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News and Commentary

Subversion of cell survival and cell death: viruses as enemies, tools, teachers and allies

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Viruses have colonized all kingdoms of life, from bacteria to plants, and from insects to humans, but are themselves at the border of the definition of living entities. Their genomic RNA or DNA embedded in protein coats of various degrees of organization and complexity, sometimes containing diverse viral enzymes and/or lipids and proteins of cellular origin, they absolutely require to be inside cells to replicate, renew, and propagate. Hence, together with the transposons and the bacterial plasmids, they represent the ultimate form of parasitism. Some viruses are rapid hijackers of the cell transcriptional and translational machinery, constantly moving from cell to cell within a host, and from host to host within one or several species. Other establish long-term infection of cells and life-long colonization of their hosts, with some DNA viruses and the retroviruses integrating their genes in the chromosomes of their cell targets.

Although many viruses cause severe, sometimes fatal, acute or chronic diseases, others induce few or no harm in their natural hosts. On an evolutionary time scale, life-long interactions of mobile genetic parasites such as retroviruses and transposons with their host have also been instrumental in shaping the genetic make-up of several species, through a process of integration in the germline of their hosts, sometimes providing them with unexpected benefits. One of the most spectacular examples of such beneficial outcome of genetic parasitism concerns the very emergence of our adaptive immune system, that allows us to discriminate between 'self' (our body) and 'non-self' (microbes and tumors). The capacity of our adaptive immune system to protect us against infections and cancers depends on the generation of several millions of different antigen receptors that are randomly expressed by lymphocytes through a process of somatic genetic recombination. *Rag1* and *rag2* (recombination-activating genes) are two of our genes whose products allows this somatic recombination process, and are essential for our survival: human newborns with *rag* loss of function have no lymphocytes and are condemned to die of infections in the absence of bone marrow transplant therapy. What is the evolutionary origin of these *rag* genes? They seem to have initially belonged to a transposon, being involved in the 'selfish' propagation of this genetic parasite in its hosts.[1] Around 400 000 000 years ago, this transposon probably become inserted in the germline of one or several common ancestors of the jawed vertebrates, and *rag1* and *rag2* progressively become involved in the recombination process that gave rise to our adaptive immune system. Thus, in a counterintuitive manner, a retrospectively welcome failure of one of our ancestors to protect itself against a genetic parasite has been instrumental in the emergence of the organ that now protects us against infections. Similar seemingly paradoxical beneficial functions are exerted by endogenous retroviral sequences that have become a significant part of our genome. For example, an envelope glycoprotein encoded by an endogenous defective retrovirus has been reported to play a crucial role in human placenta development, by allowing syncytia formation of the trophoblasts.[2] There have been other very different ways in which viruses have been instrumented by humans to become allies in their fight against disease. More than 200 years ago, the intentional use of the vaccinia virus represented the first effective tool of medicine to prevent disease, leading to the eradication of a fatal viral disease, and to the emergence of the concepts of vaccine and immunity. And the ancestral ability of viruses to insert–and express–themselves in host cells has been more recently instrumented in the form of viral (and retroviral) vectors for gene therapy approaches aimed at curing genetic diseases.[3]

But there is more to the relationship between biology, medicine and viruses than the fight against viral diseases or the use of viruses as therapeutic agents. Deciphering the complex and dynamic interplay between viruses and their hosts has led to seminal insights into life fundamental processes and into the pathogenesis of major diseases. For example, viruses were critical in the discovery of several basic functional aspects of our immune system, such as the major histocompatibility complex (MHC) restriction process[4] that allows T-cell selection, survival, and effector functions, and is the cause of allogeneic organ transplant rejection and graft *versus* host disease. It is the study of retroviruses that allowed the identification of the reverse transcriptase enzyme,[5] revealing that genetic information could also be converted from RNA into DNA and that retrotranscription was also involved in the function of a crucial cellular enzyme, the telomerase. The investigation of bacteriophages led to the discovery of the restriction enzymes that became an essential tool of molecular biology.[6] The study of the mechanisms allowing an avian retrovirus to cause cancer led to the identification of the cellular proto-oncogenes and oncogenes, opening the road towards modern cancer research.[7] During the last decade, viruses have also provided important insights into another, rapidly developing research field, that aims at elucidating

the regulation of cell survival and cell death, and its role in disease.

Programmed cell death and the evolutionary arms race between viruses and their hosts

The 'Red Queen' metaphor has provided a useful framework for understanding the selective pressures that may drive genetic and phenotypic diversification during co-evolution of predators and prey.[8] As Lewis Carroll's Alice has to keep running with the Red Queen just to stay in the same place, so do viruses and their hosts each have to keep evolving new weapons, defenses, and counterattacks just to stay in the same place, in other words to persist. One of the ancestral targets of these evolutionary arms races seem to have been the control of cell survival and cell death.[9,10] Briefly, the rapid induction of programmed cell death in response to virus and other microbe entry appears to represent an ancient and effective defense strategy. Conversely, many viruses have evolved mechanisms that repress premature death in the cells they require for their persistence and/or replication.

The 'immunity' of some bacterial colonies to infection by particular bacteriophages probably provides one of the most ancestral examples of the paradigm of self-destruction as a defense mechanism against infection.[6] In plants, the 'hypersensitivity response' is a genetically regulated process of programmed cell death that plays an important role in protection against infection.[11] In insects, the efficient propagation of baculoviruses requires the presence of two viral genes *p35* and *iap* (inhibitor of apoptosis)[12] that delay programmed death until the virus has replicated, providing the complementary paradigm that a capacity to repress self-destruction of the infected cell may be a prerequisite for the persistence and propagation of viruses in their hosts. Repression of programmed cell death is also a strategy used by viruses that colonize mammals, including humans. Schematically, mammalian programmed cell death induction involves two major pathways: (1) the extrinsic pathway triggered by ligand-mediated engagement of the CD95/tumor necrosis factor (TNF)/TRAIL receptor family, leading to the activation of the caspase cascade; and (2) the intrinsic pathway triggered by growth factor deprival, cellular stresses and p53 activation in response to genetic alterations and cell cycle dysregulation, leading to mitochondrial outer membrane permeabilization regulated by the pro-and anti-apoptotic Bax/Bcl-2 protein family, and leading to the mitochondrial release of caspase activators (cytochrome *c*, Smac/Diablo) and/or of caspase-independent death effectors (AIF, endonuclease G, htrA2).[13] Many different viral gene products repress one or more of these pathways. For instance, viral p53 repressors include the polyomavirus SV40 large T protein, the human papillomavirus E6 protein, the adenovirus E1B-55K, and the LANA proteins of the human herpes virus 8 (HHV8), the causal agent of Kaposi sarcoma. Viral Bcl-2 homologues include the adenovirus E1B-19K, the African swine fever virus LMW5-HL, the Epstein–Barr virus BHRF-1 and the HHV8 ORF16. In addition to the previously mentioned Baculovirus p35 and IAP, viral caspase inhibitors include the cowpox CrmA, the vaccinia SPI-2, the v-FLIP proteins encoded by several herpes viruses, that prevent caspase activation downstream of the engagement of the CD95/TNF/TRAIL receptor family, and downregulation of CD95 and TRAIL from the cell surface is another mechanism induced by the adenovirus E3-10.4/14.5K protein.[14,15] While most of these viral repressors of programmed cell death have cellular homologues, some seem to have none, such as the human cytomegalovirus (HCMV) UL37x1 protein[16] or the baculovirus p35.[12] The independent evolution of different viral proteins targeting a given death pathway suggests that repression of such pathways conferred a selective advantage to many viruses. Because all viruses do not seem to target the same death pathway, it is likely that such selective advantages were closely related to the particular life cycle of the viral species. On an evolutionary time scale, it is tempting to speculate that such evolutionary arms races have represented a major selective pressure on the ongoing diversification, and maybe even the emergence of programmed cell death in most if not all the infected host species.[10]

But at least in mammals, there is an additional degree of complexity involved in these battles between viruses and their hosts, that derive from the coupling of the regulation of cell cycle and programmed cell death.[17] Several viruses, and in particular DNA viruses, require entry of the infected cell into the cell cycle for viral expression and replication. Thus, viral proteins that dysregulate the cell cycle also induce programmed cell death. Such viral proteins include the adenovirus E1A, the papilloma virus E7, and the HHV8 v-cyclin. For example, the adenovirus E1A oncoprotein, the first to be expressed after infection, induces cell cycle progression, thereby causing both p53-dependent and p-53-independent Bak- and Bax-dependent programmed cell death.[18] P53, Bak and Bax, by responding to cell cycle dysregulation, act both as tumor suppressors and as antiviral effectors, but two other adenovirus proteins will prevent self-destruction of the infected cell, E1B-55K by inhibiting p53, and E1B-19K by inhibiting Bak and Bax.[18] Thus, persistent viral infection may – or not – cause cancer development, depending on the particular cell death and cell cycle pathway targeted by a given virus for persistence and replication in his host. Efficient viral propagation involves a dynamic, sophisticated and sometimes seemingly contradictory interplay with the regulation of cell survival and cell death. For example, at a late stage of infection, once viral replication has proceeded, viral proteins such as the adenovirus E3-11.6K may facilitate efficient viral release by inducing cell death. But there are still many additional layers of host- and virus-mediated interference with programmed cell death that are related to the role of the immune system in defense against infections.[19]

Programmed cell death and the interplay between pathogens and the immune system

Our first line of defense against infection is the immediate response of our innate immune system to conserved microbial molecular patterns, involving the Toll-like receptor family,

complement activation and interferon synthesis, and resulting in a complex and currently poorly understood interplay between direct anti-microbial attack and programmed cell death induction.[20,21] As a second line of defense, the T lymphocytes and natural killer cells (NK) of our adaptive immune system control viral replication either through the secretion of antiviral cytokines[22] or the killing of infected cells by the release of perforin and granzymes or the engagement of the CD95/TNF death receptors.[23] Several viral proteins, such as the HCMV US2, 3, and 11, the herpes simplex virus ICP47, the adenovirus E3/19K or the HIV Nef induce the downregulation of the MHC class I molecules that allow the cytotoxic CD8 T lymphocytes (CTL) to detect viral peptides at the cell surface.[15] NK cells conversely induce death in cells that are lacking inhibitory MHCI molecules, such as HLA-C and -E. HIV Nef provides a striking example of a viral protein that causes a selective downregulation of the MHCI molecules that allow viral detection by CTLs, while not interfering with the expression of the MHCI molecules that inhibit NK cells.[24] When failing to escape recognition of the infected cells by immune effectors, various viral proteins provide protection against these effectors by either down-regulating the CD95/TNF receptors, preventing their death signaling or blocking caspase activation, as mentioned above. While the infected cells may thus survive immune attack, uninfected bystander cell death induced by antiviral immune effectors may cause disease. For example, extensive CD95-mediated programmed death of uninfected hepatocytes seems to play an important role in the pathogenesis of acute viral hepatitis.[25] But escape of infected cells from immune defenses can also come in the form of a counterattack: several infectious pathogens induce the death of some immune effector cell populations. For instance, bacteria such as Schigella and Salmonella selectively cause programmed death in macrophages, in which they do not replicate, by injecting through their type III secretion system the IpaB or SipB protein that induces caspase-1 activation.[26] Interestingly, while physiological induction of apoptosis is non- and even anti-inflammatory,[27] these bacteria provided the first example of a coupling of apoptosis induction and inflammation, through the caspase-1-mediated processing and release of the pro-inflammatory cytokines IL1β and IL-18.[28] Several viruses cause either transient or long-lasting immunosuppression, HIV1 providing one of the most spectacular examples of a virus that causes death in both infected and uninfected immune effector cells and their progenitors,[29,30] leading to a progressive and fatal immune deficiency. Viruses may escape immune defenses by inducing death of CTLs. For example, the HIV Nef protein causes the expression of the CD95 ligand (CD95L) on the surface of infected cells, such as macrophages and CD4 T cells, leading to the death of HIV-specific CTL, and CMV induces the expression of CD95L and TRAIL on the dendritic cell surface, triggering the death of CMV-specific T cells.[15]

In summary, through the expression of some proteins that repress death of the infected cell, and others that induce death in immune effector cells, several viruses may provide the infected cell with both an 'armor' and a 'sword' in their ongoing battle against the host. The HIV1 Nef protein, which plays a crucial role in HIV replication and pathogenesis, might represent the first example of a single viral protein endowed with a dual 'armor/sword' function.[31] But pathogen-mediated induction of apoptosis in immune effector cells may not only favor immune evasion, but also, through subsequent receptor-mediated ingestion of apoptotic cells by the infected cell, metabolic changes that enhance microbe replication in its host cell.[32] Finally, the nature of the cell death phenotype (apoptotic, non-apoptotic or even necrotic) induced by either the infectious pathogen or the host may influence the induction, regulation and effectiveness of the host immune response to the pathogen.[27]

Lessons, therapeutic approaches, and unanswered questions

Preventing viral-mediated repression of cell death by selectively targeting infected cells for rapid programmed death induction may represent a novel anti-viral strategy that has shown preliminary promising results *in vitro*.[33] Conversely, preventing uninfected bystander cell death induction has shown efficiency *in vivo* by allowing animal survival in non-viral systemic infectious diseases.[34,35] But modulating programmed cell death to medicine's advantage will require a rigorous assessment of the role played by programmed cell death dysregulation in the pathogenesis of any given infectious disease. Recent findings imply that it can be difficult to predict beforehand whether cell death induction in response to infection is to the advantage of the pathogen or of the host. More generally, because the regulation of cell death is coupled with that of cell activation, it will not be easy to understand in which circumstances viral-mediated programmed cell death dysregulation may be to the advantage of the virus *per se*, or may merely represent a non-significant by-product of strategies selected for other advantages they provide in terms of cell activation, differentiation, migration or proliferation.[31] Viral-mediated subversion of programmed cell death may also tell us something novel and counterintuitive about the very concept of protection against infection. The murine mammary tumor virus is a retrovirus that induces and requires activation of a small subset of T cells for effective propagation in its host. This viral superantigen-mediated activation is rapidly followed by the death of this cell subset, thereby preventing any new infection by a similar virus.[36] Thus, 'immunity' against infection can result from a 'default' mechanism rather than from the induction of an anti-viral effector mechanism. Maybe we might one day use such a concept to our advantage. Finally, novel strategies have been recently developed, based on the use of viral-mediated subversion of cell survival and cell death as a tool for anti-cancer vaccine or therapy. For example, a self-replicating RNA vaccine, constructed from a viral gene, and containing a given antigen, has been recently shown to be more effective as a vaccine than naked DNA, through a mechanism that seems to depend on apoptosis induction by the RNA vaccine.[37] Concerning cancer therapy, several viral-based approaches are currently investigated, including the combined use of the herpes simplex virus thymidine kinase gene and ganciclovir as a suicide gene therapy, and the selection or engineering of viruses that selectively replicate and induce

death in cancer cells.[38] For example, some attenuated adenovirus strains, such as ONYX-015, that lack E1B-55K, appear to be replication defective in normal cells that express wild-type p53, but to replicate and induce death in tumor cells that lack functional p53, a hallmark of half the human cancers. Combination of ONYX-015 with chemotherapy has led to promising preliminary clinical results in recurrent head and neck cancers.[39] In such cases in which viruses are turned into allies, however, one of the paradoxical problems that have emerged is the requirement to bypass the ancestral capacity of our immune system to detect, impair or reject these foreign viral components.[40] Further deciphering the ancestral and intricate interplay between viruses and their hosts should not only extend our understanding of human physiology and pathology, but might also lead to the development of new tools and strategies for the treatment of a wide range or infectious and non-infectious diseases.

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