Introduction to immune cell signalling

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SUMMARY

The dynamic interaction of cells of the immune system with other cells, antigens and secreted factors determines the nature of an immune response. The response of individual cells is governed by the sequence of intracellular signalling events triggered following the association of cell surface molecules during cell-cell contact or the detection of soluble molecules of host or pathogen origin. In this review we will describe the general principles of intracellular signal transduction. We will then describe the signalling pathways triggered following the recognition of antigen, as well as the detection of cytokines, and discuss how the signalling pathways activated regulate the effector response.

Key words: signal transduction, BCR signalling, cytokine receptor signalling, TLR signalling.

BASIC PRINCIPLES OF CELL SIGNALLING

Cells sense, respond to and integrate a multiplicity of signals from their environment. In the context of the immune system, these include signals resulting from interactions with neighbouring cells (cell-cell contact), and the detection of soluble factors such as cytokines, which may originate at distant sites and be transported via the blood, as well as the recognition of pathogens and their products. Following ligation of cell surface receptors, intracellular signal transduction cascades are initiated, resulting in the activation of transcription factors and other proteins that regulate processes such as gene induction, phagocytosis, apoptosis, proliferation and secretion (Harnett, 1999a, b).

Signal transduction cassettes comprise specific cell-surface membrane receptors, effector signalling elements and regulatory proteins. These signalling cassettes serve to detect, amplify and integrate diverse external signals to generate the appropriate cellular response. In this review, we first discuss how cell-surface receptors sense and transduce signals by transmembrane coupling to effector enzyme systems, generating low-molecular-weight molecules termed second messengers and activating a range of key protein kinases with distinct substrates that control gene induction and other cellular responses (Harnett, 1999a, b). We will then describe the signalling cascades underlying some of the key responses of cells of the immune system to environmental stimuli of host as well as pathogen origin.

Phosphorylation is an important mechanism of control of protein recruitment and activation; kinases catalyse the transfer of a phosphate group from ATP onto specific amino acids, a process that can be reversed by the action of phosphatases. Individual kinases have distinct substrate specificities e.g. tyrosine kinases, dual specificity serine/threonine or tyrosine/threonine kinases (Bromann, Korkaya & Courtneidge, 2004; Cannons & Schwartzberg, 2004; Gambacorti-Passerini, 2004, 2005; Piiper & Zeuzem, 2004). Lipid kinases, such as phosphoinositide-3-kinase (PI3K), catalyse the phosphorylation of lipids (Cantrell, 2001, 2002, 2003; Courtneidge, 2004; Cannons & Schwartzberg, 2004; Piiper & Zeuzem, 2004). Recruitment of kinases and other enzymes, such as phospholipases, to the activated receptor following ligation triggers a cascade of enzymatic activation and the generation of second messengers such as cyclic AMP (cAMP) (Houslay, 1998; Houslay & Adams, 2003; Houslay & Baillie, 2003), inositol trisphosphate (IP3) (Irvine, 2003a, b, 2004), diacylglycerol (DAG) (Powney & Wakelam, 2002; McDermott, Wakelam & Morris, 2004) and calcium ions (Ca²⁺) (Berridge, Bootman & Roderick, 2003; Berridge, 2004), which results in propagation and amplification of the signal. Hydrolysis of phosphatidylincholine, which comprises about 40% of the total cellular phospholipid, by various phospholipases results in the generation of a range of second messengers, including arachidonic acid (phospholipase A2, PLA2), DAG (phospholipase C, PLC) and phosphatidic acid (phospholipase D, PLD), depending on the site of action of the enzyme (Wakelam & Harnett, 1998; Powner & Wakelam, 2002; McDermott et al., 2004). Arachidonic acid is a key lipid second messenger involved in the regulation of signalling enzymes, such as PLC-γ and -δ, and the α, β and γ isofoms of the protein kinase C (PKC) family of serine/threonine kinases. DAG is a cofactor required for the activation of PKC isofoms; it can...
also be metabolised to generate arachidonic acid by the action of DAG lipase. Furthermore, phosphatidic acid can be interconverted to DAG by the action of phosphatidic acid phosphohydrolase (Wakelam & Harnett, 1998; Powner & Wakelam, 2002; McDermott et al. 2004).

One of the key outcomes of intracellular signalling cascades is the activation of transcription factors, such as NF-κB, NFAT, Fos, Jun and Oct. Transcription requires the binding of RNA polymerase to the promoter region situated 5’ of the transcription start site. Transcription factors, which bind to specific regulatory DNA sequences, co-operate to permit or deny RNA polymerase access to the promoter, and thereby regulate gene expression (Cleveenger, 2004; Coffer & Burgering, 2004; Eggert et al. 2004; Smith & Sigvardsson, 2004).

**Antigen Receptor Signalling**

Recognition of specific antigens by B cells is achieved by surface-bound immunoglobulin (sIg). However, sIg is unable to transduce intracellular signals due to its short cytoplasmic tail, and hence recruits two Ig-α/β heterodimers, which possess Intracellular Tyrosine-based Activatory Motifs (ITAMs; D/E-X7-D/E-XX-YXXL-X7-YYXL/I) in their cytoplasmic tails. The earliest detectable B cell receptor (BCR) signal is tyrosine phosphorylation of proteins including the Ig-α/β ITAMs; mutations in either of the conserved tyrosines disrupts signalling. ITAM phosphorylation results in the initiation of a signalling scaffold around the active BCR (see Fig. 1). The tyrosine kinases Lyn and Syk bind to the phosphorylated ITAMs via their SH2 domains (Dal Porto et al. 2004; Gauld & Cambier, 2004; Harnett, Katz & Ford, 2005). Further recruitment of adaptors/signalling proteins occurs via SH2, SH3 (binds proline-rich sequences) and PH (binds phosphoinositides) domain interactions. The tyrosine phosphatase SHP-1 also associates with the BCR to prevent aberrant activation (Kurosaki, 2002; Cannons & Schwartzberg, 2004; Simeoni et al. 2004).

The phosphoinositide-3-kinase (PI3K) pathway is a key pathway promoting survival, gene induction and cell cycle progression following B cell activation (Fruman, 2004a, b). PI3K catalyses the conversion of phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphate (PIP3). This enables the recruitment and activation of the serine/threonine kinase protein kinase B (PKB), also known as Akt, which prevents apoptosis and promotes B cell survival, as well as the serine/threonine kinase p70 S6 kinase, which is required for the entry of cells into S phase of the cell cycle. Akt phosphorylates the pro-apoptotic protein Bad, which is then sequestered by association with the 14-3-3 protein, thereby preventing the release of cytochrome c and activation of the caspase cascade. Thus PIP3 promotes B cell survival and proliferation (Cantrell, 2001, 2002, 2003b; Fruman, 2004a).

PIP3 can also regulate gene induction via the activation of atypical isoforms of PKC (ε and ζ), which activate transcription factors such as AP-1, NFAT and NF-κB (Hao, Kurosaki & August, 2003; Guo, Su & Rawlings, 2004). Furthermore, PIP3 can recruit PLC-γ, which hydrolyses PIP2 to generate IP3 and DAG, resulting in the release of Ca2+ ions from intracellular stores and the activation of classical and novel isoforms of PKC. Ca2+ ion binding is required for the serine/threonine kinase activity of the Ca2+-/calmodulin (CaM)-dependent protein kinase (CaMII) and enables the CaM protein phosphatase calcineurin to dephosphorylate and activate NFAT. DAG is necessary for the activation of various PKC isoforms, which may also require Ca2+ binding, resulting in the activation of transcription factors such as AP-1, NFAT, NF-κB and Oct (Hao et al. 2003; Guo et al. 2004).

Another key event following ligation of the BCR is the activation of the mitogen-activated protein kinase (MAP kinase) pathways. There are three major subfamilies of MAP kinases—the extracellular regulated kinases (Erk1/2), the p38 MAP kinases and the c-Jun kinases/stress-activated protein kinases (JNK/SAPK) (Adams et al. 2004; Roux & Blenis, 2004).
Recruitment of a GTPase to the activated receptor complex is required for the activation of a kinase cascade, ultimately leading to activation of MAP kinases, which target transcription factors to regulate gene induction. For example, BCR coupling to the Erk cascade is achieved via the GTPase Ras (see Fig. 1). Inactive Ras (Ras-GDP) is anchored at the plasma membrane and is recruited by the Grb2-Sos complex. Sos, a guanine nucleotide exchange factor (GEF), promotes the exchange of GDP for GTP. Active Ras (Ras-GTP) activates Raf (a MAP kinase kinase) by binding to its N-terminal regulatory domain. Raf then phosphorylates MEK-1 (a MAP kinase kinase), which in turn phosphorylates Erk1/2. The Erk MAP kinase pathway promotes the proliferation and differentiation of B cells by targetting transcription factors such as c-Myc, Sap and Elk-1 (Cantrell, 2003a, b; Dal Porto et al. 2004; Harnett et al. 2005).

**Cytokine receptor signalling**

Cytokines and their receptors can be classified according to their structure. Most cytokine receptors belong to the class I cytokine receptor family; they are multichain receptor complexes comprising separate chains for specific cytokine recognition and signal transduction. Signalling chains may be shared by several cytokines within a subfamily. For example, IL-3, IL-5 and GM-CSF, which are structurally similar, share a common β chain, while IL-2, IL-4, IL-7, IL-9 and IL-15 share a common γ chain. gp130 is the signalling subunit of the IL-6 and IL-11 receptors, and the gp130-related IL-12Rβ1 chain is utilised by IL-12 and IL-23 (Hofmann et al. 2002; O’Shea, 2004; O’Shea et al. 2004).

Most cytokines signal through PLC, PI3K, and Ras MAPK pathways, as well as JAK-STAT pathways (reviewed in Leonard & Lin, 2000; Hofmann et al. 2002; Agnello et al. 2003; O’Shea, 2004; O’Shea et al. 2004). Binding of cytokines to their receptors triggers the recruitment of receptor-associated tyrosine kinases of the Janus kinase (JAK) family (JAK1-3, Tyk2). Activated JAKs phosphorylate tyrosine residues in the β and γ signalling receptor chains, enabling the recruitment of other proteins via their SH2 domains, in particular the cytosolic STAT proteins (signal transducers and activators of transcription; STAT1-6). STATs are then phosphorylated and released from the receptor. Phosphorylated STAT proteins homodimerise or heterodimerise via pTyr-SH2 domain interactions, and rapidly translocate into the nucleus where they act as transcription factors by binding to GAS motifs (see Fig. 2).

Different combinations of JAKs and STATs can be utilised to achieve specificity. For example, the JAK-STAT pathways play key roles in the control of polarisation of CD4+ helper T (Th) cell responses (Murphy et al. 1999, 2000; Murphy & Reiner, 2002; Agnello et al. 2003). Cytokine signals received by Th precursors determine their differentiation towards a Th1 or Th2 phenotype. Antigen recognition by the TCR in the presence of IL-12 and IFN-γ leads to the development of Th1 responses, while a Th2 bias is achieved by exposure to IL-4. Polarisation is achieved via the action of two transcription factors, T-bet and GATA-3 (Zheng & Flavell, 1997; Szabo et al. 2000; Murphy & Reiner, 2002; see Fig. 3). IL-12 and IFN-γ signal through their receptors on the surface of precursor Th cells, leading to the activation of STAT4 and STAT1 respectively, both of which induce T-bet expression. T-bet promotes IFN-γ expression by chromatin remodelling and increases IL-12Rβ2 chain expression, thereby further enhancing the IL-12 and IFN-γ signals, in part by sustaining its own expression.

Conversely, recognition of IL-4 by its receptor triggers the activation and nuclear translocation of STAT6, which rapidly induces GATA-3 expression (Zheng & Flavell, 1997; Zhou & Ouyang, 2003). GATA-3 regulates the expression of Th2 cytokines by co-ordinate regulation of the Th2 locus, which contains the genes encoding IL-4, IL-5 and IL-13, by not only inducing the expression of IL-5 and IL-13, but also by promoting chromatin remodelling to enable IL-4 transcription (Murphy et al. 1999, 2000; Lee et al. 2000; Ouyang et al. 2000; Murphy & Reiner, 2002; Agnello et al. 2003). GATA-3 thereby

![Cytokine receptor signalling via JAK-STAT pathways](image-url)

**Fig. 2.** Cytokine receptor signalling via JAK-STAT pathways. Following cytokine receptor ligation, JAKs are recruited and phosphorylate tyrosine residues in the β and γ signalling receptor chains, enabling STAT recruitment and phosphorylation. pSTAT proteins form dimers, which rapidly translocate to the nucleus where they act as transcription factors by binding to GAS elements.
TOLL-LIKE RECEPTOR SIGNALLING

Detection of pathogen products by cells of the innate immune system is mediated by pattern recognition receptors (PRR), with or without the aid of soluble host factors such as complement. PRRs include scavenger receptors, complement receptors and the recently identified family of Toll-like receptors (TLRs). TLRs, which were originally identified by their homology to the Drosophila anti-fungal protein Toll, have been demonstrated to be crucial for the recognition of a variety of pathogens and their products, including proteins, carbohydrates and nucleotides derived from bacteria, viruses, fungi, protozoa and helminth parasites (reviewed in Takeda, Kaisho & Akira, 2003).

TLRs, which are members of the Toll/IL-1R (TIR) superfamily, comprise extracellular ...

augments its own expression by positive feedback autoregulation. Furthermore, negative feedback mechanisms reinforce the fate decision. GATA-3 inhibits Th1 development by suppressing T-bet expression, at least in part by downregulating STAT4, and by increasing IL-4 production, thereby suppressing IL-12Rβ2 chain expression (reviewed in Ho & Glimcher, 2002). Similarly, T-bet may also inhibit GATA-3 expression and IL-4 production (Murphy et al. 1999, 2000; Murphy & Reiner, 2002; Agnello et al. 2003).

IFN-γ augments its own expression by positive feedback autoregulation. Furthermore, negative feedback mechanisms reinforce the Th1/Th2 bias.

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More Information
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such as IL-6 and TNF-α. Following LPS recognition, IL-1 receptor-associated kinase (IRAK)-1, IRAK-4 and TNF receptor-associated factor (TRAF)-6 are recruited to the receptor complex. IRAK-1 associates with MyD88 via the interaction of their death domains, and is phosphorylated by IRAK-4. Phosphorylated IRAK-1 interacts with and phosphorylates TRAF6, and they dissociate from the receptor to form a complex with TGFβ-activated kinase (TAK1), TAK1-binding protein (TAB)1 and TAB2. Activation of TAK1 results in phosphorylation of the IKK complex, which consists of the IκB kinases IKKα, IKKβ and NEMO/IKKγ. Phosphorylation of IκB by the IKK complex leads to its ubiquitination and degradation, releasing the transcription factor NF-κB to enter the nucleus and promote gene induction. TAK1 is also responsible for the activation of MAP kinase pathways, leading to gene induction via activation of transcription factors such as Elk, AP-1 and ATF-2.

In contrast, knockout studies indicated that MyD88 is not required for the induction of IFN-inducible genes, such as IP-10 and GARG16, following LPS stimulation (Kawai et al. 2001). These genes are induced indirectly via MyD88-independent production of IFN-β, which activates STAT1 ( Toschchakov et al. 2002). The transcription factor IRF-3, which is activated by signalling via the TRIF adaptor, regulates IFN-β production. This occurs via activation of the IKK isoform TANK-binding kinase 1 (TBK1), leading to IRF-3 phosphorylation and nuclear translocation (Fitzgerald et al. 2003; Sharma et al. 2003; Hemmi et al. 2004).

CONCLUSIONS

Activation of intracellular signalling cascades, which integrate and amplify signals received following receptor triggering, enables the controlled response of cells to multiple environmental stimuli. These highly co-ordinated processes are vital for the generation of appropriate effector responses in the immune system, to achieve effective removal of pathogens while limiting host damage. However, these communication pathways are also key targets utilised by pathogens to achieve modulation of host immune responses to promote their survival and propagation.

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**Introduction to immune cell signalling**
Evasion of innate immunity by vaccinia virus

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SUMMARY

Vaccinia virus, a member of the Poxviridae, expresses many proteins involved in immune evasion. In this review, we present a brief characterisation of the virus and its effects on host cells and discuss representative secreted and intracellular proteins expressed by vaccinia virus that are involved in modulation of innate immunity. These proteins target different aspects of the innate response by binding cytokines and interferons, inhibiting cytokine synthesis, opposing apoptosis or interfering with different signalling pathways, including those triggered by interferons and toll-like receptors.

Key words: vaccinia virus, immune evasion, signal transduction, nuclear factor kappa B, toll-like receptors.

INTRODUCTION

Vaccinia virus (VV) was the live vaccine used to achieve the global eradication of smallpox, a devastating human disease caused by variola virus (Fenner, 1988). VV is a member of the Poxviridae. Poxviruses are characterized by having a large ovoid or brick-shaped virion containing enzymes and factors for messenger RNA (mRNA) synthesis and a single linear double stranded DNA (dsDNA) genome of 130–300 kilobase pairs (kb) (Moss, 2001). Replication of poxviruses occurs entirely in the cell cytoplasm. The Poxviridae is divided in sub-families Entomopoxvirinae (insect poxviruses) and Chordopoxvirinae (vertebrate poxviruses). Generally, members of the same genus have similar virion morphology and present extensive serological cross-reaction and cross protection in laboratory animals (Fenner, 1988).

VV is the prototypic member of the Orthopoxvirus genus (sub-family Chordopoxvirinae) and remains its most extensively studied member. It was the first mammalian virus to be visualised microscopically, grown in tissue culture, titrated accurately, purified physically and analysed biochemically (Moss, 2001). VV can infect a broad range of mammalian species and can grow in many cell lines in vitro (Fenner, Witter & Dumbell, 1989). The highly attenuated VV strain modified vaccinia Ankara (MVA) was derived from strain Ankara by more than 570 passages in chicken embryo fibroblasts (Hochstein-Mintzel, Huber & Stickl, 1972; Mayr et al., 1978) and was used as a smallpox vaccine in over 120000 individuals without complications (Stickl et al., 1974; Mayr et al., 1978; Mahnel & Mayr, 1994).

In this review, we present a brief description of VV biology and the host innate response to viruses, before focusing on some of the VV genes involved in evading this host response, with particular reference to recently described VV proteins that modulate toll-like receptor (TLR)-mediated signalling.

OVERVIEW OF VV LIFE CYCLE

VV produces two forms of infectious progeny, intracellular mature virus (IMV) and extracellular enveloped virus (EEV). IMV represents the majority of infectious progeny and remains inside the infected cell until cell lysis (Appleyard, Hapel & Boulter, 1971; Ichihashi, Matsumoto & Dales, 1971). Although EEV is only a small proportion of the progeny virus, it is important biologically. It is released into the extracellular space before cell death (Appleyard et al. 1971). The EEV contains an additional lipid membrane, which contains cellular proteins and at least 5 VV-encoded polypeptides that are absent from IMV (Smith & Vanderplasschen, 1998; Vanderplasschen, Hollinshead & Smith, 1998; Smith, Vanderplasschen & Law, 2002).

Due to their large size (approximately 350 × 270 nm) VV virions are discernible by light microscopy in infected cells and were the first viruses to be observed microscopically. In traditional electron microscopy analyses of infected cells, the virions appear brick-shaped and consist of an internal electron-dense, biconcave core (containing the virus genome, major structural proteins and virion enzymes and two lateral bodies on each of its sides) (Moss, 2001).

The surface of the core contains closely packed, regularly arranged spikes, which comprise the palisade (Dubochet et al. 1994). The core structure is wrapped by a lipid membrane and additional virus proteins, which collectively constitute the IMV genome, major structural proteins and virion enzymes and two lateral bodies on each of its sides) (Moss, 2001).
membrane. Morphologically, EEV particles are similar to IMV, but contain an additional lipid membrane, which is extremely loose and fragile (Stokes, 1976; Roos et al. 1996). The presence of this extra lipid membrane and associated proteins makes EEV structurally and biologically distinct from IMV. EEV mediates the dissemination of virus within the infected host and protection is better achieved by immunity to EEV proteins than IMV, although immunity to IMV can still be helpful (Boulter, 1969; Appleyard et al. 1971; Boulter et al. 1971; Boulter & Appleyard, 1973; Payne, 1980; Law, Putz & Smith, 2005).

Like all the other members of the Orthopoxvirus genus, the VV genome is a dsDNA molecule of approximately 190 kb (length varies according to virus strain). Generally, genes from the central region of the genome are well conserved, are mostly essential for virus replication and are transcribed from either DNA strand. In contrast, genes located near the termini are more diverse, non-essential for virus replication and are generally transcribed towards the termini.

**EFFECTS OF POXVIRUS INFECTION ON THE HOST CELL**

Infection with VV results in profound changes in cell function, morphology and metabolism, which are termed cytopathic effect (CPE). Visible effects in vitro include cell rounding and detachment from neighbouring cells (Bablanian, 1970; Bablanian et al. 1978), alterations to the actin cytoskeleton (Hiller et al. 1979; Cudmore et al. 1995), microtubules (Ploubidou et al. 2000) and membrane permeability (Carrasco & Esteban, 1982). This is followed by a phase of enhanced cell migration involving the sequential elongation and condensation of lamellopodia from the cell body (Sanderson, Way & Smith, 1998). Other distinctive changes in cell morphology late during infection include the formation of virus-tipped microvilli (Stokes, 1976; Cudmore et al. 1995) as a result of actin tail extension. VV-induced CPE was traditionally viewed as a gradual degeneration of cell function, morphology and viability. Although this ultimately happens, CPE should not be viewed as a simple shut off of cell functions and random cell degeneration. Some aspects of CPE are a consequence of direct action of viral genes and may constitute a manipulation of cellular mechanisms to the advantage of the virus.

Cellular DNA, mRNA and protein synthesis are inhibited following infection with VV (Buller & Palumbo, 1991). Inhibition of host DNA replication may occur by hydrolysis of nascent single stranded DNA, by a viral endonuclease present in the incoming virion (des Gouttes Olgiati, Pogo & Dales, 1976; Dales, 1990). Concurrent with the orderly expression of VV polypeptides, host protein synthesis decreases gradually after VV infection and is completely shut off around 6 h post-infection (p.i.) (Buller & Palumbo, 1991). In the absence of VV early gene expression, IMV surface tubules (Mhuy, Morris & Bubel, 1982), the B1R protein kinase (Beaud et al. 1994) and F17R phosphoprotein (Person-Fernandez & Beaud, 1986) have been shown to inhibit host protein translation. Following expression of VV early genes, translation of cellular mRNA is inhibited selectively by small non-translated polyadenylated mRNAs (Bablanian et al. 1991; Lu & Bablanian, 1996). Additionally, the overall population of cellular mRNAs is reduced progressively, being replaced by VV mRNAs (Rice & Roberts, 1983), which contributes to the predomi-

Restriction is caused by a disruption in VV of a host range gene that is present in CPV, encoding the CP77 protein (Spehner et al. 1988). In VV WR, two genes, K1L and C7L encode proteins that enable VV replication in human cell lines. The K1L gene product is necessary for replication in RK-13 cells (rabbit kidney epithelioid cells) (Perkus et al. 1994; Bronte et al. 1997). Unlike cowpox (CPV), VV is unable to replicate in Chinese hamster ovary (CHO) cells, due to a block in the translation of intermediate mRNAs (Perkus et al. 1990). This restriction is caused by a disruption in VV of a host range gene that is present in CPV, encoding the CP77 protein (Spehner et al. 1988). In VV WR, two genes, K1L and C7L encode proteins that enable VV replication in human cell lines. The K1L gene product is also necessary for replication in RK-13 cells (rabbit kidney epithelioid cells) (Perkus et al. 1990). In a VV K1L deletion mutant, the translation of early VV mRNAs in human or RK-13 cell lines is blocked, and thus there is no VV DNA replication or further gene expression (Ramsey-Ewing & Moss, 1995). Interestingly, the insertion of CP77 into a VV WR K1L deletion mutant partially replaces K1L function, allowing VV to replicate in RK-13 cells (Ramsey-Ewing & Moss, 1996). Therefore, despite the absence of amino acid similarity, CP77, K1L and C7L all influence host range in one cell type or another.
The host immune response to viral infection is biphasic, with innate effectors such as interferons (IFNs), natural killer (NK) cells and macrophages being critical in the early phase, and with adaptive antigen-specific T and B cell responses, which are often essential for clearance of the pathogen and establishment of immunity, developing later. Here, we focus on the innate immune response to VV infection, firstly highlighting its main aspects and then concentrating on the viral strategies to subvert it.

Inflammation is characterized by pain, redness, heat and swelling at the site of infection. The complement system is a crucial innate response that may destroy pathogens directly by lysis or indirectly by opsonising pathogens for phagocytosis by macrophages and neutrophils (Janeway, 2004). Macrophages have an important function as antigen presenting cells (APC) for the activation of T cells and the initiation of a specific immune response. Mice depleted of macrophages are unable to control VV infections due to impaired virus clearance and antigen presentation (Karupiah et al. 1996). NK cells are attracted to the site of infection as part of the inflammatory response and kill virus-infected cells, especially cells with reduced levels of MHC class I on their surface (Maudsley & Pound, 1991; See et al. 1997). NK cells have a direct cytotoxic activity towards VV-infected cells in vitro (Brutkiewicz, Klaus & Welsh, 1992) and their in vivo depletion is associated with enhanced VV virulence (Bukowski et al. 1983).

IFNs are a group of secreted proteins that induce an antiviral state in infected or uninfected cells (Samuel, 1991; Johnson et al. 1994). There are two groups of IFNs: type I IFNs are secreted from leukocytes and fibroblasts and include IFN-α and IFN-β. Type I IFNs offer resistance to virus infection, stimulate the expression of MHC class I molecules on the surface of the cell and inhibit cell proliferation (Biron, 1998). Type II IFN, or IFN-γ, is secreted from macrophages, NK cells and T lymphocytes. Type II IFN is important in the activation of immune and inflammatory responses and for cell-mediated immunity (Farrar & Schreiber, 1993; Boehm et al. 1997). Both type I and type II IFNs are crucial for the restriction of poxviral infections (Schellekens et al. 1981; Werenne et al. 1985; Rodriguez, Rodriguez & Esteban, 1991; Huang et al. 1993; Muller et al. 1994; van den Broek et al. 1995; Ramshaw et al. 1997; Deonarain et al. 2000).

Mice deficient in IFN receptors are abnormally susceptible to VV infection (van den Broek et al. 1995), showing the importance of these molecules in controlling VV infection, and it is therefore not surprising that VV encodes proteins that counteract their activity. The innate immune response also includes the initial inflammatory response that consists of the infiltration of the site of infection with plasma fluids and large numbers of leukocytes. The recruitment of leukocytes to the site of infection is orchestrated by several cytokines and chemokines secreted by the infected cells or resident macrophages. Amongst the inflammatory cells are monocytes that mature into macrophages and which together with neutrophils, the major cell type of early inflammatory infiltrates, may participate in the phagocytosis of infected cells and therefore contain virus infection (Buller & Palumbo, 1991). Importantly, cells present in the inflammatory infiltrate at the site of infection secrete more antiviral and inflammatory cytokines such as tumour necrosis factor (TNF) and IFNs. The complement system of proteins present in the plasma is able to destroy enveloped virions or infected-cells via the classic (Ab-dependent) or alternative (Ab-independent) pathways, which may also have a role in controlling VV infections given that VV has a strategy to avoid both complement pathways (see Table 1).

Apoptosis, programmed cell death (Kerr, Wyllie & Currie, 1972), is a natural and complex process that occurs in response to a variety of stimuli (Osborne, 1996; Jacobson, Weil & Raff, 1997). Apoptosis can be considered as part of the innate immune response because virus infection can induce apoptosis (Everett & McFadden, 1999). This process can also be targeted by poxviruses, as reviewed in Barry & McFadden (2000).

**Table 1** shows representative mechanisms utilised by VV to evade the innate immune response, which include both secreted and intracellular proteins. Viral immune evasion strategies often target key aspects of anti-viral immunity and are tuned for maximum benefit to the virus. Therefore by studying such strategies one can predict and probe host anti-viral mechanisms. In this section, we focus on some of the strategies used by VV to evade the host innate immunity. However, it should be noted that VV possesses numerous mechanisms to counteract the host immune responses that will not be discussed here. One example is the product of the H1L gene (Fig. 1) (Najarro, Trakman & Lewis, 2001), shown to bind and dephosphorylate STAT1.

**Viral semaphorin**

Semaphorins are chemomaterials that are involved in axonal guidance during development of the nervous system. The possibility that semaphorins, with their ability to establish connections between cells, might also function in the immune system was corroborated by the finding that...