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BRIEF REVIEWS

Secreted Immunomodulatory Viral Proteins as Novel Biotherapeutics[1,2]

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***Many viruses have learned to evade or subvert the host antiviral immune responses by encoding and expressing immunomodulatory proteins that protect the virus from attack by elements of the innate and acquired immune systems. Some of these viral anti-immune regulators are expressed as secreted proteins that engage specific host immune targets in the extracellular environment, where they exhibit potent anti-immune properties. We review here viral immunomodulatory proteins that have been tested as anti-inflammatory reagents in animal models of disease caused by excessive inflammation or hyperactivated immune pathways. The potential for such viral molecules for the development of novel drugs to treat immune-based or inflammatory disorders is discussed. The Journal of Immunology,* 2004, 173: 4765–4774.**

Many viruses that successfully invade immunocompetent hosts do so by acquiring self-protective strategies to evade or subvert the consolidated forces of the innate and acquired immune responses. However, when viewed from the perspective of individual viruses faced with the challenge of surviving within specific host tissues, it is clear that each virus has acquired a unique portfolio of strategies to survive within the infected host. Thus, studies of individual viral anti-immune mechanisms tend to shed light on specific pathways that regulate the immune or inflammatory responses encountered by that particular virus. In contrast, an examination of viral strategies in general reveals that viruses as a whole can express effector molecules that target the entire gamut of immune pathways of vertebrate hosts, including some that likely remain to be uncovered. For example, a survey of the host antiviral response pathways already shown to be targeted by viruses reveals many of the key elements of modern immunology: Ag presentation, apoptosis, intracellular signaling, TLRs, cytokine pathways, serine proteinases, cytotoxic killing mechanisms, Ab generation, humoral regulators, and others. In fact, the growing collection of viral strategies that modulate these aspects of the immune system can be considered as comprising the discipline of anti-immunology and is the subject of a vast body of scientific literature (e.g., see Refs. 1–9 for some of the many excellent recent reviews).

Viruses as immunologists

The constant selection pressure from uncounted eons of coevolution between viruses and hosts has crafted virally derived anti-immune molecules of exquisite selectivity and potency. In many cases, viruses have evolved immunoregulators that no longer comply with the regulatory pressures of the host, thus endowing these molecules with highly specific anti-immune properties. Unlike the situation with commercial pharmaceuticals, viruses do not generally exploit immunomodulatory reagents that require high concentration to effectively perturb their intended immune pathways. Rather, viruses have evolved to express immunomodulators that are frequently delivered transiently at exceedingly low dosages (femtomolar to nanomolar) within a selected microenvironment of the infected tissues. Only rarely do viruses globally suppress immune responses of the host, and generally, this is only with viruses that replicate to very high titers in the blood or lymphatic system (10–13). In fact, the specific virus-encoded immunomodulators that have been examined to date require extremely low treatment dosages to regulate their target immune or inflammatory pathways without inducing generalized immunosuppression or promoting supervening infections. This combination of high potency and highly specific biochemical targeting provides a powerful platform with which to develop next-generation drugs based on viral protein immunomodulators to treat diseases based on excessive inflammation or hyperactive immune reactions (14–19).

In this review, we focus on virus-encoded immunoregulators that are secreted from infected cells and target specific pathways that regulate host immune or inflammatory responses (Fig. 1). In particular, we discuss in greater depth those virus-encoded immunomodulators that have been tested individually, in the absence of virus infection, and

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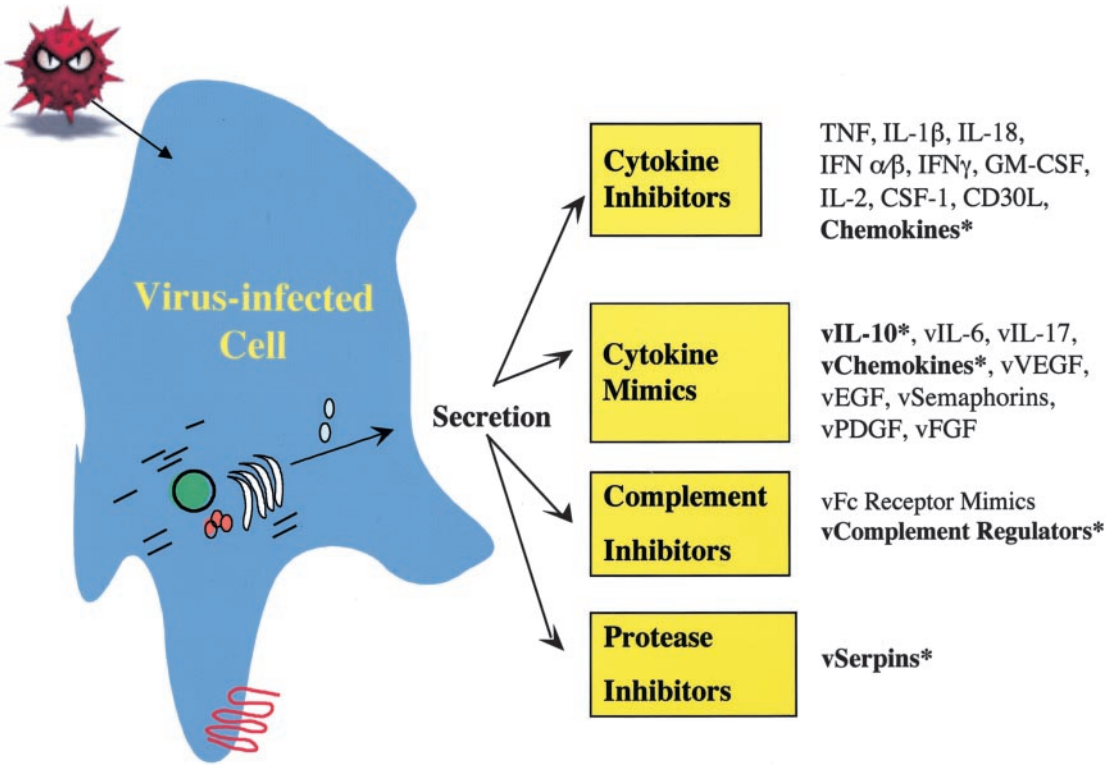

FIGURE 1. Major classes of virus-encoded secreted immunomodulators.

examined as therapeutic reagents in models of diseases associated with excessive inflammatory or immune responses (Table I). Finally, we consider the technical and regulatory challenges of using virus-derived proteins with anti-inflammatory or anti-immune properties for treatment of human diseases, and the prospects for mining the virus ecosphere for future immunomodulatory candidates.

Secreted immunomodulatory viral proteins: virokines and viroceptors

Virus-encoded immunomodulatory proteins have been identified from a wide variety of virus families, but by far the largest number come from the DNA viruses with large genomes that can express multiple genes beyond those minimally required for virus replication and propagation in tissue culture. Of these, members of the poxvirus and herpesvirus families have evolved to encode more such immunomodulators than all other virus families combined (1, 9, 14–19). In some cases, the origins of these viral genes are clearly rooted in the piracy of known host immune genes, likely by recombination with reverse-transcribed cDNA versions of expressed host genes from ancestrally infected host organisms. Presumably, any selective advantage of such host-derived genes would be manifested by increased survival of the newly recombined virus in the infected host. However, following acquisition of a host-derived immunomodulator by a given virus, subsequent evolutionary pressures can result in alterations of biologic functions of the captured modulator that are advantageous to the virus (20–22). Thus, progressive loss of modulatory functions that disfavor virus propagation can also be coupled with the acquisition of progressively increased inhibitory properties that promote virus survival, thereby allowing the virus to circumvent the host-mediated regulatory activities. As an example, the viral (v)[4] IL-10 gene of EBV (*BCRF1*) exhibits significant sequence homology to the cDNA version of human IL-10, suggesting recent acquisition from infected humans or another closely related mammalian host (23). However, the current viral version of this cytokine has lost many of the immunostimulatory properties associated with the host ligand while retaining its immunosuppressive features (23–25).

The second, and more evolutionarily mysterious, class of viral immunomodulators exhibit no obvious sequence relationship to any known host molecules. These so-called orphan viral regulators have usually been discovered empirically by the ability to bind and inhibit specific host ligands. For example, the four classes of viral chemokine-binding proteins (CBPs) were all discovered by binding and inhibition studies with known host chemokines, rather than by any sequence relationship with known immune genes (9, 14, 15, 18, 26–29). For these viral regulators, it is difficult to assess whether they represent examples of independent convergent evolution or whether their true relationship to host-derived genes will become revealed only as more genomic information from other organisms becomes available. It is also entirely plausible that some of these unique viral genes were originally derived from ancient host species that are now extinct, and their progenitor host genes may never be accurately documented.

Virus-encoded immunomodulators can function from a variety of cellular and tissue locations. For example, they can be expressed intracellularly within the infected cell, function at the surface of infected cells or virion particles, or be secreted into

[4] Abbreviations used in this paper: v, viral; CBP, chemokine-binding protein; GAG, glycosaminoglycan; MCV, *Molluscum contagiosum* virus; vCCI, viral CC chemokine inhibitor; VCP, vaccinia complement control protein; IMP, inflammatory modulatory protein; serpin, serine proteinase inhibitor.

Table I. *Treatment of inflammatory disease models with immunomodulatory viral proteins*[a]

Class	Protein (Virus)	Type	Disease Model	References
Cytokine inhibitors	M-T7 (myxoma)	CBP (type I)	Vascular hyperplasia (rat, rabbit)	53
			Renal and aortic allograft vasculopathy (rat)	54, 55
	35k/vCCI (cowpox, vaccina)	CBP (type II)	Skin inflammation (guinea pig)	40
			Airway inflammation (mouse)	56
			Peritoneal inflammation (mouse)	57
	M-T1 (myxoma)		Aortic allograft vasculopathy (rat)	55
	M3 (γ-68 herpesvirus)	CBP (type III)	Pancreatic and vascular inflammation (mouse)	58, 59
			Aortic allograft vasculopathy (rat)	55
Cytokine mimics	vMIP-II (KSHV)	Chemokine homolog	Glomerulonephritis (rat)	74
			Cardiac allograft (mouse)	66
			Spinal cord injury (rat)	73
			Cerebral ischemia (mouse, rat)	71, 72
			DTH responses (mouse)	75
	MC148 (MCV)	Chemokine homolog	Cardiac allograft (mouse)	66
	vIL-10 (EBV)	IL-10 homolog	Tumor rejection (mouse)	79
			Allograft rejection (rat, mouse)	90, 92–104
			Arthritis (mouse, SCID mouse, rabbit)	80–85, 88, 89, 91
			Pathogen responses (mouse)	115
			Venous thrombosis (rat, mouse)	107, 108
			DTH responses (mouse)	112, 113
			Sepsis (mouse)	114
			Diabetes (NOD mouse)	109, 110
			Osteolysis (mouse)	85, 86
			Autoimmune ocular disease (mouse, rat, rabbits)	87, 105, 106
			Particle inflammation (mouse)	116
			Glomerulonephritis (rat)	111
Complement inhibitor	VCP (vaccinia)	Complement inhibitor	Xenograft rejection (mouse, rat, guinea pig)	128, 131, 132, 134, 138
			Neurotrauma and spinal cord injury (rat)	140–142
			Complement inhibition (baboon)	133
			Peritonitis (mouse)	130
Inflammatory cell inhibitor	SERP-I (myxoma)	Serpin	Injury vasculopathy (rabbit, mouse, rat, rooster, pig)	154–156
			Rheumatoid arthritis (rabbit)	161
			Allograft vasculopathy (rat)	158–160

[a] KSHV, Kaposi's sarcoma herpesvirus; DTH, delayed-type hypersensitivity.

the extracellular environment. This review will focus on the secreted immunoregulators that have been independently expressed and used to treat disorders in animal models of inflammatory diseases. These secreted immunomodulators have been subdivided into virokines (ligand-like) or viroceptors (receptor-like), but note that this thematic distinction is somewhat arbitrary, because many viral regulators were identified operationally as binding proteins or inhibitors of known immune pathways, and function by still-undefined mechanisms (30–33). In any event, only a small fraction of the currently known immunoregulators from viruses have ever been tested as anti-inflammatory or anti-immune reagents in animal models (Table I), and these are the specific examples considered in greater detail in the following sections.

Cytokine inhibitors: viral CBPs

Virus-encoded cytokine inhibitors generally function as soluble receptor mimics or as secreted cytokine-binding proteins to scavenge the targeted ligand away from host receptors at the surface of immune cells. In the case of viral CBPs, four distinct protein classes of such inhibitors (termed types I to IV) have been discovered on the basis of physical chemokine-binding and inhibition assays (9, 18, 26–30). Each of these four types of CBP represent a distinctly unique protein class, and the crystal structures of two members (types II and III) reveal domain folds unrelated to any known host immune molecule (34–36). Type I CBP is represented by a single member, M-T7 from myxoma virus, a poxvirus viroceptor originally identified as a secreted 37-kDa inhibitor specific for rabbit IFN-γ but later shown to bind with low affinity to the glycosaminoglycan (GAG) domain of a broad spectrum of C/CC/CXC chemokines and inhibit leukocyte taxis in virus-infected tissues (37–39). Type II CBPs, also called viral CC chemokine inhibitors (vCCIs), have been isolated from several poxviruses (e.g., myxoma, certain vaccinia strains, rabbitpox, and cowpox) and shown to specifically bind with high affinity and inhibit a broad spectrum of CC chemokines (40–43). Type III CPB is exemplified by the M3 protein of γ-68 herpesvirus, which binds and inhibits members of all known classes of chemokines and blocks chemokine interactions with both their receptors and GAG elements responsible for chemokine gradients (35, 44–48). The type IV CBPs were only recently discovered in several α-herpesviruses, when it was

shown that particular isoforms of gpG possess the ability to bind and inhibit a wide spectrum of C/CC/CXC chemokines (49). Overall, there is considerable interest in the development of novel chemokine-modulatory drugs, and the known viral CBPs represent a potent repository of reagents with which to manipulate chemokine functions and leukocyte trafficking (50–52).

The viral CBPs tested to date in animal models have each demonstrated clearly the elegant sophistication that viruses have evolved to thwart mammalian immune defenses. Multiple studies with CBPs I–III have consistently demonstrated effective inhibition of inflammatory disorders in a range of animal disease models. M-T7, or the type I CBP, was found to block early invasion of macrophages and T lymphocytes at sites of vascular injury in rat and rabbit models (53–55). Infusion of purified M-T7 protein resulted in the inhibition of early mononuclear cell invasion postinjury and was associated with long-term reductions in atherosclerotic plaque growth (vasculopathy) after either transplant or balloon angioplasty injury (53–55). The lack of species specificity of M-T7 in a variety of animal models suggests that the inhibition of cell invasion and plaque growth was in fact the result of targeting the host chemokine circuitry rather than IFN-γ, whose inhibition by M-T7 is restricted to the rabbit species (53–55, 181). Furthermore, M-T7 protein was clinically efficacious in suppressing the vascular pathology associated with these various models even when given transiently at very low dosages (picograms to nanograms per kilogram of body weight). For example, Bedard et al. (54) demonstrated that i.v. treatment with M-T7 protein, given daily at doses up to 80 ng/kg for only the first 10 days posttransplant, markedly reduced vasculopathy and organ scarring in rat renal transplants even at 5 mo after surgery.

The viral CBP type II, M-T1 from myxoma virus, which shares close homology to the vCCI/35k from vaccinia, has also been tested in rat and mouse aortic allograft models. In the rat model, M-T1 protein (given i.v. as a single protein bolus administered immediately following vascular transplant) mediated blockade of early mononuclear cell invasion and the late development of chronic transplant vasculopathy (55). Dabbagh et al. (56) demonstrated that administration of vCCI/35k as an Fc fusion protein significantly reduced airway inflammation in a mouse model. vCCI/35k also reduced eosinophil invasion associated with eotaxin-mediated inflammation in guinea pigs (40, 55). Finally, when expressed from an adenovirus vector that was delivered by i.p. injection, vCCI/35k also reduced inflammatory cell recruitment induced by biogel in peritoneal exudates in mice (57). Like the type I and II viral CBPs, M3, a panchemokine class inhibitor, also displayed potent therapeutic activity, blocking aortic allograft vasculopathy (55) and pancreatic inflammation (58). Significantly, endogenous M3 expression from transgenic mice also blocks intimal hyperplasia (59).

The analysis of these three diverse classes of viral CBPs reaffirm the importance and impact of the chemokine system on early inflammatory responses to trauma and on long-term disease development. Whether the CBPs were administered as purified proteins (53–56), expressed through adenoviral vectors (57), or produced endogenously in transgenic mice (58), profound inhibition of inflammation was consistently observed. Although CBPs provide powerful tools to deconstruct the critical roles that chemokines play during inflammatory responses, the actual mechanisms through which these viral CBPs functionally block chemokine responses when given in such relatively low doses for very restricted time frames still require further studies. For example, the CBP type I, M-T7, inhibits inflammatory influx effectively in vivo at very low dosages (53–55), whereas this protein binds the GAG binding domain of chemokines with only low affinity in vitro (37). One clue to this paradoxical result may lie in the fact that the low-affinity GAG-binding domain of many chemokines is critical for gradient stabilization and ligand presentation to the influxing leukocytes (60–62). In any event, this is a clear example of where further studies with the viral modulator will likely shed new light on cellular chemokine-mediated response pathways in general.

Cytokine mimicry: viral chemokines and vIL-10

In the previous section, the case of CBPs as anti-inflammatory reagents was considered, and here, we review virus-encoded mimics of known chemokines or cytokines. Of the many known examples of such immune ligand mimicry, the viral homologs of chemokines and IL-10 are the only examples, to date, that have been tested in animal models of disease. In the case of viral chemokine mimics, the two examples are MC148 of *Molluscum contagiosum* virus (MCV) and vMIP-II of human herpesvirus 8/Kaposi's sarcoma herpesvirus. MC148 of MCV specifically binds human CCR8 and antagonizes the lone host chemokine ligand that signals via this receptor (I-309), whereas vMIP-II is both an agonist for CCR3 and a promiscuous antagonist for at least 10 human CC and CXC chemokine receptors (63–65). Unlike vMIP-II, MC148 does not recognize any known murine chemokine receptors, and thus is not predicted to be anti-inflammatory in mouse models, but the available data indicate that both viral ligands can nevertheless prolong cardiac allograft survival in mice (66). vMIP-II also possesses the unique ability to block Th1-polarized T lymphocytes while stimulating Th2 responses, thereby disfavoring cell-mediated immune responses (67). At present, it is not understood how MC148 acts in the murine system, but it is possible that it also targets inflammatory pathways independent of the chemokine system, or there are other still-to-be identified chemokine receptors on primary cells that are antagonized by MC148 (68).

Chemokines are expressed at elevated concentrations in the brain after mechanical trauma or chronic neuropathies such as Alzheimer's disease and multiple sclerosis (69–71). Takami et al. (72) have demonstrated that intracerebroventricular injections of purified vMIP-II protein, which can antagonize MIP-1α (or CCL3), reduced infarct size at 48 h after middle cerebral arterial occlusion, whereas, conversely, injection of MIP-1α increased infarct size in mice. Ghirnikar et al. (73) similarly found that infusion of vMIP-II protein for 7 days via osmotic minipump brain infusion after spinal cord contusion in rats decreased the number of infiltrating neutrophils (day 1 postinjury), macrophages (days 3–7 postinjury), and microglia (days 3–7 postinjury). The reduction in inflammatory cell invasion was associated with reduced neuronal loss and increased expression of Bcl-2, an endogenous apoptosis inhibitor (73). In a rat model of glomerulonephritis, i.v. infusion of vMIP-II protein inhibited CC and CX3C chemokine expression, macrophage and T lymphocyte invasion, crescentic glomeruli, and proteinuria (protein loss in the urine indicative of kidney damage) (74). Inflammatory exudates, which are believed to produce some of the $CD8^+$ T cell-mediated immunopathology associated with lymphocytic choriomeningitis virus infections,

were also reduced with vMIP-II treatment in mice (75). In a cardiac allograft transplant model in mice, gene transfer of vMIP-II and MC148 reduced CTL infiltrates and alloantibody production with associated prolonged graft survival (survival for 21 days with vMIP-II vs 13 days for control) (66). Injection of vIL-10 together with vMIP-II further enhanced graft survival, suggesting these viral immunomodulating cytokines inhibited inflammatory responses through synergistic pathways (66).

The second class of cytokine mimicry considered here is vIL-10 from EBV, which exhibits 83% identity to the human IL-10 (23–25). Despite this sequence similarity, vIL-10 exhibits primarily the immune-inhibitory properties associated with the cellular ligand (e.g., suppression of Th1-polarized responses and monocyte inhibition) and has apparently lost the immunostimulatory features normally associated with host IL-10 (e.g., activation of dendritic cells, NK cells, and some T cells) (76–79). This exacerbated inhibitory aspect of vIL-10 makes it an attractive therapeutic candidate for tolerance induction following allografts or for immunosuppression to treat disease pathologies driven by inappropriately activated lymphocytes. vIL-10 gene delivery has to date been widely tested in animal models of arthritis, allograft transplantation, osteolysis, glomerulonephritis, diabetes, uveoretinitis, venous thrombosis, and systemic inflammation associated with infection and sepsis (Table I). A reduction in proinflammatory cytokines (IL-1, IL-6, and TNF-α, among others) and a conversion from Th1 to Th2 lymphocyte response was associated with suppression of inflammation in many of the animal models tested. The extensive testing of vIL-10 in a wide range of animal models, and the large numbers of laboratories that have demonstrated effective repression of disease states in these models, strongly supports the concept that vIL-10 may provide an effective therapeutic approach to suppression of both inflammation-based and autoimmune-based disorders.

Adenovirus or adeno-associated virus delivery of the vIL-10 gene has been demonstrated to reduce Ag-induced joint inflammation in rabbits (80), mouse models of arthritis (81–84), and wear debris-initiated inflammation and osteoclastogenesis (osteolysis in joint replacements) (85, 86). The mouse model of Sjogren-like syndrome, featuring reduced tear production and ocular surface disease (autoimmune dacryoadenitis), is notable in that vIL-10 suppressed pathologic responses for a common autoimmune disorder for which few, if any, therapies are currently available (87). vIL-10 gene delivery also prevented transplanted human rheumatoid synovial tissue invasion into cartilage in SCID mice (88), indicating effective blockade of human inflammatory tissue-mediated in-joint destruction. When the gene for soluble TNFR was delivered to mice in conjunction with vIL-10, there was a greater synergistic reduction in joint inflammation (89). vIL-10 treatment induced a Th2 phenotype and was variously found to reduce the expression of IL-1, IL-2, IL-6, TNF-α, cyclooxygenase 2, and matrix metalloproteinase 3, and to increase tissue inhibitor of matrix metalloproteinase-1 (88–90). Of interest, vIL-10 suppression was transferred to contralateral untreated joints through an Ag-dependent mechanism (91).

Gene delivery of vIL-10 by retrovirus, plasmid-based gene transfer, adenovirus, or liposomes has also been shown to increase allograft survival after solid organ heart, kidney, and hepatic transplants in mice (92–96) and rats (97–100). vIL-10 has also been found in an immunosuppressed patient post-renal transplantation and was associated with preserved renal allograft function (101). vIL-10-mediated prolongation of graft survival was associated with a switch from Th1 to Th2 responses and impairing APC function with associated induction of tolerance (93, 102). Together with the altered Th2/Th1 response ratio, a reduction in costimulatory molecules (B7.1 and B7.2), cytokines (IL-2, IL-4, murine IL-10, and IFN-γ), and inducible NO synthase was detected in several models (92). Although a shift from Th1 to Th2 responses was detected, true tolerance was not seen, as evidenced by the acute rejection of both the first and second grafts, following implant of a second heart transplant into mice that had previously received a cardiac transplant with vIL-10 gene therapy (93). T lymphocyte allostimulatory activity of mouse dendritic cells and dendritic cell maturation was reduced in mouse studies of mixed lymphocyte responses in vitro (102). Conversely, vIL-10 expression from transplanted transgenic mouse hearts did not suppress graft rejection (103), whereas pretreatment of donor rat hearts with adenovirus-expressed vIL-10 did prolong graft survival (97). However, in subsequent work, expression from autologous hemopoietic stem cells did prolong allograft survival, and concomitant cyclosporin A treatment enhanced vIL-10-mediated graft survival (92, 97, 104).

vIL-10 has also been tested in animal models of autoimmune dacroadenitis and uveoretinitis (87, 105, 106), venous thrombosis (107, 108), autoimmune diabetes (109, 110), glomerulonephritis (111), delayed-type hypersensitivity (112, 113), and sepsis (114). vIL-10 significantly improved survival and reductions in pancreas and liver injury in the mouse models of sepsis induced by *Saccharomyces cerevisiae* particles or with choline-deficient diets (114). Conversely, in parasitic infection with *Schistosoma mansoni*, vIL-10 had no effect on the infestation, whereas with *Leishmania amazonensis* infestation, vIL-10 treatment led to an initial decrease in lesions followed by a later exacerbation (115). In the murine air pouch model of particle-induced inflammation, vIL-10 gene therapy decreased proinflammatory cytokine responses with reduction in IL-1α, IL-6, and TNF-α, with associated reductions in macrophage response and invasion (116).

Viral complement regulators

The regulation of complement activation at sites of inflammation is under tight control, and some viruses have specifically captured host regulatory components designed to limit complement attack of virus-infected cells (117–120). In particular, the poxviruses and herpesviruses have acquired and adapted a number of key host-derived inhibitors of the complement cascade to blunt the early stages of complement-mediated responses in infected tissues (121–123). The 35-kDa secreted inhibitor of complement expressed by some strains of vaccinia virus was, in fact, the first such viral complement regulator discovered (124–126). The vaccinia complement control protein (VCP) and the closely related version from cowpox virus, designated inflammatory modulatory protein (IMP), have been shown to structurally resemble host C4b-binding protein and to be capable of binding cellular regulators like C4b or C3b and heparin simultaneously (127). VCP and IMP have been proposed to be capable of blocking the interaction between chemokines and GAGs to inhibit chemokine gradient formation and leukocyte

chemotaxis, but this particular activity remains to be demonstrated in vivo (128). Purified VCP blocks complement activation at several stages and has been proposed to be a good candidate for the treatment of Alzheimer's disease, multiple organ dysfunction syndrome, peritonitis, and xenograft rejection (129, 130).

VCP has been tested in animal models and has shown promise for treating hyperacute rejection following xenotransplantation (131–134). Complement responses, both through the classical and alternate pathways, mediate hyperacute rejection, which markedly limits the potential for xenotransplantation (135). Chronic organ shortages have led to intensive investigations into methods that would allow for the use of alternate organ sources, such as pigs, for human transplants, and prevention of complement activation has been shown to reduce hyperacute rejection reactions (136, 137). Anderson et al. (128, 131) have demonstrated that treatment with purified VCP prolonged survival time 10-fold for xenotransplanted hearts using a heterotopic cervical mouse heart to sensitized rat transplant model. VCP treatment also inhibited xenoantibody binding to MHC class I molecules on endothelial cells and activation of the complement pathway (128). As well, VCP blocked human galactose-α-1,3-galactose Ab binding sites on pig endothelial cells, complement activation, and NK cell-mediated endothelial cell lysis (138). The cowpoxviral IMP also blocked inflammatory cell responses in mouse dermal pouch and footpad models (139).

Finally, VCP has shown promise for treatment in animal models of injury to the CNS (neurotrauma) (140–142). Interestingly, not only full-length VCP, but also a truncated version of VCP that lacks complement inhibitory properties but retains the ability to bind heparin, still provides neuroprotection, strongly suggesting multiple activities for this viral immunomodulatory protein (141).

Viral serine proteinase inhibitors (serpins)

Serpins form a large superfamily of regulatory proteins that orchestrate key pathways such as blood coagulation, fibrinolysis, complement activation, humoral responses, embryonic development, apoptosis, and inflammation (143–146). Serpins represent from 3 to 10% of circulating plasma proteins, and are suspected to have central roles in regulating the innate inflammatory responses to viruses and tissue trauma. To date, poxviruses are the only viruses known to encode biologically active serpins, and these viral serpins can be detected in either the intracellular or extracellular compartments (147, 148). The only well-characterized secreted viral serpin is Serp-1, encoded by myxoma virus, which is expressed as a 55-kDa secreted glycoprotein that binds and inhibits several human host serine proteinases such as urokinase-type and tissue-type plasminogen activators, and plasmin (149–151). When the *Serp-1* gene was deleted from myxoma virus, the resulting knockout virus was attenuated and unable to block attack and clearance by responsive inflammatory cells (152, 153). Given this potential as a potent anti-inflammatory reagent, Serp-1 was the first viral immunomodulatory protein to be purified and tested as an anti-inflammatory reagent in an animal model of inflammation (154, 155).

Studies in rabbit, rat, rooster, and swine models all revealed marked effectiveness of Serp-1 protein at reducing mononuclear cell/macrophage invasion into sites of arterial trauma, after a single picogram-to-nanogram dose given either locally into the arterial wall or systemically by i.v. injection (155, 156). Under conditions of acute infusion, Serp-1 protein behaves with pharmacokinetics that resemble other nonviral protein pharmaceuticals (157). After 4 wk, atherosclerotic plaque growth was markedly reduced in these models, indicating that even short-term Serp-1 protein dosing at the time of vascular injury can reduce longer term vasculopathy associated with chronic disease progression. Subsequent work in ApoE-deficient mice demonstrated a significant reduction in plaque growth after carotid cuff compression injury and an associated reduction in macrophage invasion, suggesting that Serp-1 treatment stabilized the arterial wall in this mouse model (158). Effective reductions in plaque growth were also observed after 4 wk in rat aortic allografts following acute treatment with Serp-1 right at the time of transplant (159). Further work in heart transplant models (rat) have also demonstrated that transient Serp-1-induced reductions in early monocyte/macrophage invasions were associated with long-term decreases in graft vasculopathy when coupled with continuous cyclosporin A dosing to inhibit T cell allograft responses (160). In keeping with the adoptive evolution of viral serpins to a uniquely inhibitory function, Serp-1 was found to block inflammatory responses and plaque growth in mouse models via the cellular urokinase-type plasminogen activator receptor, a receptor targeted by the mammalian antithrombolytic serpin, plasminogen activator inhibitor-1 (161). Finally, Serp-1 treatment has also been shown to reduce the levels of chronic inflammation in the rabbit model of Ag-induced arthritis (162).

Taken together, all of these studies reinforce the conclusion that even transient dosing with Serp-1 can reduce the magnitude of monocyte/macrophage responses to proinflammatory stimuli, and thereby mitigate the subsequent pathophysiological consequences of chronic low-level inflammation that invariably accompanies physical trauma and disease states as diverse as transplantation, angioplasty, or rheumatoid arthritis.

Immunogenicity issues

All therapeutic proteins currently licensed for use in humans, whether from human or nonhuman sources, exhibit some degree of immunogenicity in patients (163–169). The resulting Abs that are induced in susceptible patients are sometimes neutralizing but more frequently compromise the protein half-life, and only in rare cases do such induced Abs become deleterious to the patient, for example by inactivating a critical endogenous host protein such as erythropoietin (170–173). Foreign nonhuman proteins, such as bacterial streptokinase/staphylokinase, bovine adenosine deaminase, or salmon calcitonin, have been used transiently for acute therapeutic indications, but even fully human-derived recombinant proteins, such as human insulin, can be limited for long-term chronic administration protocols because of immunogenicity (166–168). As a consequence, many experimental strategies have been investigated to reduce the immunogenicity of protein pharmaceuticals, such as PEGylation, glycosylation, epitope removal, humanization, and tolerization (166–170, 174). Also, note that some clinical treatment regimens specifically induce immunosuppression, for example, cyclosporine for allograft transplantation or methotrexate for rheumatoid arthritis, which mitigates the immunogenic responses to any coadministered protein-based pharmaceuticals (166–169, 174). Thus, such treatment regimens might allow

for even chronic coadministration of viral immunomodulatory proteins with synergistic anti-inflammatory properties.

In the case of viral proteins with potent immunomodulatory or anti-inflammatory properties, the question of whether immunogenicity in humans will restrict their usage to only acute clinical indications will be properly addressed only by carefully designed human phase I and II trials, in part because of the poor prognostic capacity of animal-based immunogenicity studies (175, 176). For all biopharmaceuticals, the precise extent of immunogenicity of any given protein product will depend upon a spectrum of parameters, the most significant of which are the following: product purity, formulation, injection route, dosage, frequency of administration (i.e., acute vs chronic), and immune status of the patient population (166–168). The three principal clinical effects of concern that need to be addressed before viral proteins would be licensed for human use are, first, allergic reactions; second, the effects of any Abs to the therapeutic that could lead to treatment resistance (i.e., neutralization or changes in pharmacokinetics); and third, potential autoimmune responses induced by cross-reactive Abs against related endogenous human proteins. In fact, these concerns are equally applicable to protein biopharmaceuticals of either human or nonhuman origin, and require detailed analysis in human trials for quantification of induced Ab levels, cross-reactive profiles of any induced Abs, and efficacy parameters following multiple treatment protocols (164, 165, 168, 169, 175). Thus, the potential immunogenicity of viral protein-based biotherapeutics can be accurately evaluated only at the clinical trial level on a case-by-case basis.

Future prospects

As talented students of the mammalian immune system, viruses have developed an extraordinary range of virally mediated immunomodulatory agents. Through the unraveling of discoveries made by viruses, a new class of therapeutic agents has been revealed based upon virus-engineered immunomodulatory proteins. Viruses were the first organisms for which complete genome sequences were deduced, beginning a quarter of a century ago, and the science of "virogenomics" has been expanding rapidly ever since (177–179). The repertoire of novel viral gene products that are devoted to host modulation has also been proliferating at an astounding rate, and there are reasons to suspect that we have uncovered only the tip of the virus iceberg. For example, the discipline of virology has largely focused on viruses that cause overt pathogenesis, but the viral ecosphere is populated largely by apathogenic members that still remain to be discovered. Indeed, there are proposals to fully define the complete human "virome," or the summated sequences of all viruses that are present in the human population (180), and such genomic mining will likely uncover an even greater armamentarium of viral immunoregulators.

In some ways, the area of viral biotherapeutics mirrors the situation with snake venom peptides, which provided the basis for the development of two key classes of pharmaceuticals, angiotensin-converting enzyme inhibitors and gpIIbIIIa antagonists, which are widely used to reduce symptoms and prolong life in cardiovascular disease. In a similar fashion, we project that immunomodulatory viral proteins will establish a new pharmacopoeia for treatment of inflammation-based disorders. As more is learned about how these virus-derived drug candidates behave as pharmacological reagents, particularly in human clinical trials, we will be in better position to evaluate which human diseases have the potential to be effectively treated with this novel class of biopharmaceuticals.

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