Review article

Vaccinia virus glycoproteins and immune evasion

The Sixteenth Fleming Lecture

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Introduction

Vaccinia virus is the vaccine that was used to eradicate smallpox. This task was accomplished in 1977 and its completion certified in 1979 by the World Health Organization (WHO) (Fenner et al., 1988). Since then poxvirus infections of humans have caused little disease and have been restricted to molluscum contagiosum and rare zoonoses such as cowpox (CPV), pseudocowpox, monkeypox, orf and yaba tumour viruses. Continued interest in vaccinia virus and other poxviruses has derived in part from the development of these viruses as cloning and expression vectors (Mackett et al., 1982; Panicali & Paoletti, 1982) that have the potential as live vaccines to combat diseases other than smallpox (Panicali et al., 1983; Smith et al., 1983a, b, 1984; Paoletti et al., 1984). This approach to vaccine development has several advantages: the stability of the freeze-dried vaccine (Collier, 1955), its low cost and ease of manufacture and administration, the ability of the vaccine to induce antibody and cytolytic T cell responses (Bennink et al., 1984; Wiktor et al., 1984), the large capacity of the virus for foreign DNA (Smith & Moss, 1983) so enabling the creation of polyvalent vaccines that simultaneously express multiple exogenous antigens (Perkus et al., 1985) and the experience of using vaccinia virus in a mass vaccination campaign. On the other hand it is clear that vaccinia virus is far from an ideal vaccine due to vaccine-related complications that it may cause (Lane et al., 1969). These include skin disorders, generalized vaccinia and neurological complications. The first two conditions are largely preventable by avoiding vaccination of those with eczema or an impaired immune system, but the neurological complications are less predictable. It is recognized that for vaccinia virus to be used again as a human vaccine its safety needs to be improved (Brown et al., 1986). With this goal in mind several laboratories are undertaking a molecular characterization of the virus with particular interest in genes influencing virulence. The deletion of such genes might produce suitably attenuated viruses. This work has led to the discovery of many vaccinia virus proteins that interfere with the host immune response and to the characterization of several virus glycoproteins which affect virus dissemination and virulence. These topics are considered in this review.

Vaccinia virus biology

Vaccinia virus is the most extensively characterized member of the orthopoxvirus genus, a group of antigenically related viruses that are cross-protective (Fenner et al., 1989). These viruses are distinguished by a large and complex virion, a dsDNA genome, a cytoplasmic site of replication and many virus-encoded enzymes.

Vaccinia virus has a large virion with dimensions of 250 by 350 nm that contains more than 100 polypeptides (Essani & Dales, 1979) and which exists in two infectious morphologically distinct forms, termed intracellular naked virus (INV) and extracellular enveloped virus (EEV) (Appleyard et al., 1971; Ichihashi et al., 1971). INV represents the majority of infectious progeny and remains within the cytoplasm of the infected cell. EEV is released from the cell and has an extra lipid envelope with 10 associated proteins which are absent from INV (Payne, 1978, 1979). Strains of virus yielding higher amounts of EEV produce comets if incubated under liquid overlay in cell culture; comets represent the unidirectional spread of virus from the primary plaque (Boulter & Appleyard, 1973) (Fig. 1). Protection against
Fig. 1. Comet formation by the IHD-J strain of vaccinia virus. BSC-1 monolayers were infected with two different dilutions of vaccinia virus strains IHD-J, Lister or Tashkent and incubated under liquid medium for 2 days before staining with crystal violet.

Virus replication takes place in the cytoplasm, an unusual site for a DNA virus. Consequently, the virus encodes its own transcriptional and DNA replicative machinery. These areas have been recently reviewed (Moss, 1990; Traktman, 1991). Gene expression is regulated at the transcriptional level. There are three classes of gene (early, intermediate and late) which are transcribed in a strictly regulated cascade with expression of each class being dependent upon prior expression of proteins of the previous class. DNA replication initiates by 2 h post-infection within cytoplasmic factories and produces concatemeric molecules which are resolved into unit-length monomers.

Virus morphogenesis is a complex process that commences within the cytoplasmic factories and involves extensive wrapping with cellular membranes. The early phase of morphogenesis is recognizable by the formation of spicule-coated lipid crescents (Fig. 2). These membranes were reported to be synthesized de novo (Dales & Pogo, 1981), but recent evidence suggests that they are formed by modification of membranes of the intermediate compartment between the endoplasmic reticulum and the Golgi stacks (Sodeik et al., 1993). They become oval, acquire an electron-dense core and mature into infectious INV. Some INV is then wrapped by a
The Sixteenth Fleming Lecture

Fig. 2. Electron micrograph of a vaccinia virus factory within the cytoplasm of infected cells showing the stages of INV formation (1 to 4). Reprinted from Rodriguez & Smith (1990).

double layer of Golgi-derived membrane. These structures migrate to the cell periphery where the outer membrane fuses with the plasma membrane releasing EEV from the cell. With the WR strain, most EEV remains associated with the cell surface and has been termed cell-associated enveloped virus (CEV) (Blasco & Moss, 1992).

Glycoproteins

The number of vaccinia virus-encoded glycoproteins is uncertain but the nucleotide sequence of the virus genome predicts that there are likely to be many (Goebel et al., 1990). These may be structural components of INV and EEV, or non-structural glycoproteins which are either secreted from the infected cell or remain cell-associated.

Structural glycoproteins

INV

Early work with vaccinia virus and other orthopoxviruses used preparations of INV because the importance of EEV had not been recognized. Thus early searches for glycosylated virion proteins examined only INV and detected one glycoprotein of 40K (Holowczak, 1970; Garon & Moss, 1971; Sarov & Joklik, 1972; Payne, 1979). This is synthesized at late times during infection and contains glucosamine but not galactose, fucose or mannose. The simplicity of the glycoprotein profile of INV is in contrast to the more complex pattern of glycoproteins that are induced within infected cells and which are presumed to be virus-encoded since they are present in different cell types after infection with vaccinia virus (Moss et al., 1971; Weintraub & Dales, 1974). Trypsin or NP40 treatment removed the 40K glycoprotein from the surface of INV and tryptic peptide analyses distinguished it from the major structural proteins of similar size (Moss et al., 1973).

Surface proteins of INV which are mapped to specific genes, such as a 14K fusion protein (Rodriguez & Esteban, 1987), a 35K protein (Gordon et al., 1988), a 32K protein with homology to carbonic anhydrase (Niles & Seto, 1988; Maa et al., 1990) and a 39K protein (Maa & Esteban, 1987), are not glycosylated. Some evidence indicates that several INV structural proteins are modified by ADP ribosylation (Child et al., 1988).

EEV

Extracellular enveloped virus was identified as virus that is naturally released from cells rather than that released by mechanical cell disruption. The discovery that EEV is antigenically distinct from INV due to the possession of an extra envelope and associated antigens, and that immunity to the EEV proteins is required for protection against orthopoxvirus infection, explained why attempts to produce effective smallpox vaccines based on inactivated INV had been unsuccessful. Studies on EEV were initially hindered by its low abundance, but this problem was overcome by growing the IHD-J strain of virus in RK13 cells, a combination which yielded 25 to 40% of total virus as EEV (Payne, 1979). This system enabled the purification of milligram amounts of EEV and the detection of 10 proteins (210K, 110K, 89K, 42K, 37K and five proteins between 20K and 24K) that are absent from INV. With the exception of the 37K protein, all of these are glycosylated (Payne, 1979).

The 89K glycoprotein is the virus haemagglutinin (HA) (Payne, 1979), that is present on the surface of the infected cell and EEV (Ichihashi & Dales, 1971; Blackman & Bubel, 1972) but absent from INV. EEV from an HA- virus, IHD-W, lacks the 89K protein, and antiserum produced after infection by IHD-W does not immunoprecipitate the 89K protein from IHD-J (HA+) virus (Shida & Dales, 1981, 1982). Direct evidence that this protein is the HA was provided by the binding of the 89K protein, but not other EEV proteins, to red blood cells (Payne, 1979) and the identification of a similar sized haemagglutinating protein in the membranes of IHD-J- but not IHD-W-infected cells (Shida & Dales, 1981, 1982). The sequence of the HA gene (A56R) shows that the protein is a class I membrane glycoprotein with presumptive transmembrane signal and anchor sequences (Shida, 1986). It is very rich in serine and threonine residues (25% of amino acids), contains five potential sites for addition of N-linked carbohydrate and
has a predicted primary translation product of only 35K. The much greater size of the mature product (89K) is consistent with its extensive modification with N- and O-linked glycans (Shida & Dales, 1981). The protein is also sulphated and phosphorylated and can form disulphide-linked complexes of 220K (Payne, 1992). More extensive computational analysis identified a single domain characteristic of the immunoglobulin (Ig) superfamily near the amino terminus (Jin et al., 1989). The HA gene is transcribed both early and late during infection from separate promoters (Brown et al., 1991).

The second EEV protein to be genetically mapped was the non-glycosylated but acylated 37K protein (Hirt et al., 1986). The gene (F13L) is transcribed late during infection and the encoded protein accumulates in Golgi membranes. The protein lacks discernible membrane signal or anchor sequences but contains two internal hydrophobic regions (amino acids 130 to 157 and 175 to 192) that possibly cause its membrane association.

Two other genes, A34R (formerly called SaIL4R in the WR strain) and B5R, each encode multiple EEV glycoproteins (Duncan & Smith, 1992; Engelstad et al., 1992; Isaacs et al., 1992b). Antibody to the B5R gene product reacts with the 42K EEV protein that is absent from INV and which had been previously identified and characterized (Payne, 1978, 1992), but also reacts with higher Mr complexes of similar size to the HA (Engelstad et al., 1992; Isaacs et al., 1992b). Transcriptional analysis indicated that, like the HA gene, the B5R gene is transcribed from distinct early and late promoters (Engelstad et al., 1992). The B5R gene sequence from vaccinia virus strain Copenhagen, WR, Lister and IHD-J and from variola major virus (Goebel et al., 1990; Smith et al., 1991; Takahashi-Nishimaki et al., 1991; Engelstad & Smith, 1993) predicts that the encoded protein is a class I membrane protein. This was confirmed by: (i) the secretion of the protein after deletion of the C-terminal membrane anchor; and (ii) amino acid sequence analysis of the N terminus of the secreted product (Isaacs et al., 1992b). The gene sequence also reveals that the protein is related to the family of complement control proteins since it has four copies of a 60 amino acid motif termed short consensus repeat (SCR) typical of this protein superfamily. The mature 42K protein is modified by N-linked glycosylation at one or more of the three possible sites and is acylated (Engelstad et al., 1992; Isaacs et al., 1992b; Payne, 1992).

Gene A34R (SaIL4R) encodes three proteins of 22 to 24K that are all derived from a common protein backbone by addition of N-linked carbohydrate (Duncan & Smith, 1992). Immunoelectron microscopy showed that protein(s) encoded by this gene are present in purified EEV but not INV. These proteins are presumed to represent the very similarly sized proteins found in EEV by Payne that are also all derived from a common precursor and which are sulphated, phosphorylated and linked by disulphide bonds into aggregates of 55K (Payne, 1978, 1979, 1992). The sequence of this gene shows that there is a single hydrophobic region near the N terminus that might function as both a membrane signal and anchor sequence so that the protein has a type II membrane topology (Smith et al., 1991; Duncan & Smith, 1992). This was confirmed by immunoelectron microscopy using an antibody raised to the C-terminal region of the protein which reacts with non-permeabilized EEV (Duncan & Smith, 1992). The predicted amino acid sequence reveals a single site for addition of N-linked carbohydrate and a C-terminal region related to the carbohydrate recognition domain (CRD) of calcium-dependent (C-type) animal lectins (Smith et al., 1991; Duncan & Smith, 1992).

Genes A34R, A56R and B5R encode all the EEV glycoproteins identified by Payne with the exception of the 110K protein.

**Function of EEV proteins**

The isolation and characterization of four types of mutant virus are enabling a genetic analysis of the functions of EEV proteins. These are: (i) virus mutants that are resistant to the drug N_i-isonicotinoyl-N_2-3-methyl-4-chlorobenzoylhydrazine (ICMBH) (Schmutz et al., 1991) which inhibits plaque formation and EEV release (Payne & Kristensson, 1979); (ii) spontaneous mutants that do not express one of the EEV proteins (Ichihashi & Dales, 1971; Takahashi-Nishimaki et al., 1991); (iii) recombinant viruses in which a gene encoding an EEV protein is deleted or disrupted (Shida et al., 1988; Blasco & Moss, 1991; Engelstad & Smith, 1993); and (iv) recombinant viruses in which a gene encoding an EEV protein is inducibly regulated by the *Escherichia coli* lac repressor (Duncan & Smith, 1992). These mutants have provided a preliminary understanding of the functions of EEV proteins in virion assembly, EEV release and cell-to-cell spread of virus (plaque formation) (Table 1).

**Virion assembly and EEV release.** Studies with these and other mutants have uncoupled the formation of INV and EEV, in as much as some mutants affect EEV production while INV remains normal. This was demonstrated with the 14K fusion protein, which is present on the surface of INV, using a recombinant virus in which the 14K gene is regulated by the *E. coli* lac repressor (Rodriguez & Smith, 1990). Expression of the 14K protein is required for plaque formation and EEV production but not for production of normal amounts of infectious INV. Plainly, INV alone is insufficient for plaque formation.
The Sixteenth Fleming Lecture

Table 1. Consequences of non-expression of EEV proteins or the 14K fusion protein on plaque formation, INV and EEV production and virus virulence

<table>
<thead>
<tr>
<th>Gene*</th>
<th>Plaque type†</th>
<th>INV production</th>
<th>EEV production</th>
<th>Attenuation</th>
</tr>
</thead>
<tbody>
<tr>
<td>F13L (37K)</td>
<td>None</td>
<td>Normal</td>
<td>Very little</td>
<td>Yes‡</td>
</tr>
<tr>
<td>A28L (14K)</td>
<td>None</td>
<td>Normal</td>
<td>Very little</td>
<td>NT§</td>
</tr>
<tr>
<td>A34R (22-24K)</td>
<td>Tiny</td>
<td>Normal</td>
<td>Increased</td>
<td>NT</td>
</tr>
<tr>
<td>A56R[†] (HA)</td>
<td>Large, fusion</td>
<td>Normal</td>
<td>Normal</td>
<td>Yes</td>
</tr>
<tr>
<td>B5R (42K)</td>
<td>Small</td>
<td>Normal</td>
<td>&lt; 10%</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* Gene nomenclature as for vaccinia virus strain Copenhagen (Goebel et al., 1990).
† Over a 48 h period.
‡ R. Blasco and S. N. Isaacs (personal communication).
§ NT, Not tested.
† From IHD-W virus in which a 41K protein related to HA is expressed (Shida & Dales, 1982).

The deletion of two genes encoding EEV proteins allowed similar conclusions. A mutant virus lacking gene F13L, encoding the 37K protein, was isolated despite its inability to form plaques (Blasco & Moss, 1991). This virus produces normal amounts of INV but plaque formation and EEV formation are inhibited. The phenotype of this mutant is very similar to that of wild-type (wt) virus incubated in the presence of ICMBH and, consistent with this, a virus mutant with an amino acid substitution within the 37K protein is resistant to ICMBH (Schmutz et al., 1991).

Deletion mutants in the B5R gene of either the WR or IHD-J strain of virus also produce normal INV but plaque size is normal but the infected cells undergo polykaryocyte formation and fail to agglutinate red blood cells (Ichihashi & Dales, 1971). The HA has been called a fusion inhibitory protein due to its ability to suppress cell fusion that is otherwise induced at neutral pH by a mechanism involving the 14K protein. Thus, disruption of the HA gene (A56R) produces yet another phenotype. In this case INV production and plaque size are normal but the infected cells undergo polykaryocyte formation and fail to agglutinate red blood cells (Ichihashi & Dales, 1971). The HA has been called a fusion inhibitory protein due to its ability to suppress cell fusion that is otherwise induced at neutral pH by a mechanism involving the 14K protein. Thus, disruption of the HA gene, or addition of HA-specific monoclonal antibody (MAb), can induce cell fusion (Seki et al., 1990). In contrast, addition of 14K protein-specific MAb prevents fusion (Rodriguez et al., 1987). Some mutations in HA result in loss of haemagglutination activity but retain fusion-inhibitory activity, whilst others result in the loss of both activities (Seki et al., 1990). EEV is produced by the IHD-W virus that lacks the 89K HA protein (Payne, 1979). However, this strain of virus does produce a truncated 41K HA protein (Shida & Dales, 1982) which could function in some aspects of EEV production and plaque formation that are independent of haemagglutination and fusion-inhibition activities. This may be addressed with a mutant lacking the entire ORF.

**Plaque formation.** The role of EEV proteins and the 14K fusion protein in plaque formation are summarized in Table 1. Repression of the INV 14K fusion protein (Rodriguez & Smith, 1990) or deletion of the EEV 37K acylated protein (Blasco & Moss, 1991) allow normal INV formation, but very little EEV production and no plaque formation over a 2 day period. This suggests that EEV and not INV is required. However, several lines of
Evidence argues against EEV production being sufficient for plaque formation. First, antibodies to EEV proteins inhibit comet formation but not the size of the primary plaque (Appleyard et al., 1971; Turner & Squires, 1971; Engelstad et al., 1992). Second, the IHD-J and WR strains of vaccinia virus produce very similar plaque sizes despite forming quite different amounts of EEV (Payne, 1980; Blasco et al., 1993). Third, a reduction in plaque size results from non-expression of genes A34R (Duncan & Smith, 1992) or B5R (Engelstad & Smith, 1993) despite these mutations causing either an increase or decrease in EEV production, respectively. Collectively, these data suggest that INV envelopment and not EEV release is needed for plaque formation. Is CEV sufficient? Here too, evidence suggests not. First, plaque size is similar in IHD-J and WR virus-infected cells which have different amounts of CEV (Blasco & Moss, 1992). Second, substitution of the IHD-J A34R gene for the WR gene in a WR background increases EEV release, and presumably reduces CEV, but does not affect plaque size (Blasco et al., 1993). The A34R lectin-like protein does, however, play an important role in plaque formation since non-expression of the gene permits only a small plaque (Duncan & Smith, 1992) while increasing EEV production (A. A. G. McIntosh & G. L. Smith, unpublished results).

Attachment proteins for binding to cells. EEV and INV can bind to many cell types with similar kinetics but EEV undergoes more rapid fusion with the cell plasma membrane and more rapid penetration (Payne & Norrby, 1978; Doms et al., 1990). One or more of the EEV proteins must function as an attachment protein, but not all EEV proteins are required for infection of a single cell since HA of EEV (Payne, 1979) and A34R lectin of EEV (A. A. G. McIntosh & G. L. Smith, unpublished data) are both infectious. Nonetheless, loss of the HA protein causes virus attenuation (Flexner et al., 1987), possibly due to a diminished virus spread to different cell types in vivo. Probably each EEV glycoprotein contributes to increased virus host range.

Remarkably, each of the EEV glycoproteins has homology to a protein superfamily. The HA contains a single Ig domain at the N terminus, the A34R encoded-proteins have a single CRD of C-type animal lectins near the C terminus and the 42K protein has four copies of the SCR typical of complement control proteins. A feature common to all these protein superfamilies is that their members often mediate interactions at the cell surface, either between cells or between soluble factors and cells. It seems probable that during evolution the orthopoxviruses acquired genes from host cells encoding these superfamily members and adapted them to function as proteins mediating virus attachment to target cells. This contrasts with some other viruses which use protein superfamily members as the receptor for the virus attachment protein [e.g. poliovirus, rhinovirus and human immunodeficiency virus all bind to Ig superfamily members (Dalgleish et al., 1984; Mendelsohn et al., 1989; Staunton et al., 1989)], but do not encode the protein superfamily members themselves.

EEV glycoprotein conservation and implications for the eradication of smallpox

Immunity to the EEV proteins confers protection against orthopoxvirus infections (Boulter, 1969; Appleyard & Andrews, 1974; Payne, 1980). With knowledge of the location and sequence of the genes encoding these proteins in vaccinia virus, it was possible to ask whether similar genes exist in variola virus (the aetiological agent of smallpox) and if so, are they closely related? Genes predicted to encode the HA, the 42K protein and the lectin-like proteins are all present in the variola major virus strain Harvey genome at positions comparable to the equivalent genes in vaccinia virus (Aguado et al., 1992; Engelstad & Smith, 1993). The deduced amino acid sequence of each variola major virus protein shows a remarkable degree of conservation to the vaccinia virus counterparts. Each has the same overall structure and predicted membrane topology. The lectin-like protein has only two amino acid substitutions (98.8% identity), the 42K protein has 94% identity and the HA shows 85% identity. Although the degree of conservation of the HA is less than the other two proteins, the distribution of changes is non-random. Most changes are located close to the transmembrane anchor sequence in the external region, whilst the N-terminal Ig domain is highly conserved (97% identity). This globular region of HA is predicted to be most exposed for interaction with the host cell and for recognition by the immune system. Thus, in terms of immune recognition, the HA protein may be as highly conserved as the other two EEV glycoproteins. The high degree of conservation between these EEV proteins of vaccinia and variola major viruses explains to some degree the protection that vaccination with vaccinia virus evoked against smallpox. Genes encoding the HA, the B5R complement-related protein and the lectin-like protein are conserved in all strains of orthopoxvirus that have been examined; for B5R this includes seven strains of vaccinia virus, cowpox, rabbit-pox and variola major virus (Engelstad & Smith, 1993).

Non-structural glycoproteins

Secretory

Three glycoproteins that are secreted from vaccinia virus-infected cells have been genetically mapped and characterized. These are the vaccinia virus growth factor
(VGF), and two proteins encoded by genes B15R and B18R which are Ig superfamily members. Additionally, several other proteins, some of which are glycoproteins, are secreted from infected cells (McCrae & Pennington, 1978).

**Vaccinia virus growth factor.** The VGF is encoded by a gene expressed early during infection that maps within the ITR of vaccinia virus WR and hence is diploid in this strain (Venkatesan et al., 1982). The predicted primary translation product of this gene (19K) contains a signal and transmembrane anchor sequence and over an internal 55 amino acid region is related to the epidermal growth factor (EGF) family (Blomquist et al., 1984; Brown et al., 1985; Reisner, 1985). The mature protein is a secreted, acid-stable, 77 amino acid glycoprotein (23K to 25K) that binds to and induces autophosphorylation of EGF receptors and stimulates mitosis (Strooabant et al., 1985; Twardzik et al., 1985; King et al., 1986). The pathway of post-translational modification of this protein has been examined (Chang et al., 1988). Nonglycosylated VGF, in contrast to glycosylated VGF, does not provide a mitogenic stimulus in some cell types bearing EGF receptors (Lin et al., 1990). EGF receptor occupancy is claimed to inhibit vaccinia virus infection (Eppstein et al., 1985). However, deletion of the VGF genes does not prevent vaccinia virus infection of EGF-bearing cells, although it does reduce virus virulence in vivo and abrogate the hyperplasia characteristic of poxvirus pathology (Buller et al., 1988a, b). A more likely function of the VGF would be to stimulate metabolic activity and proliferation of uninfected cells so that as virus spreads to these cells it replicates more efficiently.

**Gene B15R (vaccinia virus interleukin-1β receptor).** The sequence of gene B15R of vaccinia virus WR suggests it encodes a secretory glycoprotein with an N-terminal signal sequence, three Ig domains and five potential N-linked glycosylation sites (Smith & Chan, 1991; Smith et al., 1991). Homology searches revealed a relationship to another vaccinia virus gene B18R (see below) and to the interleukin-1 receptor (IL-1R). Gene B15R is transcribed late during infection from an atypical TAAAATG motif at the beginning of the ORF. The gene is non-essential for virus replication and encodes a glycoprotein of 50K to 60K that is secreted from infected cells at 100000 copies per cell (Alcami & Smith, 1992). A 47K form is present within infected cells. Tunicamycin blocks secretion and reduces the intracellular protein to 37K, close to the predicted primary translation product (35K). When expressed by recombinant baculovirus the secreted protein is smaller (40K to 44K), presumably due to the incomplete glycosylation in insect cells (Alcami & Smith, 1992). Other workers reported that the gene is transcribed early during infection and that the form secreted from vaccinia virus-infected cells which binds IL-1 is 30K (Spriggs et al., 1992). The ability to bind IL-1 and the role of the protein in virus pathogenesis are considered under Immune Evasion (below).

**B18R.** This gene is predicted to encode a primary translation product that is slightly larger than B15R but very similar in structure, with a signal sequence followed by three Ig domains, five N-X-S/T glycosylation motifs and no transmembrane anchor (Ueda et al., 1990; Smith & Chan, 1991; Smith et al., 1991). Transcriptional analyses showed that the gene is expressed early during infection (Ueda et al., 1990; Alcami & Smith, 1992). In vitro translation of B18R mRNA produces a 40K protein very similar to the predicted size (40-7K) (Ueda et al., 1990). When expressed from recombinant baculovirus the protein is present on both the cell surface and in the culture medium and has a size of 48K to 50K (Alcami & Smith, 1992; Morikawa & Ueda, 1993). The protein is present in vaccinia virus-infected cells as 52K and 60K to 65K forms and the larger polypeptide is also secreted into the medium (Alcami & Smith, 1992). Immunofluorescence showed that the protein is located on the cell surface (Ueda et al., 1990). The gene is non-essential for virus replication and the gene product has been termed the poxvirus surface antigen (Ueda et al., 1969, 1972; Alcami & Smith, 1992; Morikawa & Ueda, 1993). The function of this protein is unknown, although it contributes to virus virulence (A. Alcami & G. L. Smith unpublished data).

**Other secretory proteins.** Proteins of 12K, 14-5K, 21K to 27K, 25K, 33K, 31K to 35K, 35K and 36K to 38K are secreted from cells infected with vaccinia virus strain Evans (McCrae & Pennington, 1978). These are likely to be virus-encoded, rather than virus-induced, because similar patterns are observed in the supernatants of different cell types infected with the same virus. The most abundant protein (35K) is secreted both early and late during infection but is neither glycosylated nor sulphated. However, the 36K to 38K (early), 33K and 21K to 27K (late) proteins are all sulphated and glycosylated and an early protein 31K to 34K is glycosylated. None of these proteins have been genetically mapped. Subsequently, the abundant 35K protein was shown to be immunologically related to a 35K secretory protein expressed by vaccinia virus strain Lister but not by strains WR, Wyeth and Tian Tan (Patel et al., 1990). The gene encoding this protein maps within the ITR and is expressed from the constitutively active 7.5K promoter that has been widely used in vaccinia virus expression vectors (Mackett et al., 1984). In vaccinia virus strain WR this gene encodes only a 7.5K protein due to a 19 nucleotide deletion within the region encoding the signal peptide (Patel et al., 1990). Although the protein in strain Lister contains a site for N-linked carbohydrate at-
tachment, it is unknown whether the protein from Lister is glycosylated and the immunologically-related protein from strain Evans is not labelled with glucosamine (McCrae & Pennington, 1978). Vaccinia virus strain WR expresses a different secretory 35K protein which is related to complement control proteins and which is not glycosylated (Kotwal & Moss, 1988). Other proteins produced by vaccinia virus strain WR are known to be secreted but not glycosylated, such as the 13.8K protein encoded by gene N1L (Kotwal et al., 1989).

Vaccinia virus nucleotide sequence data predict that several other genes encode secretory proteins that may be glycosylated (Goebel et al., 1990; Howard et al., 1991; Smith et al., 1991).

**Cell-associated glycoproteins**

Two-dimensional PAGE identified at least 13 virus-induced, glycosylated proteins in vaccinia virus-infected cells (Carrasco & Bravo, 1986). Some of these are the glycoproteins of INV and EEV, others are glycoproteins that are both cell-associated and secreted from the cell (such as B18R; Ueda et al., 1972; Alcami & Smith, 1992) and several may be present only in infected cells. Most cell-associated virus-induced glycoproteins do not copurify with INV but are associated with membranes (Moss et al., 1971). Some of these are present on the cell surface and coexist with, rather than replace, host components (Weintraub & Dales, 1974). The major surface glycoprotein is detectable after infection with HA+ but not HA− virus (Ichihashi & Dales, 1971; Blackman & Bubel, 1972) and is the HA (see above). Membranes from cells infected in the presence of rifampicin contain the same glycoproteins (89K, 42K and 20K to 23K) as those in the infected cell plasma membrane and in EEV (Payne & Kristensson, 1990).

The presence of virus glycoproteins in the plasma membrane may account for the altered membrane permeability of infected cells (Carrasco & Esteban, 1982), but does not explain the basis for the recognition and lysis of infected cells by syngeneic cytotoxic T lymphocytes (CTLs) (Mallon et al., 1985), since CTLs recognize peptides bound with major histocompatibility molecules. The targets for vaccinia virus-specific CTLs are unknown.

**Immune evasion**

Orthopoxviruses are in many respects susceptible to immune recognition and elimination: they generally cause acute infections and are unable to establish latent or persistent conditions; they are unable to undergo antigenic variation to escape existing immunity (possibly because there are many targets for immune recognition and DNA viruses have lower mutation frequencies than RNA viruses); they are large virus particles that are well recognized by phagocytes; and infection by these viruses induces long-lasting immunity that is broadly cross-protective within the orthopoxvirus genus. These properties, together with the lack of an animal reservoir for variola major virus, the plentiful supply of potent vaccine and the determination of the WHO, contributed to the success of the smallpox eradication campaign. Why then are these viruses able to replicate efficiently in the mammalian host and ensure transmission to other susceptible hosts? Part of the answer lies in the possession by poxviruses of many genes which interfere with specific parts of the host immune system such as, complement, interferon (IFN), inflammation, cytokines and CTL recognition. These are illustrated in Fig. 3. Other virus-encoded secretory proteins contribute to virulence by unknown mechanisms but probably involve some aspect of the immune system, so that with time the array of defences recognized in vaccinia virus is likely to increase.

**Interferon**

Vaccinia virus has at least three genes that interfere with IFN action. Strategies employed include the expression of a soluble receptor for IFN-γ to block the binding of this cytokine to its receptor and intracellular proteins that counter the antiviral state induced by IFN treatment.

Interferons are a complex group of cellular proteins that are secreted in response to various stimuli such as virus infection (for review see Samuel, 1991). These proteins bind to cell receptors and trigger signal transduction and transcription of IFN-responsive genes. The encoded proteins prime the cell for an antiviral state but may require additional stimuli for activation such as binding to dsRNA which is often produced following infection.

![Fig. 3. Summary of vaccinia virus immune evasion strategies.](image-url)
Two IFN-responsive pathways are well characterized, the 2',5'-oligoadenylate (2',5'-A) pathway and the IFN-induced P1 protein kinase. 2',5'-A is produced by the enzyme oligo-2',5'-A synthetase, a protein normally resident in cells but induced by IFN to higher levels and which is inactive without the presence of dsRNA. In the presence of dsRNA, 2',5'-A is synthesized and activates ribonuclease L. This RNase then cleaves RNA 3' to the sequence 5'-UpXp-3' and translation is arrested. The P1 protein kinase (sometimes called p68 or DAI) is also IFN-induced and is activated by binding to dsRNA. The activated protein kinase catalyses autophosphorylation and transphosphorylation of translational initiation factor eIF2\(\alpha\) at serine 51. This modification decreases eIF2\(\alpha\) affinity for eIF2\(\beta\) and results in a failure to assemble functional translation initiation complexes.

Early work established that vaccinia virus is relatively resistant to IFN and, surprisingly, coinfection of vaccinia virus and vesicular stomatitis virus (VSV) overcame the sensitivity of VSV replication to pretreatment of cells with IFN (Thacore & Youngner, 1973a, b). The vaccinia virus gene(s) encoding the protein(s) mediating resistance to IFN are localized near the left ITR since virus infection in the presence of IFN prevents the spontaneous deletion of DNA from this region (Paez & Esteban, 1985). A factor responsible for the rescue of VSV is a virus-specified early protein that inhibits the P1 protein kinase-mediated phosphorylation of the eIF2\(\alpha\) (Whitaker-Dowling & Youngner, 1983, 1984; Paez & Esteban, 1984b; Rice & Kerr, 1984). Partial purification of this specific kinase inhibitory factor (SKIF) showed that the protein directly blocks the activation of P1 protein kinase by low concentrations of dsRNA (Akkaraju et al., 1989). Further purification identified SKIF as a 25K dsRNA-binding protein (Watson et al., 1991) and enabled the determination of a partial amino acid sequence (Chang et al., 1992). Comparison of this sequence with vaccinia virus ORFs, deduced from the complete DNA sequence of vaccinia virus strain Copenhagen, identified the gene encoding this protein as E3L (Chang et al., 1992). Expression of the E3L gene produces a 25K protein that binds dsRNA and which competes with the P1 protein kinase for this ligand. Consequently, the E3L protein restricts P1 protein kinase activation and the resultant eIF2\(\alpha\) phosphorylation and inhibition of translation. Since the 2',5'-A system is also activated by dsRNA, the E3L protein may also reduce activation of RNase L.

A second vaccinia virus protein (10.5K), encoded by gene K3L, interferes with the P1 protein kinase-induced inhibition of translation. This 88 amino acid protein has homology with eIF2\(\alpha\) over the N-terminal region which includes Ser (51) that is phosphorylated by P1 protein kinase, although the vaccinia virus protein has lysine at this position (Beattie et al., 1991). The K3L protein acts competitively with eIF2\(\alpha\) as a substrate for the P1 protein kinase, without being phosphorylated itself, so that phosphorylation of the functional translational initiation factor is blocked and translation may continue (Davies et al., 1992). Deletion of the K3L gene from the virus genome greatly increases the sensitivity of protein synthesis and vaccinia virus replication to IFN (Beattie et al., 1991). A comparison of the relative efficiency by which the E3L and K3L proteins interfere with the function of IFN in an in vitro system showed that the E3L protein is more effective than K3L (Davies et al., 1993). However, a comparison of the virulence and IFN sensitivity of viruses that have lost either gene is needed in vivo.

Vaccinia virus infection also influences the 2',5'-A system (Paez & Esteban, 1984a; Rice et al., 1984). It is possible the E3L-encoded 25K dsRNA-binding protein may reduce activation of 2',5'-A synthetase. However, increased levels of 2',5'-A are observed following vaccinia virus infection of IFN-treated cells (Rice et al., 1984). Moreover, cells infected with wt vaccinia virus in the presence of the anti-poxvirus drug isatin-beta-thiosemicarbazone, or cells infected at the non-permissive temperature with temperature-sensitive (ts) mutants mapping to gene A18R have elevated concentrations of 2',5'-A (Cohrs et al., 1989). Infections under these conditions have a late abortive phenotype characterized by normal DNA synthesis but the degradation of rRNA and cessation of late protein synthesis (Condit & Motyczka, 1981; Pacha & Condit, 1985). The sequence of the A18R gene (Pacha et al., 1990) predicts that the protein may be an RNA or DNA helicase, prompting speculation that the elevated levels of 2',5'-A observed after infection with A18R ts mutants may be attributable to a failure to unwind dsRNA and thereby prevent activation of 2',5'-A synthetase (Koonin & Senkevich, 1992). Recent data show that although there is an increased level of dsRNA late during infection with A18R ts mutants, this is attributable to aberrant late transcription (Bayliss & Condit, 1993).

A third mechanism that vaccinia virus and other poxviruses use to block IFN action is by expressing a soluble receptor for IFN-\(\gamma\) (see under soluble cytokine receptors, below).

**Complement**

The complement system is a complex family of plasma proteins that when activated by the classical or alternative pathway mediates defence against pathogens, including viruses, by promoting inflammation, phago-
cytosis and lysis of micro-organisms or infected cells. Activation of the classical pathway requires interaction of antigen and specific antibody whereas the alternative pathway may be activated by antigen alone such as lipopolysaccharide.

Vaccinia virus encodes two proteins related to the family of complement control proteins. The 42K protein encoded by gene B5R is part of the EEV envelope and has been described above. The amino acid sequence shows that it is most closely related to factor H, a regulator of the alternative pathway of complement activation, but the ability of this protein to bind complement components is unknown.

The second protein is encoded by gene C21L located near the left ITR (Kotwal & Moss, 1988). Like B5R, the C21L protein has four SCRs but it differs from B5R in that it lacks a C-terminal hydrophobic transmembrane anchor and consequently is secreted into the medium. The gene is likely to be expressed late but not early during infection since there is a late transcriptional initiation site (TAAATATG) at the beginning of the ORF and the early transcriptional terminator sequence (TTTTTNT) is present within the coding region. The predicted primary translation product is 28.6K but the secretory form lacking the signal peptide is larger, 35K. This increased size is not due to N-glycosylation since there are no N-X-S/T motifs within the protein and the protein is reported not to be labelled with glucosamine. The amino acid sequence of C21L shows it is most closely related to human C4b-binding protein and it interacts with cells bearing C4b on the surface and prevents C4b binding to the receptor for C4b and C3b (complement receptor 1, CR1) (Kotwal et al., 1990). The protein also prevents antibody-dependent complement-enhanced neutralization of virus infectivity and contributes to virus virulence since a deletion mutant lacking the gene is attenuated in vivo (Isaacs et al., 1992a).

Soluble cytokine receptors

Vaccinia virus strains WR and Copenhagen each contain ORFs with amino acid homology to the external domain of three different cytokine receptors. Vaccinia virus strain WR gene B15R (B16R in vaccinia virus strain Copenhagen) is related to the IL-1R, gene A53R (formerly called SalF19R in vaccinia virus strain WR) and genes C22L and B28R (Copenhagen) are related to the tumour necrosis factor receptor (TNFR) and gene B8R (WR and Copenhagen) is related to the IFN-γ receptor. Neither virus has functional receptors for all three cytokines. In Copenhagen all three ORFs related to TNFR and the ORF related to the IL-1R are disrupted by termination or frameshift mutations. Similarly, in WR the TNFR homologue is disrupted into three fragments. However, a functional receptor for each cytokine is expressed in at least one poxvirus.

Interleukin-1β receptor. Interleukin-1 is a key cytokine in the inflammatory and immune response and exists in three structurally related forms IL-1α, IL-1β and IL-1 receptor antagonist (IL-1ra). All three cytokines bind to both the type I and type II IL-1 receptors but only IL-1α and IL-1β trigger signal transduction. IL-1α and IL-1β may induce beneficial responses to infection or tissue injury but be harmful if unregulated or over-expressed.

Vaccinia virus strain WR has two ORFs (B15R and B18R) showing amino acid homology to the external domain of the type I and type II IL-1R, with the closest relationship being between B15R and the type II IL-1R (McMahan et al., 1991; Smith & Chan, 1991). B15R encodes a 50K to 55K secreted glycoprotein that is expressed late during infection and which binds IL-1β but not IL-1α or IL-1ra. B18R encodes an early, 60K to 65K, secretory glycoprotein that is also present on the cell surface but does not bind IL-1 or IL-6 (Alcami & Smith, 1992). The specificity of B15R protein for IL-1β was confirmed by competition studies in which unlabelled IL-1β but not IL-1α, IL-1α nor IL-6 competed with the binding of 125I-labelled human IL-1β for the vaccinia virus receptor. The vaccinia virus IL-1βR has a high affinity for IL-1β (Kd 234 pM), comparable to that of the external domain of the cellular receptors. However, the vaccinia virus protein differs from the cellular receptor in the number of copies expressed per cell. Whilst the cellular receptors are usually expressed at between 10 and 100 copies per cell, more than 105 copies of B15R are expressed per cell during a 24 h infection (Alcami & Smith, 1992). This high level is consistent with the proposed function of the soluble receptor, namely to bind and sequester free IL-1β and to prevent the cytokine reaching the cellular receptors and triggering signal transduction. The inability of the vaccinia virus IL-1βR to bind IL-1ra is logical since IL-1ra cannot trigger signal transduction by cellular receptors and competes with IL-1α and IL-1β for these receptors. In fact, binding of IL-1ra by the vaccinia virus IL-1βR would be counter-productive because it would compete with IL-1β for the soluble receptor. The inability to bind IL-1α is more interesting and suggests that during poxvirus infections IL-1β is more important. Consistent with this, CPV expresses a serine protease inhibitor (serpin) which inhibits in vitro the IL-1β precursor converting enzyme (ICE) (Ray et al., 1992), in addition to expressing the soluble IL-1βR. The ICE converts the intracellular precursor of IL-1β into the secreted and active form. Vaccinia virus strain WR expresses a very similar serpin-like protein (92 % amino acid identity) encoded by B13R (Kotwal & Moss, 1989; Smith et al., 1989).

There is conflicting evidence about the role of the...
vaccinia virus IL-1R in virus pathogenesis. Two groups constructed a deletion mutant in the WR strain of vaccinia virus and tested the virulence of the mutant virus after either intracranial or intranasal infection of mice (Alcami & Smith, 1992; Spriggs et al., 1992). Intracranial inoculation indicates that the deletion mutant is attenuated compared with wt, whilst in the intranasal model, the virulence of the deletion mutant is increased compared to wt and animals suffer a more rapid and extensive weight loss and more rapid death. Elevated systemic levels of IL-1β produced following infection in the absence of the soluble IL-1βR might be responsible for these changes. Evidently the model of infection determines whether the vaccinia virus IL-1βR is either beneficial or detrimental to the host. Not all vaccinia virus strains express the IL-1βR and it is interesting that the safer human vaccine strains of vaccinia virus (Wyeth and Lister) express the receptor, whilst those vaccine strains that caused a higher frequency of post-vaccinial complications (Copenhagen, Tashkent and Tian Tan) either do not express the receptor, or express a receptor which binds human IL-1β very poorly (Alcami & Smith, 1992). Thus in humans as well as mice there is a correlation between increased virus virulence and the absence of the IL-1βR, although there may be many other genes which differ between these virus strains and which affect virulence.

Tumour necrosis factor receptor. TNF-α and -β are related cytokines with a pivotal function in inflammation and the immune response and have potent antiviral activity. There are two cellular TNFRs which each have an external domain consisting of four repeats of a cysteine-rich motif followed by a transmembrane anchor sequence and different cytoplasmic tails for signal transduction. The sequence of vaccinia virus strain WR shows that ORF SalF19R (A53R in Copenhagen) is related to the external domain of TNFR but the repeated domains are fragmented by frameshift and nonsense mutations (Howard et al., 1991). Similarly, the complete sequence of vaccinia virus strain Copenhagen (Goebel et al., 1990) shows an ORF identical to WR SalF19R and another ORF related to TNFR within each ITR (C22L and B28R) (Upton et al., 1991). The predicted 28K peptide encoded by the latter ORFs has a signal peptide but, like A53R, the cysteine-rich domains are fragmented. Although vaccinia virus strains Copenhagen and WR lack a functional TNFR, poxviruses of the Leporipoxvirus genus [myxoma virus, Shope fibroma virus (SFV) and malignant rabbit virus] possess a complete TNFR that is secreted from the cell and which binds TNF (Smith et al., 1991; Upton et al., 1991). The SFV TNFR binds both TNF-α and -β and the TNFR of myxoma virus increases virus virulence (Upton et al., 1991).

Interferon-γ receptor. Vaccinia virus gene B8R is predicted to encode a protein related to the product of gene T7 from the ITR of SFV (Howard et al., 1991) and myxoma virus (Upton et al., 1992). Each protein contains a signal peptide and sites for addition of N-linked carbohydrate within a relatively hydrophilic domain but no anchor suggesting that the protein is secreted. Computational analyses show that these proteins are related to the external domain of the receptor for IFN-γ (Upton et al., 1992). The myxoma virus gene product is an abundant, secretory 37K protein that accumulates in the culture supernatant early and late during infection and which binds IFN-γ (Upton et al., 1992). The protein abrogates the inhibition of VSV plaque formation caused by pretreatment of cells with IFN-γ. IFN-γ is a key cytokine that activates macrophages, induces expression of major histocompatibility complex (MHC) antigens and directs the development of a cytolytic rather than humoral response to infection. All these effects are triggered by binding to a cellular IFN-γ receptor followed by signal transduction and, therefore, may all be inhibited by a virus-encoded soluble receptor. Such suppression is a valuable asset for a virus.

A common feature of each poxvirus-encoded cytokine receptor is that only the external domain containing the cytokine-binding site is expressed and the transmembrane anchor sequence and cytoplasmic tails are absent. Thus the receptor is released from the infected cell so that it may bind the cytokine in the extracellular space and prevent this binding to the cellular receptors. The abundance of the poxvirus soluble receptors suggests that they are designed to compete with the cellular receptors for free cytokines. All three cytokines (IL-1β, TNF and IFN-γ) are molecules with important and overlapping functions in the inflammatory and immune response to infection. The acquisition by poxviruses of soluble receptors to all of these cytokines emphasizes the important role of these proteins in the host defence against virus infections and the benefit to the virus in combating more than one cytokine simultaneously. Other proteins are secreted from vaccinia virus-infected cells into the supernatant and contribute to virus virulence by unknown mechanisms. Some of these proteins have no homology to entries in protein databases. An exciting possibility is that these proteins bind to growth factors, cytokines or adhesion molecules for which the cellular receptors have not yet been identified.

Cytotoxic T lymphocytes

Although class I MHC-restricted CTL are produced following vaccinia virus infection there is evidence that the virus can modulate (i) the efficiency with which CTLs
are induced to specific epitopes during infection (Coupar et al., 1986) and (ii) the recognition by CTLs of specific epitopes when these are expressed from vaccinia virus-infected cells (Coupar et al., 1986; Townsend et al., 1988). Proteins expressed early during infection are in some cases more efficient at inducing CTL responses and to being recognized by CTLs than if the same protein is expressed from a late virus promoter (Coupar et al., 1986). However, this late blockage in antigen presentation is antigen- and epitope-specific and is not absolute (Townsend et al., 1988). The mechanisms causing virus interference with CTL induction and the presentation blockage of certain epitopes to CTLs are unknown. However, the observation that defective presentation of certain peptide epitopes to CTLs is overcome by expression of rapidly degraded protein suggests that the blockage is at the level of antigen proteolysis (Townsend et al., 1988). It was proposed that a family of vaccinia virus-encoded serpins might mediate this virus-induced inhibition of proteolysis (Townsend et al., 1988; Smith et al., 1989). However, experimental evidence for this proposal is lacking.

### Inflammation

In addition to soluble cytokine receptors vaccinia virus has other mechanisms to suppress inflammation that include the expression of a steroid synthesizing enzyme, 3β-hydroxysteroid dehydrogenase (3β-HSD), and a family of serpins. An ORF (SalF7L or A44L) within the genome of the WR and Copenhagen strains of virus shows homology to 3β-HSD (Goebel et al., 1990; Blasco et al., 1991; Smith et al., 1991). The gene is transcribed early during infection and encodes an active 38K enzyme that converts pregnenolone to progesterone in vitro. The enzyme is non-essential for virus replication but contributes to virus virulence in vivo (Moore & Smith, 1992). The nature of the steroid hormones that are synthesized in vivo is unknown but if these are glucocorticoids they might be immunosuppressive and account for the observed attenuation.

Vaccinia virus expresses a family of serpins (genes K2L, B13R and B22R) (Boursnell et al., 1988; Kotwal & Moss, 1989; Smith et al., 1989) and, by analogy with the situation in CPV, it is proposed that one or more of these serpins inhibits inflammation. Cowpox virus expresses a 38K protein that is required for the haemorrhagic pock phenotype of CPV strain Brighton Red (CPV-BR) on the avian chorioallantoic membrane (CAM) (Pickup et al., 1986). Spontaneous CPV deletion mutants lacking this serpin produce a raised, white pock rather than the flat, haemorrhagic phenotype. Since serpins control biological cascades such as blood coagulation and complement activation it was suggested that the serpin might cause the haemorrhagic phenotype by interfering with blood coagulation. However, histological examination of the white pocks show that the lesions are heavily infiltrated with heterophils (neutrophils), whilst the red pocks are not, indicating that the serpin directly or indirectly inhibits the migration of these cells to the infected locus (Palumbo et al., 1989). Probably the presence of these inflammatory cells prevents haemorrhage by clearing the virus infection. Extracts from cells infected with strains of CPV producing red but not white pocks actively inhibit cell migration in vitro (Chua et al., 1990). Potent neutrophil chemoattractants, such as leukotriene-B4 and dihydroxyeicosatetraenoic acid (dihETE), are byproducts of arachidonic acid metabolism. Poxvirus infection induces alterations in arachidonic acid metabolites (Palumbo et al., 1993) and inhibitors of arachidonic acid metabolism specifically block orthopoxvirus replication (Palumbo & Buller, 1991). In cells infected with CPV lacking the 38K serpin, the level of diHETE is elevated compared with infection with CPV-BR expressing the 38K serpin (Palumbo et al., 1993), showing that the CPV 38K protein alters lipoxygenase metabolites and consequently affects the inflammatory response to infection (Palumbo et al., 1993). Another activity of the CPV 38K serpin is the in vitro inhibition of ICE and hence IL-1β production (Ray et al., 1992).

In vaccinia virus strain WR serpin B13R shares 92% amino acid identity with the CPV 38K protein and yet this virus does not produce red pocks on the CAM (Kotwal & Moss, 1989; Smith et al., 1989). The gene is non-essential for virus replication since a deletion mutant lacking the gene is viable (Zhou et al., 1990) and in vaccinia virus strain Copenhagen the gene is disrupted by a frameshift mutation (Goebel et al., 1990). The function of the second serpin near the right end of the genome of the WR strain of vaccinia virus (B22R) is unknown although it is a non-essential protein which reduces antibody responses to a foreign antigen expressed from vaccinia virus (Zhou et al., 1990). In vaccinia virus strain Copenhagen the same gene is present at the opposite end of the virus genome (C12L) (Goebel et al., 1990). The third vaccinia virus serpin (K2L) prevents virus-induced cell fusion (Law & Smith, 1992; Turner & Moyer, 1992; Zhou et al., 1992). Serpins are encoded by fowlpox virus (Tomley et al., 1988) and Leporipoxviruses (Upton et al., 1986) in addition to CPV and vaccinia virus.

In summary, vaccinia virus displays a great diversity of strategies for immune interference that are providing new insights into virus pathogenesis and immunology and may provide proteins with therapeutic value. In view of this extensive immune interference it is perhaps surprising that the frequency of complications resulting from vaccination with vaccinia virus was not higher than
that observed. It seems probable that deletion of some or all of the genes interfering with the host’s immune system may simultaneously improve vaccine safety and immunogenicity.

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