-γ) and perforin-mediated killing are responsible for their requirement (Karupiah et al., 1996). CD4 T cells, in an apparently cytolsis-independent manner, are required for full clearance of virus, especially from skin, probably through cytokines such as IFN-γ. Depletion of CD4 T cells does not affect mortality (Karupiah et al., 1996).

In addition to secretion of cytokines, the cytolytic activity of immune-effector cells contributes to the control of virus replication and recovery from infection. Granule-mediated apoptosis is a key mechanism in the elimination of infected cells by CTL and NK cells. The result of CD8^{+} Tc-cell activation through TCR engagement by antigen presented by a target cell is the synthesis of cytokines, such as IFN-γ and TNF-α, and release of granule contents, primarily perforin and granzyme (gzm). Studies using gene-knockout mice have helped to elucidate the specific contributions of the cytotoxic proteins perforin, GzmA and GzmB. Perforin appears to be more important in recovery from ECTV infection than in recovery from other orthopoxvirus infections. The LD_{50} in perforin-deficient B6 mice is 6 logs lower than that in wild-type mice, and deficient mice are also sensitive to the less virulent strain Hampstead-egg (Müllbacher et al., 1999a; Wallich et al., 2001). This finding is dramatically different from that obtained with VACV, which shows no difference in perforin-knockout mice compared with wild type, or CPXV, which shows only a slight reduction in morbidity/mortality in perforin-deficient B6 mice (Müllbacher et al., 1999a). GzmA-deficient mice are slightly more susceptible to ECTV infection, and GzmB-deficient mice are even more so; however, double-knockout mice show a more pronounced defect, with accelerated spread of the virus from the inoculation site and death as early as 4 days post-infection. GzmA and GmzB therefore apparently act synergistically to destroy infected cells.

CTL and NK cells can also kill target cells via the Fas/Fas-L pathway, which signals through caspase 8. Because of the capacity for inhibition of caspase 8 by CrmA/SPI-2, this apoptotic pathway can be inhibited in infected cells (Müllbacher et al., 1999b; Turner et al., 2000). Cells infected with CPXV and RPXV lacking CrmA/SPI-2 are more susceptible to Fas-mediated killing and slightly more sensitive to gzm-mediated alloreactive CTL lysis (Mächen et al., 1996), but ECTV-infected cells are not protected from major histocompatibility complex (MHC) class I-restricted killing, suggesting that ECTV serpin does not protect from gzm- or perforin-mediated killing (Müllbacher et al., 1999b). In support of this, SPI-2 does not inhibit GzmB activity in vitro (Turner et al., 2000).

Role of mouse genetics in ECTV susceptibility and resistance

Disease severity and outcome are influenced by multiple factors, including virus strain, dose and route of infection. Importantly, the mouse genetic background is another consideration, indicating that critical host factors are involved in regulating susceptibility and resistance. Wild mice show variable susceptibility (Buller et al., 1986), whilst laboratory strains have been useful in identifying host determinants of resistance. Overall, a polarized type 1 cytokine response, with emphasis on the importance of IFN-γ, and a potent cell-mediated immune response are characteristic of resistant mice (C57BL/6), whilst a type 2 polarized response (or lack of Th1 response) is associated
with susceptible strains (BALB/c, A/J and DBA/2) (Chaudhri et al., 2004).

Many genetic factors that control disease appear to be involved in limiting virus spread during the first few days of infection, more so than limiting virus replication itself (Brownstein et al., 1993). By crossing DBA/2 and C57BL/6 mice, loci that confer a B6-like response to infection, and therefore contribute to host resistance to severe mousepox, could be identified. The resistance to mousepox (rmp-1) locus on chromosome 6 controls replication of virus in the liver and may map to the NK gene complex NKR-P1, which functions in signal transduction and activation of NK cells (Delano & Brownstein, 1995; Brownstein & Gras, 1997). DBA/2 mice do not express members of this gene complex, whereas C57BL/6 mice do. This finding is consistent with the probable importance of the NK-cell response in defence against ECTV. Interestingly, genetic mapping of resistance to another mouse pathogen, mouse cytomegalovirus (MCMV), led to the same region of the chromosome and identification of the NK cell-activating receptor, Ly49H (which stimulates perforin release), as a critical gene for resistance to infection (Forbes et al., 1997; Arase et al., 2002). NK cells from MCMV-resistant C57BL/6 mice express Ly49H and are activated directly by ligation with the MCMV M157 protein, whilst cells from susceptible BALB/c mice lack Ly49H and thus are not activated by this mechanism during MCMV infection. The rmp-2 locus on chromosome 2 maps near the complement component C5 gene and protects females more than males (Brownstein & Gras, 1997). C5-deficient mouse strains, such as DBA/2, display impaired recruitment of circulating leukocytes to the site of infection. Rmp-3 is also gonad-dependent and is linked to the MHC (H-2) (Brownstein et al., 1992). The rmp-4 locus, located on chromosome 1, confers 100-fold higher resistance in male mice over a fully susceptible background and 500-fold higher resistance in female mice (Brownstein & Gras, 1995). Animals with the rmp-4 locus showed restricted virus replication in the spleen and, to a lesser extent, the liver. rmp-4 maps to the selectin gene complex, members of which encode type II membrane proteins expressed widely on different cell types, and may also contribute to leukocyte recruitment (Brownstein & Gras, 1997).

The cytokine-inducible nitric oxide synthase (NOS2) is another innate resistance gene. NOS2-deficient mice are highly susceptible to ECTV infection, although they display normal cellular and IFN-γ responses (Karupiah et al., 1998). The potent anti-poxviral activity of nitric oxide was established previously both in vivo and in vitro (Karupiah et al., 1993b).

Conclusions

The study of poxviruses is of great interest, due to the repertoire of proteins that they encode to modulate the host response, and presents an opportunity to enrich our understanding of the virus–host relationship. The genomic sequence of ECTV paired with the mouse model of infection has provided insight into mechanisms of disease and the complex network of cells and mediators that comprise an effective host defence. Through genetic manipulation of both the virus and host, the specific contributions of these factors to virus replication, host defence, disease and virus transmission are being determined. Furthermore, the similarity of ECTV genome to that of other poxviruses – 40 genomes of different poxvirus genera, species and strains have been sequenced – and the impact of poxviruses on human and animal health underscore the value of the ECTV–mouse model.

References


Ectromelia virus


