How viruses evade host responses

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Humans have evolved a highly complex defence system to combat a wide variety of pathogens. Among the pathogens, developing a remedy for most of the viral infections still remains a great challenge as viruses evolve very rapidly as well as produce a variety of proteins. These features provide viruses with strategies to evade almost any hurdle imposed by the host defences to block their replication. It is important to understand the mechanisms of virus–host interactions, as this information can prove useful in symptomatic treatment of viral infections and aid in the design of antiviral vaccines. Further, since viruses rely almost entirely on the host cell machinery for their propagation, these studies educate us about our own cells and the basic mechanisms of gene expression and immunity.

IN a recent essay[1], Nobel Laureate Joshua Lederberg writes, 'Our relationship to infectious pathogens is part of an evolutionary drama. Here we are; here are the bugs. They are looking for food; we are their meat. How do we compete? They reproduce so quickly, and there are so many of them. They tolerate vast fluctuations of population size as part of their natural history; a fluctuation of 1% in our population size is a major catastrophe. Microbes have enormous potential mechanisms of genetic diversity. We are different from them in every respect. Their number, rapid fluctuations, and amenability to genetic change give them tools for adaptation that far outpace what we can generate on any short-term basis'.

Viruses form a large part of the pathogens we encounter daily. Mention the word 'virus' and it evokes responses generally associated with a fear of the unknown – as if it were an alien from outer space out to conquer our bodies. The truth is that viruses are simple organisms that have evolved with us and are constantly educating us about our own complex biological systems.

What then, is a virus? Well, it is small and it is not a bacterium. While an average bacterium or a human cell is about 5 microns, the smallest virus is about 30 nm and the largest one about 500 nm (1000 nm = 1 micron). Unlike bacteria, viruses are not susceptible to antibiotics (that is why flu takes seven days to go without medicines and one week with them!!). There are two other questions commonly asked about viruses. Are they living or non-living? A virus is not strictly alive… it is not strictly dead either. When inside a living cell, it can make copies of itself (reproduce) and is considered living. Outside a living cell it cannot multiply on its own, and is thus non-living. There is not a single living system, be it the lowly bacterium or the complex *Homo sapiens*, that does not have a virus infecting it. Where do viruses come from? We cannot say for sure. But, at least in the case of eukaryotic viruses, they are believed to have originated from some kind of cellular organelle, perhaps ultimately from nuclear DNA, perhaps from other organelles. Many would have undergone enormous changes, but we are still ignorant of the order in which those changes may have taken place. Basically, we know very little about the origins of viruses.

Apart from their small size, all viruses have only two common features: (i) a genetic programme (either DNA or RNA), and (ii) a protein coat to protect this genetic information and aid in its transfer from one host to another. Small size necessitates a small genome, which is the reason for its obligate parasitism. This absolute dependence upon the host has, however, taught us much about living systems more complex than the virus itself[2]. In the early days of molecular biology, bacteriophages (viruses that live in bacteria) taught us, amongst other things, about DNA and its role in the inheritence of genetic traits. Today, the more complex eukaryotic (animal) viruses are teaching us how our immune system works, how our cells divide and die (apoptosis), and how signals received at the surface of our cells are transmitted downstream and translated into genetic responses (signal transduction).

This review will discuss how viruses have adapted to deal with the host in attaining their goal of multiplication and transfer from an infected to a naïve host.

Viruses and the diseases associated with them are the major cause of human morbidity and mortality. When a virus infects a host, the latter tries to eliminate it by stimulating both antigen-specific immune effector mechanisms (antibody and T cell responses) and non-specific defence mechanisms (natural killer cells and interferons). Simultaneously, the virus tries to subvert one or more of these mechanisms to prolong its own lifespan.

Despite their restricted genome size, a number of viruses have been found to encode proteins that interfere at various levels with specific or nonspecific host de-

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fences. These proteins enable the virus to replicate more efficiently amidst antiviral host defences by evading them. The evasion of host immunity is advantageous to the virus at several levels. In case of widespread immunity in a host population, a new virus can evade the pre-existing immunity and thus persist in the population without getting eliminated. Evasion of immunity also increases viral pathogenicity (or its ability to cause disease) in the individual host. For example, the virus may grow to higher titres in the host and thus increase its chances of transmission to a naïve recepient. Several viruses avoid detection and elimination by the immune system and are able to persist in the host[3].

This brings us to the point of acute versus persistent viral infections, situations important for understanding the pathogenic nature of different viruses. There are many infections, e.g. herpes, hepatitis B and C, in which the virus is not eliminated from the host, but persists for months, years or even the lifetime of the host. This is in contrast to acute infections, such as poliomyelitis or influenza, in which the virus is eliminated from the host within a few weeks. In a persistent infection, the virus either (a) continues to multiply and hence remains demonstrable, resulting in a chronic infection, or (b) may survive in some non-infectious form, as a latent infection with a potential for future reactivation.

The sole purpose of a virus is to make more of itself. A prolonged infection offers a better chance of finding a naïve host. Further, almost every pathogen and virus es for certain, reach a dead end if they kill the host. As Lederberg[1] puts it, '... our microbial adversaries have a shared interest in our survival. ... Truly severe host pathogen interactions historically have resulted in the elimination of both species. We are contingent survivors of such encounters because of this shared interest'. Viruses have evolved various strategies to evade host defence mechanisms in order to establish a persistent or at least a protracted state of viremia.

Viral evasion of the host immune response

The host immune response to infection is generally played at two levels – non-specific, targetting all invading pathogens with the same 'bullet' or specific, targetting each pathogen with one made to order[4]. The non-specific mechanisms include mechanical barriers (e.g. skin), body fluids (e.g. stomach acid, tears) and enzymatic pathways (e.g. complement and interferons). This is the *innate immunity*. Specific mechanisms or *adaptive immunity* includes components of the antibody and cell-mediated pathways, and to some extent the complement pathways as well.

Antibodies developed by the host in response to infection by a pathogen form an important line of defence against the primary infection as well as subsequent attacks by that pathogen[5]. The repertoire of antibody-producing B lymphocytes is vast and is genetically programmed to respond to a very large number of pathogens. The T lymphocytes are also an important part of adaptive immunity. While the helper T (T_h) cells aid in expansion of the pathogen-specific B lymphocytes, the cytotoxic T lymphocytes (CTLs) target and destroy host cells that are already infected by the pathogen. Such co-operative action and rapid mobilization of forces in the event of future attacks (secondary infection) by the same or closely-related pathogens necessitates a memory in the host directed towards specific recognition sites on the pathogen[5]. Proteins of the pathogen that are either part of the structure or proteins that are expressed following infection, generally offer these recognition sites (epitopes). The pathogen-specific epitopes are seen and remembered by the host immune system. Since epitope recognition, antigen-specific lymphocyte expansion and antibody production form the basis of adaptive immunity, these are the very steps targeted by pathogens for successfully persisting in the host. We will see how viruses achieve this.

Strategies to induce immunosuppression

A large number of viruses evade immune response by causing generalized immunosuppression. This can be achieved in many ways.

Infection of immunocytes: Many persistent viruses infect lymphocytes (B or T cells) and monocytes and either destroy these immunocytes by cytolytic mechanisms or alter their functions[4]. Examples are the herpes simplex virus (HSV), Epstein-Barr virus (EBV), poliovirus, measles virus, etc.

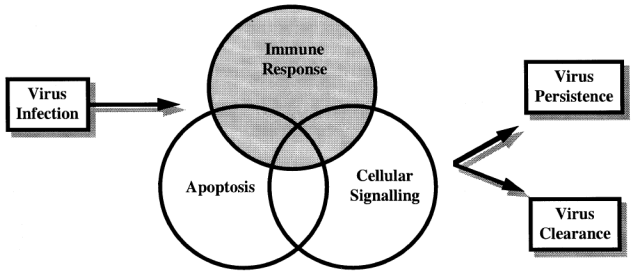

Figure 1. Viruses interact with three basic host responses. On virus infection, the host responds by activating immune mechanisms and by sacrificing a few virus-infected cells (apoptosis) in the interest of the whole organism. Cellular signalling plays a central role in both these processes. Whether an infection becomes persistent or is cleared depends largely upon how effectively the virus is able to modulate these host responses.

Destruction of specialized antigen-presenting cells: Mononuclear phagocytes (blood monocytes, tissue macrophages and dendritic cells) comprise a principal cellular element in the clearance and inactivation of most viral pathogens. These cells are a major target and infectious reservoir for many persistent viruses, e.g. lentiviruses (such as HIV), cytomegaloviruses (CMV), etc. The macrophage–virus interactions dramatically affect the ability of these cells to act as scavenger cell, immune effector cell, or susceptible target cell for virus replication. Destruction of macrophages following viral infection results in a generalized immunosuppression against most immunogens, affecting both antibody and T cells[6]. This mechanism appears to be the reason for late immune failure in AIDS.

Induction of tolerance: Some viruses can infect the thymus and cause clonal deletion of maturing virus-specific T cells[7,8]. This results in the virus being viewed as self by the immune system, without any generalized immunosuppression. Such a mechanism for viral persistence has been demonstrated experimentally for lymphocytic choriomeningitis virus (LCMV) and hepatitis B virus (HBV).

Strategies to evade antibody responses

Viruses carry proteins on their surface that are critical for binding to target cells and for entry into these cells. These proteins, which are so important for the viral life cycle, are obvious targets for the host antibody response. The host produces neutralizing antibodies targetted to these proteins. Many persistent viruses respond by altering immunogenic regions of these proteins to escape the neutralizing antibodies. At least three different mechanisms are known through which this is achieved by viruses.

Antigenic variation: The polymerases of RNA viruses lack proof-reading function, resulting in high mutation rates and the gradual accumulation of minor mutations in viral proteins. Point mutations in the envelope glycoprotein result in escape from neutralizing antibodies, e.g. neuraminidase and haemagglutinin of influenza virus and gp120 of HIV. In HIV-1 three distinct groups are recognized: the major (M) group, the outlier (O) group and a newly-recognized (N) group. Of these, viruses in the major (M) group are further divided into ten different subtypes, A through J, based on the antigenic properties of the envelope proteins[9]. These are also reflected in epitope changes in intracellular proteins such as Gag, Pol and Nef.

Antigenic shift: This strategy is used by viruses with segmented genomes. For example, the influenza virus genome is made up of 8 RNA segments. During coinfection of a single cell by two viruses, the segments from both infecting viruses can reassort. This can result in the emergence of a new (reassorted) strain which will not be recognized by previously-formed antibodies, and can thus establish infection in individuals previously exposed to influenza virus[10]. Many influenza pandemics, such as the Spanish Flu of 1918 and the more recent Hong Kong Flu have resulted from such antigenic shifts in the virus.

Down-regulation of viral protein expression: The intracellular localization of a virus facilitates it to escape antibody responses. Although antibodies can detect viral antigens expressed on the cell surface, many viruses delay displaying their proteins on the cell surface until late in the infective cycle. Others downregulate the expression of surface glycoproteins, in comparison with other nonsurface viral proteins[11].

Strategies to evade cellular responses

T lymphocytes play a crucial role as they can recognize a virus-infected cell early in the infectious process. This is accomplished by recognizing the complex of viral peptide and major histocompatibility complex (MHC) proteins expressed on the cell surface. But, viruses have developed strategies to evade T cell recognition at several different levels, emphasizing the importance of T-cells in virus clearance.

Infect cells lacking class I MHC: Herpes viruses infect neurons which express little or no MHC class I molecules[12]. Moreover, there is very little expression of viral RNA and protein which enables the virus to establish latent infection in the individual. Other viruses that follow this strategy for persistence are measles, LCMV and alphaviruses.

Mutation of viral protein T-cell epitopes: In many viruses, the rapid rate of replication (e.g. in HIV: 10^9 virions per day *in vivo*) coupled with the error-prone nature of the replicase results in a diverse population of viruses that are likely to contain a mutation within any CTL epitope[13]. These mutations enable the virus to evade T cell responses. Such mutations could act at any one of the several steps involved in the process of antigen presentation. It could prevent proteosome cleavage, TAP transport to the endoplasmic reticulum, binding to MHC or recognition by T cells (Figure 2).

MHC class I restricted antigen presentation and its evasion: Virus-infected cells are targets for $CD8^+$ cytotoxic T lymphocytes (CTL), which are MHC class I restricted. The infected cells express class I-peptide

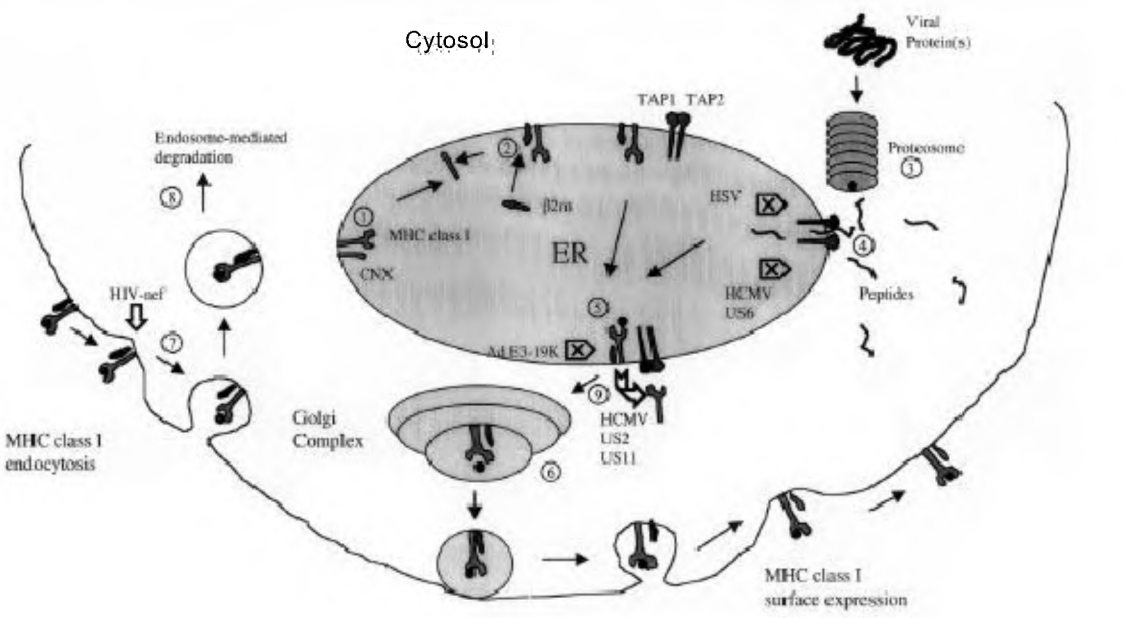

Figure 2. MHC class I pathway and its modulation by viruses. The cell surface MHC class I complex comprises heavy and light (β 2-microglobulin) chains and a peptide bound to the heavy chain. Newly-synthesized heavy chains are associated with the ER-resident chaperone, calnexin (CNX) (1), which is displaced on light chain association (2). Proteins are degraded in the cytosol through the proteosomal pathway (3); the resulting peptides are presented to ER-resident MHC class I molecules by the MHC-encoded peptide transporter TAP (4). Once loaded with peptide (5), the MHC I complex is allowed to leave the ER for the cell surface through the golgi pathway (6). During regular turnover, cell surface MHC I complex undergoes endocytosis (7) and endosome-mediated degradation (8). ER-resident heavy chains can also be dislocated back to the cytosol for proteosome-mediated destruction (9). A number of viral proteins are known to target different stages of the MHC class I pathway. Some examples are shown. Boxed arrows indicate enhancement and cross (X) indicates blockage. See text for details.

(pathogen derived) complex on their surface which results in stimulation of antigen-specific CTLs. Antigen presentation involves not only the expression and processing of viral antigens, but also the biosynthesis and intracellular trafficking of MHC molecules[4].

The peptides to be loaded onto class I molecules are generated by destruction of pathogen-derived proteins through ubiquitin-mediated proteosome cleavage. The peptides are then translocated by an MHC-encoded peptide transporter, TAP, which is physically linked to the class I molecule through the ER-resident protein tapasin. Only when they are properly assembled and loaded with peptide, class I molecules are allowed to exit the ER and enter the secretory pathway, to be displayed on the cell surface. Most of the class I molecules on the surface of normal cells are occupied with peptides derived from the cell's own proteins. When a pathogen infects the host cell and its proteins enter the cytosol, these self-peptides are replaced by pathogen-derived peptides during turnover of the MHC molecules.

The importance of MHC class I pathway in the generation of antiviral immunity can be gauged from the fact that viruses are known to target every single step in the assembly and trafficking of the MHC class I complex[14]. These can be categorized as follows.

(i) Regulation of MHC class I genes: Some viruses encode proteins that can inhibit the transcription of class I genes[15] and other components of class I presentation pathway[16] such as TAP. For example, the HIV-1 Tat protein downregulates the transcription of MHC class I genes.

(ii) Interference with cytosolic proteolysis: In human cytomegalovirus (HCMV) infected cells, expression of the viral phosphoprotein, pp65, inhibits the generation of HCMV-specific T cell epitopes[17]. In EBV, the Gly–Ala repeats present in the viral EBNA-1 protein interfere with its proteolysis within the proteosome[18].

(iii) Blockage of peptide transport: Herpes simplex virus type 1 (HSV-1) and HSV-2, each encode a polypeptide inhibitor of TAP. The viral proteins compete with peptides for binding to the TAP complex[19]. Similarly, the HCMV protein US6 is an efficient inhibitor of the TAP transporter.

(iv) Retention and dislocation of class I molecules: Adenoviruses encode the E3-19K type I membrane glycoprotein which has an ER-retrieval signal in its cytoplasmic tail. The binding of this protein to MHC class I molecules forces their retention in the ER, preventing

transport to the cell surface[20]. The HCMV products US2 and US11 can bind to class I molecules and redirect heavy chains to the cytosol (a process referred to as dislocation), where they are destroyed by the proteosome[21].

(v) Internalization of class I molecules: The HIV Nef protein downregulates MHC class I by increasing endocytosis of class I molecules present on the cell surface[22]. This results in escape of HIV-infected cells from $CD8^+$ CTL-mediated killing.

Strategies to evade cytokine action

Cytokines (also called lymphokines or monokines) are secretory proteins made by cells of the immune system, following their activation[23]. These are simple polypeptides or glycoproteins, with a molecular mass of ≤ 30 kDa, but for many the functional forms are homodimers or homotrimers. As soluble factors, they act as messengers on cells of the immune system to promote regulation of the immune response and elimination of the invading pathogens. Cytokine production is transient and their action radius is usually short, making the effect autocrine and paracrine, but rarely endocrine.

The immune responses to antigens are often distinguished by a relatively distinct pattern of cytokines produced by $CD4^+$ T-helper cells (Figure 3). This in turn drives the immune system towards either a predominantly cell-mediated (Th1) or a humoral (Th2) immune response. The Th1 cells secrete interleukin-2 (IL-2), interferon-gamma (IFN-γ) and other mediators to promote CTL and other delayed-type hypersensitivity reactions frequently required for the clearance of intracellular pathogens. The Th2 cells secrete IL-4, IL-5, IL-6, IL-10 and IL-13, all of which promote allergic type responses and stimulate the differentiation of antibody producing B-cells.

Cytokines produce their actions by binding to specific high affinity ($K_d \sim 10^{-9} - 10^{-12}$ M) receptors on target cells[23]. This binding sets off a series of intracellular events that result in the activation of specific proteins through phosphorylation and dephosphorylation, and in the formation of protein–protein interactions. The signal transduction pathway set in motion by cytokine binding at the cell surface culminates in altered gene expression in the target cells. Phenotypically, cytokine action leads

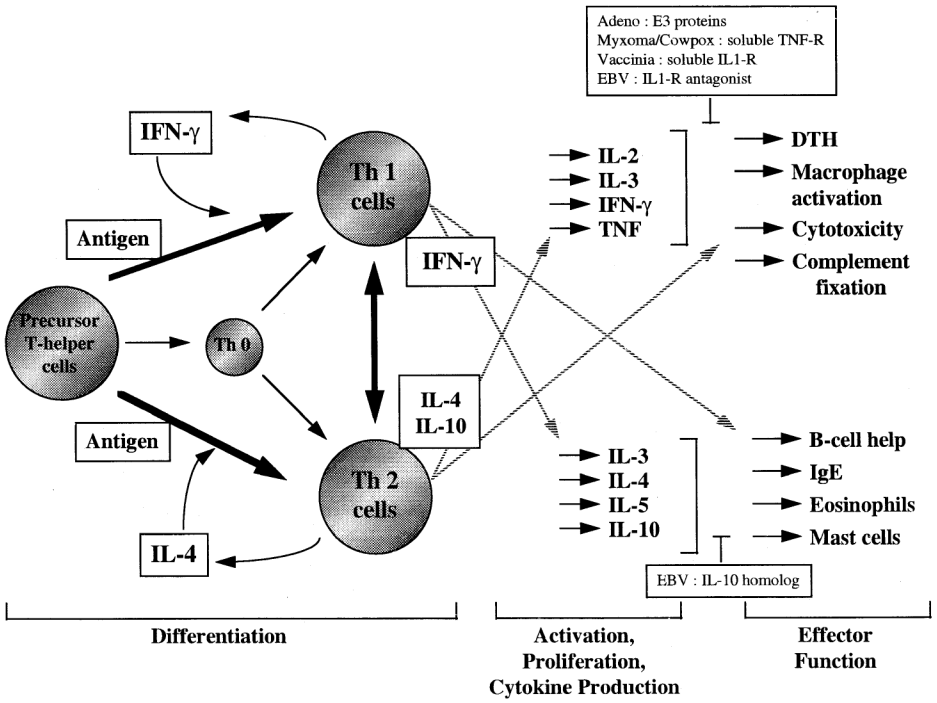

Figure 3. $CD4^+$ cell subsets, cytokines and effector functions. The T-helper ($CD4^+$) precursor cells differentiate into the Th1 or Th2 subsets and each undergoes clonal expansion through an autocrine loop in the presence of antigen. The Th1 cells require interferon-γ (IFN-γ) and Th2 cells require interleukin-4 (IL-4) for this expansion. These cytokines are also required to cross-regulate the activation and effector functions of each subset. Following activation, the $CD4^+$ lymphocyte subsets proliferate and produce other cytokines that are required for their effector functions. While Th1 cells provide help to $CD8^+$ cells (CTLs) for cell-mediated immunity, the Th2 cells provide help to B-cells for antibody production. A number of viral proteins are known to interfere with these effector functions. Solid arrows represent activation and dotted arrows represent repression. See text for details.

to an increase (or decrease) in the rate of cell proliferation and/or changes in the state of cell differentiation. Cytokines often act by regulating the production of other cytokines (cytokine cascade) or by modulating the receptors for other cytokines. Further, since body cells and tissues are rarely exposed to a single cytokine at a given time, cross-talk (either synergism or antagonism) between cytokines is extremely important for the end result[23].

How do cytokines protect cells from viruses? Besides regulating the immune response to the host's advantage, some cytokines show more direct antiviral actions. The interferon-induced antiviral pathways involve: (i) the induced synthesis of a family of enzymes called 2-5-oligoadenylate synthetases, which polymerizes ATP into 2′-5′-linked oligoadenylates that in turn activate cellular ribonucleases[24] to degrade viral RNA, and (ii) the induced synthesis of a protein kinase PKR[25], that phosphorylates and inactivates the translation elongation factor 2 alpha (eIF2α). Both pathways require double-stranded RNA (dsRNA) produced during viral replication as a cofactor, and the net effect of both pathways is inhibition of cellular (and therefore also viral) protein synthesis. Other IFN-induced mechanisms are known to directly inhibit viral replication[26], or promote lysis of infected cells by enhancement of MHC class I expression and viral peptide presentation[27]. Other cytokines such as tumour necrosis factor (TNF), transforming growth factor-beta (TGF-β) and IL-1 have also been shown to inhibit viral replication through IFN-like mechanisms or through the upregulation of MHC class I or class II antigens[28].

Since cytokines are so important in regulating immunity to pathogens, it is no surprise that many viruses have developed mechanisms to evade cytokine action. These are done at two different levels.

Antagonism with interferon-mediated actions: Viruses have evolved mechanisms to counteract the inhibitory effects of IFN on virus replication. Some highly virulent viruses (e.g. poliovirus) rapidly inhibit cellular RNA and protein synthesis, and thus interfere with the ability of cells to produce IFN; others offer more subtle regulation. The known mechanisms of inhibition of IFN action by viruses are summarized in Table 1. An overview shows that DNA viruses generally target the IFN-mediated signal transduction[29,30]; the cellular PKR and RNaseL enzymes that form the effector arm of IFN responses are also popular targets with many viruses[29,31].

Table 1. Molecular mechanisms developed by viruses to inhibit interferon actions

Virus	Virus product	Specific target	Mechanism of action
Adenovirus	Ela protein	Signal transduction	Blocks IFN signalling
	VA RNA	PKR	Blocks activation
Vaccinia	E3L protein	PKR	Binds dsRNA
Epstein-Barr	EBNA-2 protein	Signal transduction	Blocks signalling
	EBER RNA	PKR	Blocks activation
Myxoma	M-T7 protein	IFN-γ	Neutralizes IFN-γ
Hepatitis B	Terminal protein	Signal transduction	Blocks signalling
Herpes simplex	2-5(A) analogues	RNase L	Blocks activation
HIV-1	Tat protein	PKR	Degrades PKR
	TAR RNA	PKR	Blocks activation
Reovirus	Sigma 3 protein	PKR	Binds dsRNA
Hepatitis C	E2 glycoprotein	PKR	Blocks activation

Table 2. Proteins encoded by viruses that inhibit cytokine action

Virus	Product	Mechanism of action
Epstein-Barr	BCRF1 (viral IL-10, which binds to and signals through the human IL-10 receptor)	Inhibits synthesis of IFN-γ, IL-2, IL-1, TNF; it facilitates EBV replication by promoting B cell growth and transformation.
Poxvirus	Cytokine response modifier (crmA, a protease inhibitor)	Inhibits proteolytic activation of IL-1β by IL-1β converting enzyme (ICE) and thus blocks apoptosis induced by CTL, TNF or Fas
Vaccinia and cowpox virus	Array of cytokine receptors	Bind and block the activity of α-, β and γ-IFN, TNF, IL-1

Antagonism with other cytokines: Interferons are not the only cytokines targetted by viral products. Viruses have developed elaborate forms of molecular mimicry[32,33] whose only purpose appears to be to circumvent cytokine-mediated host defence (Table 2). Many viruses can direct the synthesis of proteins that can either neutralize cytokines[34] or inhibit their synthesis.

Viral modulation of cell death

Programmed cell death (PCD) or apoptosis is a process in which cells are induced to commit suicide[35]. It occurs during embryogenesis, tissue differentiation, aging or tumour regression. Apoptosis can be triggered by a variety of external and internal stimuli which include viral infection, growth factor withdrawal and DNA damage[36]. It is an effective way for the organism to sacrifice a few infected cells to limit the spread of infection, or cells with damaged DNA to preserve the overall genetic integrity. Cells undergoing apoptosis show DNA laddering, chromatin condensation and break-up into small, membrane-wrapped pieces (apoptotic bodies). These apoptotic bodies are engulfed by nearby phagocytic cells, e.g. macrophages and thus the entire scavenging action takes place without induction of an inflammatory response. A simplified view of the apoptotic programme is presented in Figure 4; it can be divided into four phases[36].

Stimulus: The provocation phase. This may be (i) an external signal delivered through surface receptors, e.g. CD95 ligand/CD95 (also called FasL/Fas), TNF/TNF receptor, etc. or (ii) an intracellular signal from the action of a drug, toxin or radiation, e.g. reactive oxygen species, DNA damage, etc.

Detection and transduction: The signal is detected by transmembrane proteins or the metabolic state of the cell, and is transduced through signal transduction pathways to the death effector machinery. In the FasL/Fas pathway, for example, proteins such as FADD (Fas-activated death domain) are involved in signal transduction.

Effector: The death signal activates cellular proteases (also called caspases; cysteine proteases that cleave at

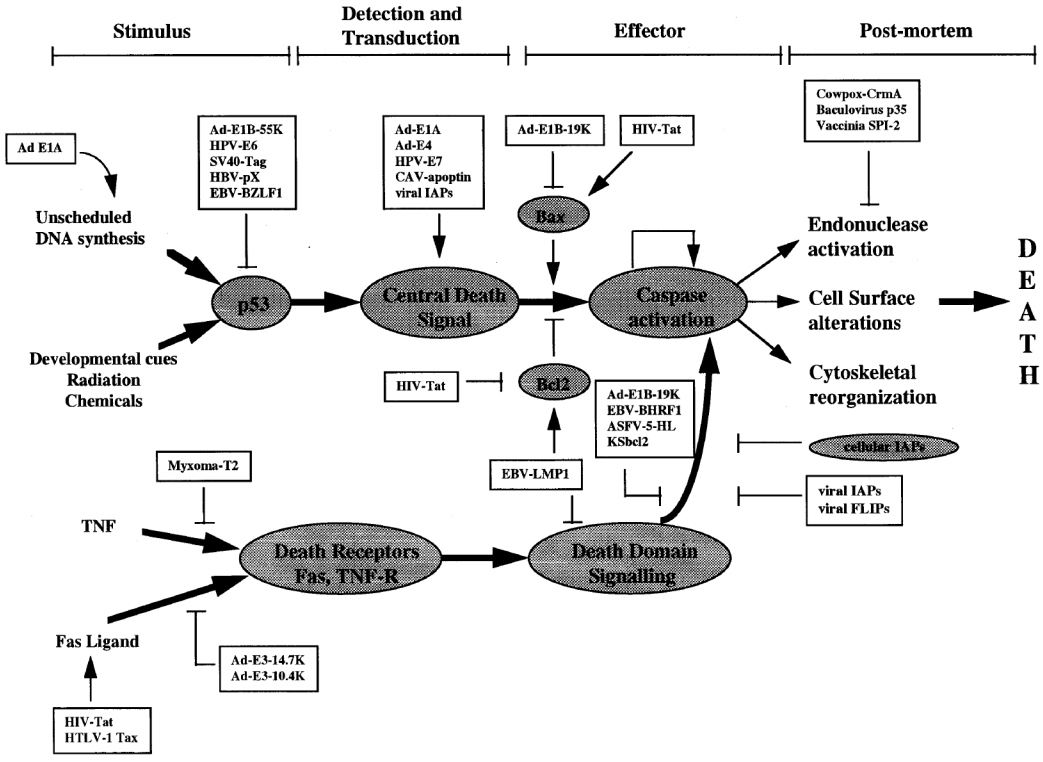

Figure 4. The apoptotic pathway and its modulation by viral proteins. Apoptosis is a physiological death process triggered by internal or external stimuli. The process can be divided into four phases: stimulus, detection and transduction, effector and post-mortem. The activation of effector proteases (called caspases) is controlled by an as yet undefined central death signal and by cellular proteins of the Bcl-2 family. Many signal transduction pathways, either p53-dependent or independent of p53, can lead to caspase activation. The caspases can activate themselves and each other resulting in the initiation of cell death. In the post-mortem phase, caspase-activated endonucleases cause DNA destruction; proteolysis results in cell surface and cytoskeletal alterations. The apoptotic cell is not lysed, but removed by phagocytosis. The cellular components are shown in shaded ovals; the viral proteins interacting with the pathways are shown in boxes. Thick arrows indicate pathways, thin arrows indicate activation and bars indicate repression or blockage.

aspartate residues) which are effectors of the death response. In the case of external stimuli, caspase 8 (also called FLICE) gets activated, while for internal stimuli caspase 9 present on the external surface of mitochondria is activated. This phase also includes a number of positive (pro-apoptotic) and negative (anti-apoptotic) regulators of the caspases. While proteins such as Bcl-2 and Bcl-x are anti-apoptotic, others such as Bax, Bad or Bak are pro-apoptotic.

Post-mortem: Cellular endonucleases are activated, the chromatin condenses and the cell's DNA is degraded. This is accompanied by changes in the cell surface and cytoskeleton. *In vivo*, dying cells are recognized and engulfed by other cells (phagocytosis).

Apoptosis is an important mechanism through which the host tries to reduce or eliminate the spread of progeny virus by eliminating virus-infected cells at an early stage. Thus, it is not surprising that many viruses have evolved mechanisms to suppress or delay early cell death (Figure 4) to allow production of high yields of progeny virus[37,38]. But the viral effects are not limited to suppression of apoptosis. Many viruses also produce proteins which induce apoptosis[37,38]. The induction of apoptosis late into the viral replication cycle enables the dissemination of progeny virions without causing an inflammatory response or exposure to host enzymes and neutralizing antibodies.

Viral inhibition of apoptosis

Small DNA tumour viruses have the ability to activate the DNA synthetic machinery in infected cells, which is then used for replication of viral DNA. However, this unscheduled DNA synthesis also activates p53, resulting in the transcriptional activation of p53-responsive genes (such as p21 and Bax) and p53-dependent apoptosis of cells. Several viruses have therefore developed strategies to block p53-induced apoptosis. The SV40 large T antigen binds directly to the p53 DNA binding domain[39], preventing its binding to and activation of cognate promoters. The E1B protein of adenovirus binds to the activation domain of p53 and blocks the activation of p53-responsive genes[40]. The E6 protein of papilloma viruses also binds p53, but marks it for degradation via the ubiquitin pathway[41].

Infection by many viruses also induces p53-independent apoptosis. Many viruses encode Bcl-2 homologues which inhibit p53-independent apoptosis. The E1B-19K protein of human adenovirus interacts with Bax and Bak[42,43] which are positive regulators of apoptosis and inhibits their action. The adenovirus E3 protein acts at multiple levels – it enhances clearance of Fas from the cell surface[44], thus inhibiting Fas-induced apoptosis, and it blocks TNF-mediated apoptosis[45] by interfering with downstream signalling from the TNF receptor. It is likely that these functions promote viral infection by preventing CTL-induced apoptosis of infected cells.

There are examples of other mechanisms as well. A number of viruses encode homologues of Bcl-2, which is an inhibitor of apoptosis. For example, the BHRF1 protein of EBV protects B cells from apoptosis[46], thereby giving EBV a selective advantage for replication in these cells. The cowpox virus CrmA protein acts as a specific inhibitor of proteases belonging to the ICE family, e.g. caspase 1 and caspase 8 and thus prevents or delays apoptosis. This inhibition of important effector molecules also results in inhibition of apoptosis caused by CTLs[47] and Fas signalling[48]. The vaccinia virus encodes a protein (SPI-2), highly homologous to CrmA which inhibits its apoptosis induced by TNF-α or Fas[49]. The HSV T-1γ134.5 protein prevents host protein shut-off through the PKR pathway and thus promotes virus survival in the infected cell[50]. The net effect of all these mechanisms is protection of virus-infected cells and a selective replication advantage to the intracellular viruses.

Viral activation of apoptosis

During the late stages of infection, many viruses encode proteins that overcome the effect of apoptosis-inhibiting viral proteins, allowing the cell to die and viral progeny to be disseminated to neighbouring cells. For example, the adenovirus E3-11.6 K protein (ADP; adeno death protein) is expressed in large amounts during the very late stages of infection[51]. It is proposed to block the anti-apoptotic action of the adenovirus E1B-19 K protein. The poliovirus 2A protease, earlier known to shut off translation by destroying the elongation factor 2α (eIF2α) has recently been shown to be sufficient for inducing apoptosis in poliovirus-infected cells[52]. The regulatory protein Tat, thrown out from HIV-infected cells can be spontaneously taken up by uninfected cells where it upregulates the expression of Fas ligand and activation of caspase-8, resulting in apoptosis[53]. Overexpression of Fas ligand also leads to the death of uninfected T cells in the proximity of infected cells. Tat expression downregulates the expression of Bcl-2 and increases the expression of apoptosis accelerator Bax[54]. The net effect is loss of far greater numbers of immunocytes during HIV infection than those infected by HIV, or the so-called 'bystander effect'.

During the post-mortem phase of apoptosis, the contents of the dying cell are not released into the interstitial fluid. Instead, cells undergoing apoptosis are encased in membrane-bound apoptotic bodies which are neatly absorbed into neighbouring cells. Thus, in the case of virus-infected cells, apoptosis represents a very

efficient mechanism by which the virus can induce cell-death and disseminate progeny, while limiting the induction of inflammatory and immune responses. Enveloped viruses generally exit infected cells by budding at the plasma membrane. However, for nonenveloped and nonlytic viruses there is no proper mechanism to explain their exit and dissemination from infected cells. Apoptosis might just be the answer.

Viral regulation of signal transduction

In eukaryotes, cellular homeostasis is directed by extracellular signals. These signals received at the cell surface are transduced through various cellular pathways to the nucleus where regulation of gene expression results in phenotypic changes. The entire process, known as the signal transduction pathway, is critical for cells to respond to their environment. It is therefore not surprising that in their quest for survival and control of the host cell, viruses have evolved numerous genes that target this pathway (Figure 5). The two signalling networks most frequently targetted by viruses are cell growth and immunoregulation.

Cell growth

Tumour viruses are the best-studied examples and adopt several strategies to stimulate the proliferation of their host cells, thereby ensuring their own replication. Some induce the synthesis of cellular DNA replication proteins, others inactivate tumour suppressor proteins such as p53 and pRB (the retinoblastoma protein), that act as cell cycle checkpoints to inhibit proliferation[55]. Common examples of these include the p53-interfering viral proteins, SV40 T antigen, adenovirus Elb and papillomavirus E6 proteins, or the pRB-interfering proteins, SV40 T antigen, adenovirus E1a and papillomavirus E7 proteins. Viruses can also activate cellular pathways that normally stimulate cell proliferation by encoding homologues of growth factors, growth factor receptors and downstream components of growth factor signalling pathways[55]. Another class of viral transforming proteins which bears no resemblance to cellular proteins can activate growth factor pathways by either activating components of the signalling pathway or by mimicing the structure of an activated component[55]. Here we will discuss only these viral proteins and categorize them as signal activators and signal interceptors.

Signal activators: Viral proteins are known to target various intracellular signalling pathways. The net effect is either constitutive or prolonged activation of these pathways, resulting in increased cell proliferation. The pathways targetted and selected examples of viral activators are outlined here.

(i) *Viral growth factors:* Poxviruses encode proteins with growth factor-like activity. The vaccinia virus 19 kDa protein, called vaccinia growth factor (VGF) shows significant homology to members of the epidermal growth factor (EGF) family[56]. EGF and related molecules, including VGF, bind to the EGF receptor and initiate cellular signalling cascades that include tyrosine phosphorylation and activation of second messenger systems, resulting in cellular proliferation. This mitogenic signalling is proposed to be beneficial to virus replication[57].

(ii) *Receptor tyrosine kinases:* Integral membrane proteins that transmit growth factor signals are targetted by DNA viruses and retroviruses. The bovine papillomavirus (BPV) E5 protein is a 44 amino acid hydrophobic protein localized to the ER and golgi apparatus in transformed cells. It induces platelet-derived growth factor-beta (PDGFβ) receptor dimerization in the absence of ligand which inhibits receptor turnover, resulting in persistence of activated receptors at the cell surface[58].

(iii) *Non-receptor protein tyrosine kinases (PTKs):* These proteins associate with signal-transducing plasma membrane receptors and are targets for viral intervention[59]. The polyoma middle T antigen (PymT) associates with and activates the cellular tyrosine kinases c-Src, c-Yes and Fyn[60], possibly by dephosphorylating an inhibitory C-terminal tyrosine (Tyr527 in c-Src). The activated kinase phosphorylates PymT, creating binding sites for other signalling molecules such as SHC, PI3 kinase and PLCγ. These interactions transduce mitogenic signals normally triggered by growth factors (Figure 5).

(iv) *Mitogen-activated protein kinase (MAPK):* The MAPK network is the major pathway that transduces mitogenic signals from the plasma membrane to the nucleus. MAPKs, also known as extracellular signal-regulated kinases (ERKs), are protein serine/threonine kinases that are rapidly activated upon stimulation of a wide variety of cell surface receptors. Activation results in their translocation to the nucleus, where MAPKs control the expression of genes essential for many cellular processes, including cell growth and differentiation[61]. Some oncogenic viruses target the MAPK pathway. The herpesvirus samiri (HVS) STP-C488 protein forms a complex with p21ras, resulting in Ras activation and constitutive activation of MAPK[62]. Similarly, the hepatitis B virus X protein (HBx) prevents turnover of the Ras–GTP complex resulting in activated Ras and the downstream MAPK pathway[63]. Since HBx also inhibits apoptosis by directly interacting with p53 (Figure 4), the combined effect of decreased apoptosis and increased mitogenic signalling is an increase in hepatocyte proliferation. This likely explains the development

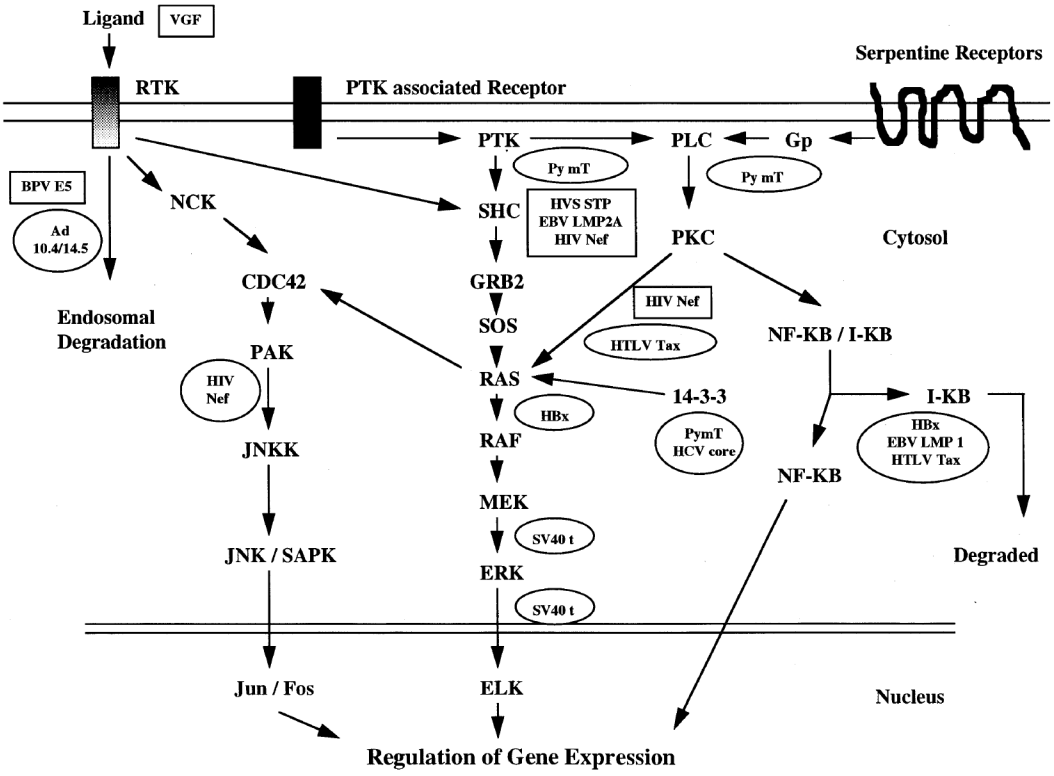

Figure 5. Cell growth signalling and its regulation by viral proteins. Signals such as growth factors, hormones, cytokines, etc. received at the cell surface are transduced through a cascade of events to the nucleus. This results in regulated expression of genes and the associated phenotype. Three major pathways originate at the cell surface through receptors with intrinsic kinase activity (RTK), receptors associated with protein tyrosine kinase (PTK) and serpentine (also called 7-transmembrane or 7-TM) receptors. Protein phosphorylation and dephosphorylation, and protein–protein interactions through a variety of domains are responsible for signal transduction. Various cellular proteins involved in the pathways are shown. Examples of some viral proteins that either activate (ovals) or inhibit (rectangles) steps in these pathways are also shown. See text for details.

of tumours with long latency following chronic infection by the hepatitis B virus.

(v) *Phosphatidyl inositol/protein kinase C (PKC):* This is another major pathway known to transmit mitogenic signals. The phosphoinositide-specific phospholipase C (PI-PLC) is a receptor controlled family of enzymes that catalyses the splitting of phosphatidyl inositol-4,5-biphosphate into 1,2-diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP3)[64]. IP3 induces release of intracellular calcium; the elevated Ca^{2+} together with DAG induces translocation and activation of PKC, which is essential for cell proliferation and differentiation. A number of viruses target this pathway of signal transduction. The PymT protein forms a complex with PLC-γ1 resulting in its activation[65]. The human T-lymphotropic virus type 1 (HTLV-1) Tax, which is a viral regulatory protein, associates with PKC resulting in its autophosphorylation in the absence of co-factors and its translocation from cytosol to membranes[66].

(vi) *14-3-3-proteins:* The 14-3-3 family comprises of at least seven isoforms in mammalian cells[67]. The interactions of these proteins with cellular proteins such as Raf-1, PKC, Bad and others, suggest that they are connected to signal transduction and cell proliferation pathways. The PymT[68], and more recently the HCV core proteins[69] have been shown to bind the 14-3-3 proteins and activate the Raf-1/MAPK pathway. The physiological effect of this interaction is likely to be increased cell proliferation, beneficial to small DNA viruses as well as the transforming HCV.

(vii) *Transcription factor NFkB:* Nuclear factor-kB has been implicated in many cellular regulation pathways, such as transformation, inhibition of cell death, immunoregulation and activation of viral gene expression[70]. In resting cells, NFkB is present as a cytoplasmic complex with an inhibitor, IkB. Cellular activation triggers phosphorylation of IkB, its release from NFkB and subsequent translocation of the latter to the nucleus where it can regulate gene expression. Many viral proteins

involved in inducing cellular proliferation target the NFkB–IkB complex. The HTLV-1 Tax protein causes persistent nuclear expression of NFkB by phosphorylation and degradation of IkB[71] and also by physically interacting with IkB[72]. Tax activates the NFkB binding sites present in the IL-2 and IL-2 receptor promoters, thereby setting up an autocrine loop that results in cell proliferation without exogenous growth signals. The HBV targets this pathway through the HBx protein, as described earlier, and also through the middle surface antigen (MBHs), an ER-resident transactivator that activates NFkB signalling through a radical-mediated pathway[73].

Signal interceptors: Apart from the viral proteins that activate signalling pathways, there are also proteins that bind cellular signalling proteins and inhibit their activity. Persistent or latent viruses may benefit from this interception since induction of cell proliferation or activation pathways may lead to apoptosis of host cells or activation of the lytic replication pathway in latently infected cells. $p56^{Lck}$, a Src family non-receptor tyrosine kinase is a common target for signal interceptors since it is associated with cell surface receptors such as CD2, CD4, CD5, CD8, the IL2 receptor and a number of downstream effectors[74]. Lck has been implicated in T-cell receptor (TCR)-mediated or HIV-induced T-lymphocyte apoptosis[75]. The HVS ORF1 (tip) forms a complex with Lck and may be responsible for its sequestration; similar effects are seen with an EBV protein called LMP1 (ref. 76). However, both these viruses are transforming viruses – HVS transforms T-cells and EBV transforms B cells. Thus the inhibition of Lck by viral proteins may appear counter-intuitive. However, these viruses encode other proteins such as STP (by HVS) and LMP2A (by EBV) that promote mitogenic signalling and cell proliferation. These strategies may allow cellular transformation by HVS and EBV to proceed without the potentially adverse effects of an activated Lck, such as untimely virus replication and apoptosis.

Inhibition of cell proliferation and activation pathways has also been reported for non-oncogenic viruses. The HIV Nef protein has been shown to interact with cellular signalling at different points. Nef downregulates the cell surface CD4 receptor via endocytosis[77] and forms a complex with Lck, thus inhibiting its kinase activity[78] and protecting the virus-infected cells from reinfection and activation through the TCR. Nef also associates with a member of the p21-activated kinase (PAK) family[79]. The recruitment of PAK to the membrane by Nef might facilitate PAK activation pathways, leading to the activation of c-Jun kinases (JNK; a stress response kinase). This may result in inhibition of HIV-mediated apoptosis of infected cells. But, Nef is also known to get into uninfected CD4/CD8 cells and cause apoptosis, suggesting that it may act differently when present endogenously or acquired exogenously.

Immunoregulation

In the section on evasion of the host immune response, we dealt with various mechanisms of immunoregulation. To reiterate, host antiviral defences are at two levels: directed against free virus and directed against virus-infected cells. We have already discussed viral strategies to evade antibody and CTL responses, as well as cell death. It must be emphasized that all these strategies rely heavily upon signalling pathways.

Before getting to its target cell, the virus must first pass extracellular immune defences such as the complement cascade and antibody neutralization. Both directly mediate lysis or phagocytosis of free virus and virus-infected cells. Therefore, the most successful viral evasion strategies are those that inhibit the association with complement and/or antibody-dependent phagocytes. Vaccinia and herpesviruses have evolved a number of proteins that bind to C3b, C4b or Fc to inhibit complement activation and phagocytosis[80].

One common viral strategy to evade CTL responses is to downregulate MHC class I expression[14]. However, this makes virus-infected cells prone to natural killer (NK) cell-mediated lysis, an activity that dominates cellular cytotoxicity early after infection. Persistent viruses such as herpesviruses have developed strategies to counter this. The CMV UL18 (human) and m144 (murine) proteins are MHC class I homologues. These inhibit NK cytotoxicity, possibly by binding the inhibitory NK receptors[81]. The human CMV glycoprotein, gpUL40, has recently been shown to enhance the surface expression of HLA-E, an inhibitor of NK cells[82]. Thus, by modulating classical and non-classical HLA alleles, CMV is able to evade CTL as well as NK cell cytotoxicity.

Cytokines, as discussed earlier, are soluble proteins that play key roles in the induction and maintenance of inflammation, immune response, differentiation and embryonal development. Different strategies are followed by viruses in regulating the cytokine network[83]. The inflammatory action of cytokines such as IL-1 and TNF are targetted by many viral proteins. The most direct approach is taken by Shope fibroma/myxoma virus and cowpox viruses that encode secreted forms of the TNF receptor, and by vaccinia virus which expresses a soluble IL-1β receptor. The EBV gp350 viral envelope protein induces expression of an IL-1 receptor antagonist which competitively inhibits IL-1 activity. Since IL-1 and TNF are both inducible cytokines, their expression and maturation are also targetted by viruses such as cowpox and vaccinia.

Many of the effects of cytokines are transmitted through signalling pathways which are initiated when they bind to their receptor. These are also targetted by a number of viral proteins. The EBV BCRF1 protein, which is a homologue of the IL-10 cytokine, binds to and signals through the IL-10 receptor[32,33]. This protein likely facilitates EBV replication by activation of resting B cells and assists EBV in escaping immune surveillance by downregulating inflammatory cytokines. The cytotoxicity of TNF is also modulated by some viruses at the signalling level. The adenovirus E3-14.7 K protein and the E3-10.4/14.5 K heterodimeric protein block TNF-induced arachidonic acid release[45], a crucial signalling event. Further, arachidonic acid and its metabolites are potent mediators of inflammation. The adenovirus E1B-19 K prevents TNF-mediated apoptosis by inactivating Bax[42], a pro-apoptotic cellular protein. Other proteins such as the HSV ORF16 are Bcl-2 homologues[84] and thus enhance cell survival.

Signalling through the interferon type I (for IFN-α or β) or type II (for IFN-γ) receptors activates the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) signal transduction pathway[23]. The only known viral interceptor of the JAK/STAT pathway is the adenovirus E1A protein, which acts at two different levels. It represses the expression of a critical intermediate, the IFN-stimulated genes factor (ISGF)[85], and binds the transcription factors p300/CBP required by the STAT proteins[86]. The varicella zoster virus (VZV) has recently been shown to modulate the MHC class II pathway by downregulating expression of Stat1 and Jak2 proteins involved in IFN-γ signalling[87].

Concluding remarks

The goal of an invading pathogen, like the rest of us, is to propagate itself. It must find a niche in the host that it can occupy for the time required to reproduce. A limited genome also demands that it is able to salvage the required metabolites from the host. And while all this is happening, the pathogen must evade the defence mechanisms of the invaded host. This is a finely balanced act. On the one hand, the pathogen should be protected against rejection, but on the other hand, the host must not be so incapacitated that it perishes before the pathogen has reproduced.

The immune system and the pathogen it deals with have co-evolved for millions of years. As we have seen here for viruses, this has given rise to the development of very complex and intimate interactions between the two. Vertebrates have developed immunity; the invading viruses have discovered interesting and elegant ways to circumvent the immune mechanisms. Some of these interactions clearly favour the invading virus and were probably evolved by it for that purpose. Other interactions promote the survival of the host and destruction of the virus, and therefore, probably evolved in the host.

How have viruses gained these functions? One alternative is 'molecular piracy'. A large number of viral proteins involved in regulating cellular signalling, modulating cell death or immunoregulation, have clear homology to cellular proteins. Perhaps they were acquired from hosts. Acutely transforming retroviruses and the oncogenes they carry are excellent examples of such acquisition. It is interesting to note that sequence homology between cellular and viral proteins is very strong for RNA viruses (~80% amino acid identity in most cases), but weak for DNA viruses (~40% amino acid identity). This could be the result of generally high replication rates and a lack of proof-reading function in RNA replicases, leading to far more stringent requirements for survival. DNA viruses that successfully evade host responses generally have low replication rates and therefore might be able to get away with low affinity (efficacy) interactions. The second group of proteins used by viruses bears no obvious sequence similarity, but performs a function similar to their homologues in the host. This might be the result of convergent evolution, a potent selective force behind the generation of protein diversity. However, since the virus mutates far more rapidly than the host, the absolute dependence of the virus on the host may ultimately limit the diversity in viral proteins.

Viral strategies to evade host immune responses are played out at three different levels: (i) evasion of humoral (antibody) responses, (ii) evasion of cytotoxic cell responses, and (iii) evasion of cytokine responses. Viral evolution plays a central role in the development of these strategies. Random mutations in viral genomes are selected if they offer an advantage in getting away from antibodies or CTLs. We have also seen how suicide of a virus-infected cell (apoptosis) is an effective host response. The importance of this mechanism is evident from the wide range of strategies viruses use to inhibit cell death. Finally, we have seen that signalling is a process central to cell growth and regulation. It therefore comes as no surprise that in their quest for control, viruses have developed many strategies to regulate the signal transduction pathways in cells.

Here, we have highlighted only those interactions that have evolved in the virus. There will also be interactions whose reasons and evolutionary basis will not be very clear. This will, in all probability, be the result of our limited understanding of these systems. As we enter a new era in biology, dominated by genomics and a holistic approach to biological riddles, it is hoped that we will discover some of the not-so-obvious pathways of interactions between viruses and the host cells they invade. It is in this maze that we will find ways to control viruses and conquer the diseases they cause.

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