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A SKEPTICAL LOOK AT VIRAL IMMUNE EVASION

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1. ABSTRACT

In the past several years, many viral gene products have been found to encode proteins which interfere with immune defense mechanisms. Whether these interactions between virus and immune system components are actually evasion mechanisms used during viral infections in their natural hosts remains to be proven. *In vitro* studies do, however, reveal several tactics which may aid viral replication and dissemination by interfering with components of both the innate and adaptive immune systems. In this manuscript, we discuss the more intensively studied of these putative *in vitro* evasion tactics and ponder their relevance in *in vivo* situations.

2. INTRODUCTION

The raison d'être of the adaptive immune system has been a topic of discourse amongst biologists. Some suggested the system evolved to preserve tissue integrity and eliminate cancer (1) but others might advocate that the driving force was the need by long-lived animals to control invasion and residence by agents which can rapidly evolve ways to bypass the innate defenses. The adaptive immune system, as we see it today (in mammals such as ourselves) successfully defends the body against all but a few microbes. We know that the absence or malfunction of certain components of adaptive immune defenses widens the spectrum of agents which can cause disease. As such, agents which are successful invaders and achieve residence may teach us valuable lessons on how the immune system itself functions (2). In addition, a careful analysis of a microbe's properties may reveal tactics which permit them to deal with host immunity, a strategy known as "immune evasion". So far, most such strategies have been identified only by *in vitro* analysis and few have been shown to act similarly within the body of their natural hosts.

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Prominent among agents which succeed in establishing stable long-term relationships with their hosts are herpes viruses (HV). Humans can be infected with at least eight such viruses. Some HV achieve persistence, existing in a latent state, in 80% or more of the population and have minimal consequences in immune competent individuals. HV have numerous candidate evasion mechanisms and have been favorite subjects of study by many investigators. Table 1 lists some of the better studied immune evasion strategies of herpes as well as other viral pathogens.

In this brief review, we outline some of the more prominent mechanisms representing immune evasion as measured *in vitro* and comment as to whether such putative evasion measures actually function similarly *in vivo*. Our objective is to evaluate if immune evasion in the complex environment of the body actually occurs or whether these tactics are misleading *in vitro* phenomena.

3. EVASION OF INNATE DEFENSE MECHANISMS

Most invading viruses have a window of opportunity to establish infection before the adaptive immune response becomes effective. In the case of HV, this is ample time for them to secure permanent residence in a state often refractory to subsequent immune recognition (3). To counteract initial infection, the host possesses an array of innate defenses which could minimize replication. Among the leading innate defenses are natural killer (NK) cells, macrophages, and several humoral molecules, particularly the cytokines and the complement cascade. Evading the action of one or more of these activities may ensure the success of an invading virus. Specifically, animals with genetic defects of NK cells or which have been artificially depleted of NK cells are more susceptible to murine cytomegalovirus (MCMV) and HSV (reviewed in 4). Recently cytomegalovirus (CMV), an infection also affected by NK cell function (5), was shown to encode a gene product which counteracted NK cell

Table 1: Selected Methods of Viral Evasion

VIRUS	GENE/gene product	EVASION MECHANISM	REFERENCE
Human cytomegalovirus	UL-18	Resist NK cell killing	6
Murine cytomegalovirus	M144	Resist NK cell killing	7
Myxoma	MT-7	IFN-γ Receptor homologue	13
Vaccinia	B18R	IFN Receptor homologue	11
Vaccinia	E3, K3	Interfere with IFN induced intracellular signalling	10
Myxoma	T2	TNFRII (p75) homologue	9
Cowpox	crmB, crmC	TNFRII (p75) homologue	9
Cowpox	CrmA	Inhibitor of IL-1β converting enzyme	12
Herpes simplex virus	gC-1	Inhibit complement function	17, 18
Herpes virus saimiri	CCPH	Inhibit complement function	15
Herpes virus saimiri	HVS-A15	Inhibit complement membrane attack complex	16
Adenovirus	E3-19K	Interfere with MHC I antigen presentation	35
Herpes simplex virus	ICP47	Interfere with MHC I antigen processing	24
Human cytomegalovirus	US2, US11, US6, US3	Interfere with MHC I antigen processing	25, 26, 27, 28
Murine cytomegalovirus	M152/gp40	Interfere with MHC I antigen processing	29
Human immunodeficiency virus	nef	Interfere with MHC I antigen presentation	38
Epstein-Barr virus	EBNA1	Interfere with MHC I antigen presentation	31
Murine cytomegalovirus	M04/gp34	Alter surface expression of MHC I	30
Adenovirus	E1B-55K, E4orf6	Protection against apoptosis via p53 inactivation	42
Adenovirus	E1B-19K, E3-14.7K, E310.4K/14.5K	Protect against TNF induced apoptosis	43
Equineherpes virus-2	E8	Protect against death receptor induced apoptosis	44
Herpesvirus saimiri	ORF71	Protect against death receptor induced apoptosis	44
Molluscum contagiosum virus	ORF159L	Protect against death receptor induced apoptosis	44
Bovine herpesvirus-4, Human herpes virus-8	unidentified	Protect against death receptor induced apoptosis	44
Epstein-Barr virus	LMP1, BHRF1	Protection against apoptosis via bcl-2 upregulation and homology	46, 47
Epstein-Barr virus	EBNA-5, BZLF1	Protection against p53 induced apoptosis	48, 49
Herpes simplex virus-1	γ34.5, ICP4	Protection against apoptosis	50
Epstein-Barr virus	BZLF2	Prevention of surface expression of MHC II	56
Herpes simplex virus	US7, US8	Fc receptor homologues: prevent complement neutralization and ADCC	65

destruction of virus infected cells *in vitro*. The gene product, UL18 in the case of human CMV and m144 for murine CMV, encodes a protein which acts as a molecular mimic of MHC Class I (6,7). The presence of this 'decoy' MHC I molecule essentially instructs NK cells to not kill thus protecting the virally infected cell from the consequences of viral induced MHC Class I down regulation. Infection of immune intact and NK cell depleted mice with MCMV m144 negative mutants gives convincing evidence that this viral decoy protein indeed plays a role in virulence since the mutant virus showed substantially increased replication in NK cell depleted mice (6). However, it is not yet certain whether UL18 defends effectively against NK cell killing during initial infection *in vivo* or whether this gene product is the reason patent infection can be established and maintained in humans.

NK cells effect the innate immune response in two ways; by killing infected cells directly or by generating humoral factors such as interferons (8). The interferons protect uninfected cells and modulate the protective actions of other components of immune defense. Blocking the activity of interferon represents a promising evasion strategy since it should both increase the pool of susceptible target cells as well as disarm potential antiviral effectors. Some viruses do possess homologues of interferon receptors providing them the ability to tie up and impede the function of interferons. Many orthopoxviruses (myxoma virus, vaccinia virus, cow- and rabbitpox viruses) have receptor mimics not only for interferons alpha, beta and gamma but also for other cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF)-alpha (9). Additionally, the vaccinia virus E3L gene product, through its binding of double stranded RNA, effectively inhibits the interferon (IFN)- induced intracellular signaling cascade which follows receptor triggering (10). Studies on poxvirus mutants *in vivo* make a strong case that the possession of these receptor mimics accord higher virulence. In particular, mice infected intranasally with a B18R (type 1 IFN receptor mimic) deleted vaccinia virus showed no signs of illness whereas mice infected with either wild-type or B18R rescued virus almost uniformly died (11, 12, 13). Indeed, the poxvirus cytokine receptor story provides the best evidence *in vivo* supporting the immune evasion hypothesis. Curiously, it appears that poxviruses engage almost in overkill since they have multiple potential strategies (table 1) seemingly designed to overcome host resistance. It is peculiar, therefor, that poxviruses have not adopted a lifestyle of *in vivo* persistence but survive in the environment outside their vertebrate hosts.

Viral interference of host chemokine function may prove to be another useful viral evasion strategy. Kaposi's sarcoma-associated herpesvirus (KSHV or human herpesvirus 8, HHV-8) encodes two viral proteins from open reading frames (ORFs) K4 and K6. These have sequential homology to the human beta-chemokines MIP-1 alpha, MIP-1 beta and RANTES (14). The beta-chemokines prevent HIV-1 entry to susceptible CD4+ cells by binding to the surface CCR5 chemokine receptor which is also the HIV-1 coreceptor. The viral MIP (vMIP) displays *in vitro* functional similarity to the human chemokines by reducing HIV entry to HHV-8 infected cells. While it is interesting to speculate that HHV-8 may protect against infection with HIV, the significance of this interaction *in vivo* is currently merely speculative.

One of the most potent protective elements of the innate defense system is the complement cascade. Although perhaps most useful for protection against bacteria, the complement system also generates components which serve to destroy invading viruses. Complement activation either succeeds in disrupting viruses or virus infected cells directly or by stimulating the antiviral cells and molecules of the inflammatory response to achieve the same purpose. Some viruses such as HSV and herpesvirus saimiri (HVS) appear to have adopted mechanisms of evading complement mediated activities. HVS encodes a protein homologous to the complement regulatory protein, decay-accelerating factor (DAF). This viral protein is called complement control protein homologue (CCPH). It may be either membrane bound on or secreted from infected cells and potentially binds host complement components C3b and C4b thus minimizing the membrane destructive effects to infected cells (15). HVS encodes a second gene, HVS-A15, with significant homology to the terminal complement control glycoprotein, CD59. *In vitro* studies have shown the HVS CD59 homologue prevents deposition or action of the membrane attack complex on the surface of infected cells (16). However, whether these complement evasion strategies of HVS play any role *in vivo* remains to be determined.

HSV binds via its protein gC-1 to one of the pivotal components of complement, C3b, which protects cell-free virus from complement lysis (17). Evidence for this comes from observations that gC deleted viral strains are more susceptible than are wild-type viruses to complement mediated neutralization *in vitro* and, similar to all HSV mutants, less virulent *in vivo*. Whether the necessity to use gC *in vivo* to bypass complement activation explains the fact that gC minus mutants are rarely found in nature (18) requires further investigation.

4. EVASION OF ADAPTIVE HOST IMMUNITY

Viruses are usually recognized as foreign to the host and the consequent immune response attempts to rid the body of infection. RNA viruses do not integrate into the host genome. Persistence for them demands continual replication and replacement of virions lost to a successful immune response. RNA viruses have the ability to undergo rapid mutation with the consequent antigenic variation putting them one step ahead of the inevitable immune response (19, 20).

Some agents persist in immune hosts presumably due to their evasion tactics. Several DNA viruses become virtually invisible by hiding in the host cell's genome and expressing few, if any, genes. However, for transmission to occur, this gene shut-off must be reversed and latency must be periodically interrupted. Advocates of immune evasion focus on these DNA viruses which persist inspite

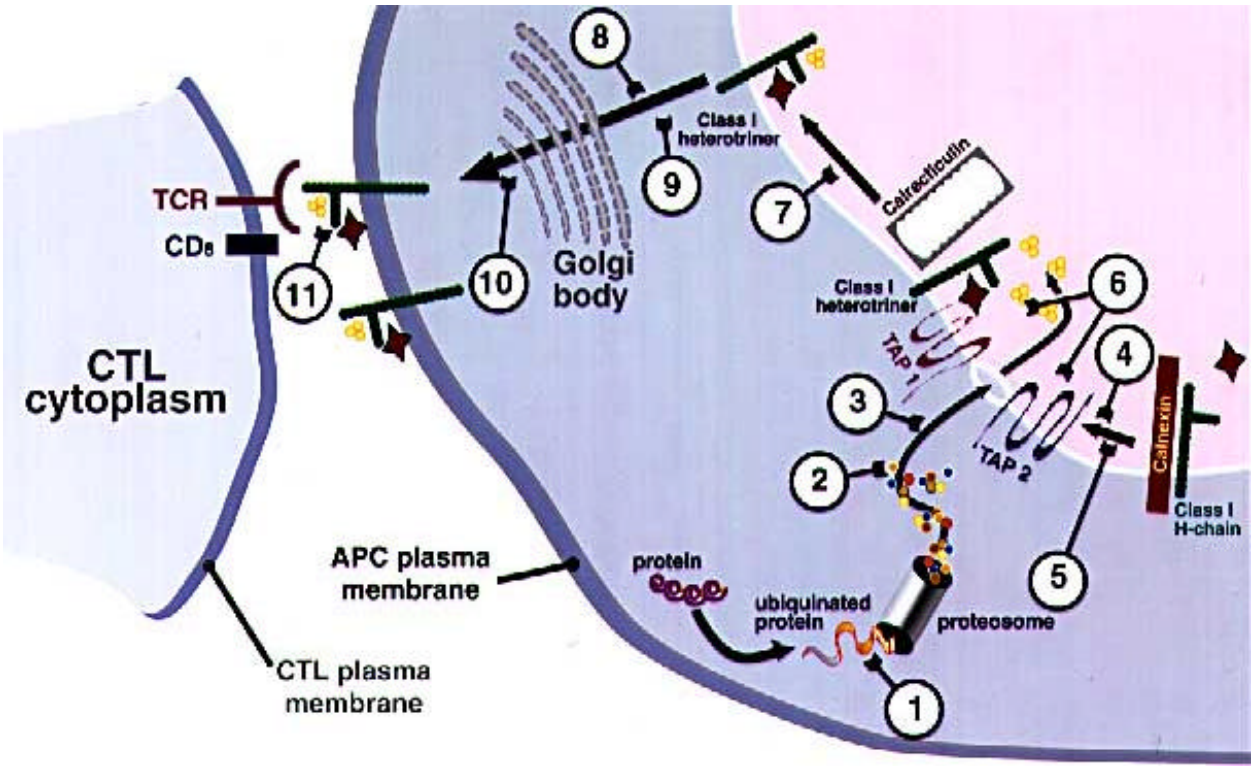

Figure 1: Viral interference with MHC Class I processing and antigen presentation: Viruses and their gene products are numbered as follows: 1-EBV EBNA1; 2- BHV-1; 3- HSV ICP47; 4- CMV US2; 5- CMV US11; 6- CMV US6; 7- CMV US3; 8- Adenovirus E3-go19K; 9- murine CMV m152 gene product; 10- HIV nef; 11- murine CMV m04 gene and gp34 protein. Viral proteins are degraded by cytoplasmic proteosomes following ubiquitination. Following TAP transport to the endoplasmic reticulum, viral peptide associates with the MHC I heavy chain and beta 2-microglobulin. It is this trimeric complex which is transported through the Golgi to be expressed on the APC cell surface. See text and table 1 for further details and references.

of dangling their potentially immunogenic proteins at the immune system. The immune elimination of viruses is primarily the domain of T cell activity (21). Consequently, circumventing T cell function appears to be the most commonly used approach by successful viral pathogens to achieve evasion. Not surprisingly, a large number of mechanisms have been identified, at least by *in vitro* analysis. Few, however, have been proven to function in a similar manner *in vivo*.

4.1 CD8+ T cells

Most T cell evasion mechanisms appear directed at minimizing the recognition by and effector function of CD8+ cytotoxic T lymphocytes (CTL). This arm of the immune response is assumed critical for viral clearance although rarely do such cells act alone *in vivo* to effect immunity. The CD8+ T cells are frequently assisted by, or in certain viral infections even surpassed by, CD4+ T cells (22, 23). Candidate evasion tactics against CTL are recognized which act at the level of the CD8+ antigen receptor, the essential coreceptor or cell adhesion machinery, antigen processing and presentation for CTL recognition, as well as at the process of infected cell killing (usually apoptosis) (See table 1 and figure 1). Especially abundant are the mechanisms which compromise antigen presentation. These have mainly been observed in HV, particularly CMV (24-31).

One bonus from the study of viral evasion of antigen processing has been a better understanding of the basic biology of this process. Still missing, however, are convincing observations proving that a postulated evasion mechanism has crucial importance *in vivo* especially within the host that the virus naturally infects. For example HSV has the protein ICP47 which binds to the transporter associated protein, TAP, thus inactivating TAP and ultimately preventing full assembly of the MHC Class I complex. Failure of infected cells to expresses viral peptides leaves these cells refractory to CTL mediated recognition and allows viral replication and spread (24). CMV uses at least 5 gene products which independently inhibit various steps of the MHC Class I processing pathway (25-30). Consequently, one might expect such viruses to abrogate CTL induction, or at least be refractory to CTL action but there is no evidence that this actually occurs *in vivo*. With HSV for example, it is now known that a vigorous CTL response occurs in man and that recurrence may actually correlate with a change in CTL functional efficiency (32). In other words, HSV infected cells *in vivo* do not escape CTL recognition.

The relevance of the human CMV MHC Class I inhibitory effects have not so far been evaluated *in vivo*, but CMV infection in man is usually inconsequential except in those suffering significant immunosuppression (33). Thus only in the absence of effector T cell function does infection with this virus become clinically significant. Viewed in this respect, the 'normal' host immune system appears to function quite well at maintaining CMV at very low levels inferring that this virus's potential evasion strategies truly play little or no role. Alternatively, CMV may actually be a successful opportunist possessing evasion strategies which work to its benefit only during severely attenuated host immune responses but not during the robust responses of immune competent hosts. This conundrum may only be resolved following *in vivo* studies.

The adenovirus system, which was the first to reveal CTL processing and recognition escape mechanisms, provides further evidence of *in vitro* evasion of CTL function. One of the earliest studies exploring viral escape mechanisms investigated an induced tumor model using human adenovirus in a mouse system. In this model, reduced MHC associated antigen correlated with enhanced tumorigenesis (34). Currently, however, there is no evidence that MHC Class I proteins are down regulated during natural human infections with adenovirus and fortunately adenoviruses have not been implicated as a cause of human cancers.

The adenovirus gene E3 encodes a 19 kilodalton glycoprotein which localizes to the endoplasmic reticulum (ER) membrane where it's cytoplasmic tail binds to newly formed MHC I complexes, preventing their terminal glycosolation and thereby retaining them within the ER (35). Again, through *in vitro* transfections with viral deletion mutants, the E3 gene product significantly decreases MHC Class I expression and CTL killing of infected cells (35).

Evading CTL recognition could also be achieved by interference with T cell receptor (TCR) function, or with the many molecules involved as coreceptors or coadhesins, during CTL function. Viruses could conceivably undergo mutation and become CTL escape variants. In fact, such a strategy was shown to occur with the RNA virus LCMV although only in the highly contrived circumstance of a T cell repertoire handicapped transgenic mouse system (36).

CTL are thought to play a crucial role in the control of HIV infections and some have hypothesized that the emergence of CTL escape variants serves an important part of HIV pathogenesis. Evidence for such ideas remains equivocal but the topic remains under intense investigation (37, 38). The herpesvirus, Epstein-Barr virus (EBV), also has an evasion mechanism directed at CTL TCR function (39, 40). In particular, in EBV isolates from two distinct populations, the natural mutation of just 2 amino acids in the dominant CTL epitope rendered infected cells invisible to CTL recognition. Nevertheless, it is as yet unclear whether these epitope loss variants play a role in the establishment of persistent infections in those human populations in which they predominate. Apart from avoiding appropriate contact with the CTL TCR, EBV also possesses a means of down regulating the cell adhesion molecules ICAM1 and LFA3 which are involved in stabilizing the CTL TCR: MHC I complex (41). The mechanisms involved in achieving these effects, however, are not known.

Apoptosis would seem to benefit the invading virus in terms of the development of strategies to resist the final outcome of the cytotoxic immune response against virally infected cells. Some viruses have evolved means of preventing or delaying apoptosis by both p53-dependent and -independent mechanisms (reviewed in 42). This strategy provides more time for viruses to complete their replication cycle and be transmitted to other cells and hosts. Adenoviruses encode by far the most extensively studied genes implicated in these processes with protein products involved at different steps in the apoptotic pathway. For instance, E1B-55K and E4orf6 bind to separate loci of p53 thus achieving inactivation and protection from cell death. (42). Adenovirus E1B-19K, E3-14.7K, and E3-10.4K/14.5K proteins function to inhibit TNF induced apoptosis *in vitro*, perhaps by preventing TNF-activation of phospholipase A2. Animal studies using E3-14.7K deletion mutants have confirmed a counteractive effect to TNF anti-viral effects (43).

Several viruses have recently been shown to encode genes whose products inhibit apoptosis signaled through death receptors. In this decoy system employed by several gamma-herpes viruses, virally encoded proteins bind to an intermediate of the death cascade thus preventing the activation of interleukin-1 beta converting enzyme (ICE)-like proteases. These viral proteins, termed vFLIPs, are not only involved in the facilitation of viral spread but may contribute to persistence and transformation (44). The inactivation of ICE is also a strategy used by the cowpox virus. The product *crm*A has been shown to be a successful inhibitor of ICE and thus apoptosis by *in vitro* analysis (45). Epstein-Barr virus encodes 4 gene products with different anti-apoptotic effects. The EBV LMP1 and BHRF1 proteins not only have homology to bcl-2 but also upregulate the expression of this endogenous anti-apoptotic protein (46, 47). The EBV EBNA-5 and BZLF1 proteins interact with p53 and may be involved in the shift from latency to lytic infection (48, 49). Finally, HSV-1 also has been shown to encode 2 genes which demonstrate cell-type specific suppression of apoptosis by *in vitro* analysis (50). Apparently, these gene products achieve this inhibition by inactivating the eukaryotic translation initiation factor eIF-2 alpha thus inhibiting the synthesis of proteins essential for the DNA fragmentation characteristics of apoptotic cells (51). Experiments have shown that whereas wild-type viruses which retain the apoptosis defeating machinery are virulent, deleting genes responsible provides mutants of lesser virulence (42). Based on these *in vitro* studies, it would appear that the prevention of apoptosis is an important viral evasion tactic particularly if those gene products involved significantly influence virulence. However, without relevant *in vivo* data, any

association between viral virulence, apoptosis, and the identified viral gene products is merely speculative.

4.2 CD4+ T cells

CD4+ T cells can also effect antiviral immunity and with some agents, this defense mechanism may assume greater importance than the CD8+ mediated immunity (52). Evading CD4+ T cell recognition or the processes such cells orchestrate during their effector function represents a useful survival property. Processes that interfere with MHC II antigen presentation may achieve evasion of CD4+ T cell function. However, there is sparse direct evidence to support the existence of such strategies. One possible supporting example is the observation that the pathogenesis of HSV-1 and HSV-2, following intraocular infection in a mouse model, show differences which correlate with decreased expression of MHC II as well as IL-6 and IFN-gamma by HSV-2 infected microglial cells (53). The resultant decrease in CD4+ T cell activation during the course of viral infection has a speculated role in disease progression (54). It is worth pointing out, however, that HSV is not a mouse pathogen and observations on the pathogenesis of this agent in mice can provide misleading insight (55).

EBV gene BZLF2 encodes a product which specifically binds to the beta chain of the MHC II molecule and can either retain it intracellularly, thus preventing its surface expression, or bind to it at the cell surface thus blocking interaction with the appropriate T cell TCR and effective antigen presentation (56). Both known mechanisms of specific viral interference of MHC II expression appear to inhibit CD4+ T cell activation and perhaps serve to prevent the development of T help for the establishment of an antiviral humoral response.

CD4+ T helper cells may mediate effector function by the elaboration of cytokines. Some viruses interfere with cytokines either by encoding decoy receptors which interfere with cytokine function, or by producing homologous molecules which inhibit the activity of protective cytokines. The possible role of several decoy receptors was discussed above as a means of evading innate immunity.

Currently, there is not an array of virally encoded proteins which appear to counter the normal process of MHC II antigen processing. This situation may reflect the neglect of the subject by cell biologists and perhaps the misconception that antiviral immunity depends principally on CD8+ T cell function. It is known, however, that the effectiveness of MHC II processing is enhanced by IFN-gamma action (57). Consequently, viruses encoding the IFN-gamma decoy receptor will minimize CD4+ T cell function and there is some *in vitro* evidence that this process occurs.

Considerable interest was raised by the observation that the EBV BCRF1 gene product has high structural and functional homology *in vitro* to the human cytokine, IL-10 (58). This cytokine, a product of the Th2 T cell subset and a potent inhibitor of Th1 cytokine production (59), may both enhance B cell proliferation and suppress immunity, thus facilitating pathogen dissemination and survival early in the infectious process. However, the BCRF1 gene product is a late protein produced only during the lytic cycle, a brief event with EBV, and it has not been proven functionally important during this period *in vivo*. Persistence and the need for evasion are also necessary during the latent cycle. *In vitro* studies suggest latency of EBV is maintained within resting B cells presumably by the LMP-2 gene product (60). The interaction between BCRF1, which promotes a Th2 environment, and LMP-2, which allows EBV infected cells to escape immune clearance, is unclear. Although speculative, interaction may be vital for the maintenance of chronic or latent states and perhaps in the development of tumors associated with EBV, all states which require evasion. A convincing scenario and *in vivo* data, unfortunately, are currently lacking.

HVS provides another example of a virally encoded cytokine with Th2-like function. The HVS-13 gene product has structural homology to human IL-16 and has been shown *in vitro* to upregulate IL-6 production from virally infected human cell lines. It may also function as a costimulatory molecule for T cells (reviewed in 61). Although the *in vivo* role of this viral homologue remains to be determined, it appears to promote a humoral immune response.

Kaposi's sarcoma associated herpesvirus (KSHV or human herpesvirus 8) ORF K2 encodes a homologue of human IL-6 which has *in vitro* anti-apoptotic activity comparable to that of the human cytokine (14). The role of this viral protein may be in the development of neoplastic tissue in infected individuals, a state which requires constant immune system evasion.

Finally, the adenovirus E1A gene product interacts at the IL-6 promoter to effectively suppress the transcription of IL-6 in virally infected cells (62). Given the other known viral evasion strategies of adenovirus, it is unclear what benefit the virus may gain by suppressing IL-6 during acute infection, particularly with no *in vivo* evidence of the virulence role played by this gene product. However, given that IL-6 is important in both B cell maturation and functional immunoglobulin class switching, it is interesting to speculate whether the E1A gene product functions to limit the humoral response during adenovirus infections. The MCMV system may provide another mechanism, however. Here it was found that virally encoded IL-6 synergizes with IL-1 alpha to induce optimal elaboration of viral protein products which influence glucocorticoid production (63). The immune suppressive effects of glucocorticoids are well known. Could the elaboration of viral protein products which influence glucocorticoid production be yet another means of viral immune evasion? This, of course, remains to be elucidated.

4.3 Humoral Immunity

CD4+ T cell recognition depends upon the processing and presentation of peptides in the context of MHC II molecules. The eventual outcome is the

production of viral peptide specific antibodies which can then neutralize virus particles or kill infected cells through antibody-dependent cell-mediated cytotoxicity (ADCC). Since it seems critical that antibody recognize viral particles, one likely evasion strategy would be the variation, reduction or complete elimination of expression of viral peptides. This has been accomplished, in part, by influenza and HIV viruses via antigenic drift and by those viruses which undergo latency. HSV genes US7 and US8 encode two protein products, gI and gE, which are surface expressed as an Fc receptor complex (64). This complex binds the Fc portion of both monomeric IgG and antigen-antibody complexes. The proposed model suggests that antigen specific IgG binds to a surface expressed viral glycoprotein on infected cells and is itself bound by the surface expressed viral Fc receptor. This prevents association of the IgG Fc portion with an effector cell's Fc receptor and development of an effector immune response. The HV Fc receptor protects virus from complement neutralization due to inhibition of C1q complement binding. It also protects virus from antibody neutralization as well as protecting infected cells from ADCC (65). It would appear that HSV has developed a foolproof method of avoiding elimination by innate defenses as well as cell-mediated and humoral defenses. Unfortunately, the *in vivo* system in which gE and gI mutant strains were tested failed to prove the decrease in virulence was due to lack of viral Fc receptor activity (66), thus, the importance of this evasion mechanism awaits clarification. However, these viral protein products may be critically important in allowing cell-to-cell spread of HSV to occur without encountering the extracellular milieu.

5. CONCLUDING REMARKS

The notion that viral immune evasion strategies exist is both sensible and credible. Verification appears at hand by *in vitro* studies as well as from a few, often contrived, *in vivo* animal model systems. The study of immune evasion has taught important lessons in the fundamental mechanisms of antigen processing and immune system recognition of viral proteins. As put so nicely by Rolf Zinkernagel, "viruses can teach us immunology" (2). However, since confirmatory *in vivo* data for most of these putative evasion mechanisms remains scarce, becoming overly enthusiastic about these potentially seductive hypotheses is cautioned against. Currently, the reality is that there are precious few examples where a speculated immune evasion measure has been shown to function as such *in vivo* following infection in the virus's natural host. Of course, the results of further experimentation may reveal examples which are vital *in vivo* evasion strategies. It is likely that many mechanisms will be proven to be interesting *in vitro* curiosities unrelated to pathogenesis. The next few years of experimentation will undoubtedly yield valuable clarification as to the true relevance of viral evasion strategies.

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