CHAPTER 22

Viral Pathogenesis, Modulation of Immune Receptor Signaling and Treatment

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Abstract

During the co-evolution of viruses and their hosts, the latter have equipped themselves with an elaborate immune system to defend themselves from the invading viruses. In order to establish a successful infection, replicate and persist in the host, viruses have evolved numerous strategies to counter and evade host antiviral immune responses as well as exploit them for productive viral replication. These strategies include those that target immune receptor transmembrane signaling. Uncovering the exact molecular mechanisms underlying these critical points in viral pathogenesis will not only help us understand strategies used by viruses to escape from the host immune surveillance but also reveal new therapeutic targets for antiviral as well as immunomodulatory therapy. In this chapter, based on our current understanding of transmembrane signal transduction mediated by multichain immune recognition receptors (MIRRs) and the results of sequence analysis, we discuss the MIRR-targeting viral strategies of immune evasion and suggest their possible mechanisms that, in turn, reveal new points of antiviral intervention. We also show how two unrelated enveloped viruses, human immunodeficiency virus and human cytomegalovirus, use a similar mechanism to modulate the host immune response mediated by two functionally different MIRRs—T-cell antigen receptor and natural killer cell receptor, NKp30. This suggests that it is very likely that similar general mechanisms can be or are used by other viral and possibly nonviral pathogens.

Introduction

Facing the destructive consequences of microbial infections, the human immune system has evolved two arms of host defense designed to discriminate foreign agents and mount appropriate effector responses: the innate and adaptive immune systems. Differing primarily in their receptors and receptor specificities, the innate immune system functions as the early and immediate defense mechanism and recognizes a broad set of conserved and invariant properties of nonself agents, such as viruses, through a diverse set of germ-line encoded pattern recognition receptors (PRRs), including members of the toll-like receptor (TLR) family and the retinoic acid inducible gene 1 (RIG-1)-like helicases.[1-3] In contrast, the adaptive arm of the immune system is the more slow-responding defense mechanism but the more pathogen-specific; infectious antigens are

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processed in antigen-presenting cells (APCs), presented in the context of major histocompatibility complex (MHC) class I or II molecules and are recognized by somatically generated receptors on antigen-specific T-cells that are ultimately activated and perform effector functions. Collectively, the innate and adaptive immune systems work cooperatively to defend against infection, pathogenic proliferation and disease.

In order to persist in an immunocompetent host, viruses in particular have been described to have developed intricate strategies to evade the innate immune system.[4-11] Following viral infection and recognition of viral components by PRRs,[1,12-16] innate immune cells, such as dendritic cells and macrophages, normally respond robustly with secretion of type I interferons (IFNs), a group of pro-inflammatory cytokines that upregulate numerous interferon-stimulated genes (ISGs);[8,17-20] overexpression of ISGs initiates a series of antiviral, antiproliferative and immunoregulatory responses against the infected cell.[2,8,20-23] A number of viruses, including influenza and herpesvirus, employ diverse counteracting mechanisms to disrupt the IFN regulatory pathway at nearly every step, including blocking IFN induction/expression, intercepting binding of IFNs to their natural target receptors, modulating intracellular IFN-mediated signaling pathways and finally downregulation of ISG expression.[24-27] By disrupting the IFN regulatory pathway, viruses are able to attenuate the antiviral properties of type I IFNs and survive recognition by the innate immune system.

Because type I IFNs also upregulate expression of MHC class I and II proteins,[28-35] virus-mediated disruption of normal IFN activity has been suggested to not only interrupt innate immunity but adaptive immunity as well.[2] Other unrelated viruses, namely human immunodeficiency virus (HIV), human T-cell lymphotrophic virus (HTLV) and human cytomegalovirus (HCMV), have also developed strategies that modulate innate and adaptive immune processes, but do not involve type I IFNs nor IFN regulatory pathways. In contrast, HIV, HTLV and HCMV target members of the family of multichain immune recognition receptors (MIRRs) found on immune cells and either disrupt or surprisingly augment MIRR-mediated activation signaling as required for self-preservation. Predicted and explained by the signaling chain homooligomerization (SCHOOL) model (see also Chapters 12 and 20),[36] numerous unrelated viruses employ viral proteins either to (1) disrupt intermolecular transmembrane (TM) interactions between recognition and signaling subunits of MIRRs in an effort to disarm the receptor or (2) cluster the signaling subunits to activate or augment MIRR-triggered signaling. More interesting, these viruses have exquisitely incorporated targeting and manipulation of MIRR signaling in viral processes essential to the viral life cycle: viral entry, membrane targeting and viral escape and replication. By overlapping multiple functions in a single viral protein product, the virus is able to maintain a simple genome conducive to rapid replication but have the added benefit of diverse functionality.

In this chapter, we discuss an intriguing principle of convergence for a number of divergent viruses in their strategic choice to uniformly target MIRRs. Our investigation of how seemingly disparate viruses target a single family of membrane receptors exposes a redundancy in viral strategies exploiting the host innate and adaptive immune systems. MIRR-targeted strategies disrupting the MIRR TM architecture from the extracellular space as well as virus-induced clustering of MIRR signaling subunits from the cytoplasmic space (Fig. 1) will be described for a select group of viruses that are functionally disparate, target different host cells and differ in their replication strategies. We will also display the power of the SCHOOL model-guided primary sequence evaluation for a number of additional viruses and its ability to predict additional MIRR-targeting viral agents not previously conceived. Furthermore, by understanding the mechanisms viruses have developed over centuries of evolution to modulate MIRR-mediated triggering in the immune response, we gain insight into the fundamental details of the mechanisms underlying normal MIRR-mediated immune activation processes and can begin to learn how to take advantage of these optimized processes. Finally, the learned viral strategies and newly developed concepts of MIRR signaling can be translated towards new lines of rational drug design efforts targeting MIRRs and modulation of immune activation (see also Chapter 20). MIRR-targeted strategies stretch beyond the specific viruses discussed in this chapter and represent a surprising junction in viral strategies. Whether

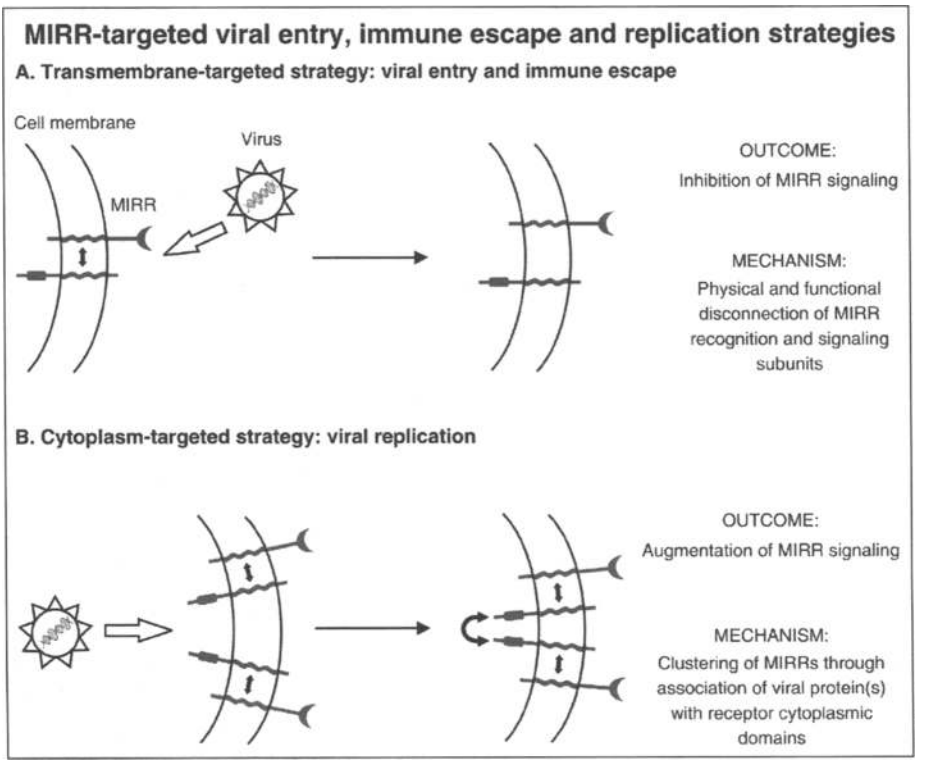

Figure 1. Targeting MIRRs: suggested immunomodulatory strategies used by viruses to entry target cells, survive and replicate. Transmembrane interactions between MIRR recognition and signaling subunits are shown by black arrows. Immunoreceptor tyrosine-based activation motifs (ITAMs) are shown as gray rectangles. Circular arrow indicates viral agent-induced receptor clustering.
Abbreviation: MIRR: multichain immune recognition receptor.

this strategy represents a convergence in evolution of disparate viruses or hints towards a similar evolutionary origin from which viruses have diverged remains to be determined.

Viruses: Classification and Pathogenesis

One of the quandaries encompassing virology and virologic discovery has been the difficulty in the classification or grouping of viruses. Although a single taxonomy governing the naming of viruses has been well-established, numerous classification methods have been suggested, highlighting similarities in virion structure, target organ systems or genomic composition. Here we describe the principles underlying the development of the Baltimore classification method and its application towards segregating viruses based on replication methods and pathogenesis. However, as a consequence of viral classification and the strict segregation of viruses from one other, universal viral strategies linking differentially classified viruses have been tragically overlooked. We postulate that a number of viruses that lie in different classifications are only seemingly different and that generic immunomodulatory strategies targeting MIRRs serve as a surprisingly common tactic shared by them.

Viral Classification

Viruses represent a collection of infectious, obligate intracellular parasites that require a living host cell to replicate. They are comprised of either DNA or RNA, a virion capsid comprised of proteins encoded by the viral nucleic acid and depending on the specific virus, a surrounding envelope. Due to the high genetic, morphologic and pathogenic variability found among different viruses, classification has proven difficult. Early attempts to organize viruses were based on their structural organization, highlighting differences in nucleic acid (DNA vs RNA), virion symmetry, presence of an envelope and number of capsomers.[37,38] For example, one system of viral classification[37,38] developed by Lwoff, Horne and Tournier, the LHT system, merged all viruses under one phylum, Vira, then divided into two subphyla, subphylum Deoxyvira (DNA viruses) and Ribovira (RNA viruses) which then divided into classes based on virion symmetry and finally segregated by number of capsomers present in the infecting virus.

The most recent and widely accepted virus classification system is based on functional characterization that differentiates viruses based on their replication strategies and chemical nature of its nucleic acid. Coined the "Baltimore classification",[39] viruses are grouped into seven groups or classes, termed the "Baltimore Classes I-VII" (Table 1). Each group of viruses uses a different replication strategy, such as exploitation of the host polymerases (Group I) or direct translation of injected positive-sense RNA (Group IV). Although each viral group contains viruses with the same type of nucleic acid (i.e., positive-sense single stranded (ss)RNA, double stranded (ds)DNA, etc.), there is remarkable variation in virion symmetry and presence or absence of an envelope surrounding the virus (Table 1). Therefore, viral architecture and morphology don't necessarily correlate with function and structurally different viruses unexpectedly share common functions and strategies. In this section, we further describe how three seemingly unrelated viruses (Table 1; HIV, HCMV, HTLV) share a common targeted approach in their mutual ability to modulate the immune system to enhance viral entry, replication and pathogenesis: uniform exploitation of the architecture and function of different MIRRs to directly suppress or augment immune activation (Fig. 1).

Viral Pathogenesis

Despite the vast diversity in viruses and target cells in the human host, there is a common sequence of processes that serve as the foundation for all viral infection. First, the infecting virus must migrate to the primary site of infection, usually through direct inoculation, or through the respiratory, gastrointestinal or genitourinary route. The virus then undergoes a process of viral entry, including attachment, a physical connection of the virus to the target cell through a viral cell recognition protein-host receptor interaction and penetration, exit from the extracellular space and entry into the cellular environment. Once inside the target cell, the virus particle uncoats and releases its viral contents, including its nucleic acid genome, in preparation of viral replication. Depending on the nature of the nucleic acid and the Baltimore group classification, viral genes may be translated directly by the host cell translation machinery (i.e., Group IV positive-sense ssRNA viruses) or incorporated into the host genome (i.e., Group I dsDNA viruses). Regardless of whether the expressing transcript originates from the viral particle itself or integrated viral genes, mRNA transcripts are translated, localize to the site of maturation and assemble into virion particles, encapsulating the viral genome in the process. Depending on the enveloped property of the infecting virus, the viral particle either surrounds itself in host membrane during budding and release (enveloped viruses) or releases without an envelope (non-enveloped viruses). Released viral progeny are then free to infect other host cells and proliferate in the host organism.

Collectively, these processes represent the fundamental stages in viral pathogenesis shared amongst members of virtually every group and class of viruses. However, inside the fine details of each stage lay intricate subprocesses that aid in enhancing viral persistence and virulence. Unexposed until recently (see also Chapter 20)[36,40,41] is the universal targeting of MIRRs that multiple viruses have surreptitiously concealed in several viral processes, including viral entry, membrane targeting and viral replication. In particular, HIV and HCMV specifically target different receptors within the MIRR family during viral entry through extracellular targeting mechanisms (Fig. 1A)

Table 1. Baltimore classification of viruses

Family	Capsid	Envelope	Genome Size (kb)	Representative Virus*	Primary Target Cell/Organ System
Group I: dsDNA					
Adenoviridae	Icosahedral	No	26-45	Adenovirus 1	Epithelial tight junctions: heart, pancreas, nervous system, prostate, testis, lung, liver, intestine
Herpesviridae	Icosahedral	Yes	125-240	Human cytomegalovirus (HCMV)	Fibroblasts
Papovaviridae	Icosahedral	No	7-8	BK Virus (polyomavirus)	Kidney epithelium, lymphocytes
Poxviridae	Ovoid	Yes	130-375	Vaccinia virus	Broad tropism
Group II: positive-sense ssDNA					
Circoviridae	Icosahedral	No	2	Transmitted transfusion virus (TTV)	Oral and intestinal mucosa
Parvoviridae	Icosahedral	No	4-6	Adeno-associated virus (AAV)	Broad tropism
Group III: dsRNA					
Bornaviridae	Icosahedral	No	5-6	Borna disease virus	Broad tropism, neuronal cells
Reoviridae	Icosahedral	No	19-32	Human rotavirus	Small intestine enterocytes
Group IV: positive-sense ssRNA					
Astroviridae	Isometric	No	6-7	Astrovirus 1	Jejunum, ileum
Calciviridae	Icosahedral	No	7-8	Norwalk virus	Upper GI tract, jejunum
Coronaviridae	Helical	Yes	28-31	SARS coronavirus (SARS-CoV)	Upper airway, alveolar epithelial cells
Flaviviridae	Spherical	Yes	10-12	Hepatitis C virus	Hepatocytes

Continued on next page

Table 1. Continued

Family	Capsid	Envelope	Genome Size (kb)	Representative Virus*	Primary Target Cell/Organ System
Picornaviridae	Icosahedral	No	7-9	Hepatitis A virus	Hepatocytes, intestinal mucosa
Togaviridae	Icosahedral	Yes	10-12	Rubella virus	Nasopharynx, lymph nodes
Group V: negative-sense ssRNA					
Arenaviridae	Helical filaments	Yes	11	Lymphocytotic choriomeningitis virus (LCMV)	Broad tropism, hilar lymph nodes, lung parenchyma
				Lassa virus (LASV)	Dendritic cells, macrophages and other immune cells, hepatocytes, endothelial cells
				Mopeia virus (MOPV)	Dendritic cells, macrophages, endothelial cells
				Tacaribe virus (TACV)	Dendritic cells, macrophages
Bunyaviridae	Helical filaments	Yes	11-19	Hantaan virus	Lung parenchyma, lymph nodes, hematopoietic cells
Filoviridae	Helical filaments	Yes	19	Ebola virus	Broad tropism, mononuclear phagocytic system, mucosa
				Zaire Ebola virus (ZEBOV)	Mononuclear phagocytic system
				Sudan Ebola virus (SEBOV)	Mononuclear phagocytic system
Orthomyxoviridae	Helical filaments	Yes	10-15	Influenza virus A	Upper and lower respiratory tract
Paramyxoviridae	Helical filaments	Yes	13-18	Parainfluenza virus 1	Lower respiratory tract epithelium

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Table 1. Continued

Family	Capsid	Envelope	Genome Size (kb)	Representative Virus*	Primary Target Cell/Organ System
Rhabdoviridae	Helical filaments	Yes	11-15	Vesicular stomatitis virus	Oral mucosa
Group VI: positive-sense ssRNA-RT					
Retroviridae	Spherical	Yes	7-13	Human immunodeficiency virus (HIV)	Intestinal mucosa, T-cells, dendritic cells, macrophages, microglia
				Human T-cell lymphotropic virus type 1 (HTLV-1)	T-cells
Group VII: dsDNA+RT					
Hepadnaviridae	Icosahedral	Yes	3-4 kb	Hepatitis B virus	Hepatocytes

*Underlined are the viruses discussed in this chapter.
Abbreviations: ds: double-stranded; kb: kilobase(s); RT: reverse transcriptase; SARS: severe acute respiratory syndrome; ss: single-stranded.

whereas HIV and HTLV target MIRRs from the intracellular environment (Fig. 1B) during viral replication. Although these viruses selectively inhibit or augment MIRR-mediated activation of the target cell during different viral stages, viral persistence is universally enhanced either through disarmament of the immune response or enhancement of the replicative environment. By overlapping multiple processes in each viral stage, viruses have demonstrated a remarkable efficiency in their life cycle that emphasizes their advanced evolution. Intriguingly, MIRR-targeted functions enacted by viral proteins seem to be present at multiple checkpoints in viral pathogenesis, coinciding with several viral stages. Therefore, it is not unreasonable to propose that MIRRs represent a key component in the host cell that multiple viruses have ubiquitously evolved to target, disrupt or activate as desired (Fig. 1).

Viral Entry and Membrane Targeting

In order for a virus to proliferate, it must first undergo a process of attachment to the target host cell and then penetration either through fusion or direct access; collectively, these two processes comprise viral entry and are often actuated by a single protein molecule. Viral attachment has been a subject of intense investigation and several details regarding the necessary specificity of viruses for their host cells have emerged. Interestingly, disparate viruses overlap in their specificities for their primary natural receptors. For example, members of the coronaviruses (OC43),[42] orthomyxoviruses (Influenza A, B)[43,44] and reoviruses (T3)[45-47] contain surface receptors that are specific for sialic acid residues found on the host cell receptor whereas members of the picornaviridae (rhinoviruses, polioviruses)[48-51] and retroviruses (HIV-1)[52-56] bind surface receptors that adopt the canonical immunoglobulin fold such as intercellular adhesion molecule-1 (ICAM-1), the immunoglobulin G (IgG) superfamily and CD4, respectively. Although there is little sequence or structural similarity in their envelope or capsid proteins, these viruses exhibit redundancy in receptor specificity.

Following attachment, the virus penetrates the host cell either through fusion in the case of enveloped viruses or direct entry for non-enveloped viruses. Although the steps and strategies non-enveloped viruses use to enter cells are largely unknown, the events leading to viral fusion have been studied in great detail. Membrane fusion of enveloped viruses is mediated by fusion proteins that exist primarily as homo- or heterodimeric type I integral membrane proteins found embedded in the surrounding envelope.[57,58] Concealed in the fusion protein is the fusion sequence or fusion peptide (FP), a short hydrophobic sequence ranging from 3-6 to 24-36 amino acids, that serves as the primary mediator of virus-host cell membrane anchoring. Depending on the location of the FP and the structural nature of the fusion protein, fusion proteins are segregated in three types. Type I fusion proteins found in such viruses as influenza are comprised of alpha-helix coiled-coil domains that contain FPs at the N-terminus. Type II fusion proteins contain primarily beta-sheet structures and contain internal FP sequences. The third group of fusion proteins do not fall in the type I and I classifications and are found in such viruses as coronaviruses and herpesviruses.

After translation in the host cell, type I and II fusion proteins are fusion-incompetent and require processing by viral proteases in order to be fusion-competent, or primed for fusogenic activity. Once the mature, processed and primed virus encounters a target cell, fusion events are mediated either by direct recognition and binding of the virus to its receptor on a target cell or a pH trigger commonly found in viruses that fuse within the endosome and not the outer membrane.[57-64] Once fusion is initiated, the fusion protein undergoes irreversible conformational changes that result in exposure of the FP. The hydrophobic peptide then embeds into the target host membrane, directly linking the virus and target cell. Previous investigation has attributed the embedding properties of the FP as a conclusion of predicted secondary sequences that FPs adopt amphipathic helices with hydrophobic residues on one face and polar residues on the opposing face.[65] However, recent work (see also Chapter 20)[41] has suggested that FPs from HIV and HCMV not only have generalized hydrophobic sequences, but sequences that specifically target host receptors, namely members of the MIRR family. If in fact MIRR-targeted strategies are conserved in a number of viruses and overlap with viral entry, sequence analysis of FPs from viruses other than HIV and HCMV should identify those viruses that share in their immunomodulatory specificities for MIRRs.

Human Immunodeficiency Virus

Viral entry of HIV is mediated by the product of HIV *env* expression, the type I fusion protein gp160, that is processed by HIV protease to yield the viral receptor gp120 (aa1-511) and fusion protein gp41 (aa512-684), found associated as heterohexameric complexes $[(gp120)_3\text{-}(gp41)_3]$[66-68] on the surface of HIV particles. Following encounter of a target T-cell, gp120 first binds the CDR2 loop of the CD4 coreceptor. CD4 induces a conformational change in gp120 that enhances binding to a coreceptor, namely CXCR4 or CCR5, to form the ternary CD4-CXCR4/CCR5-gp120 complex.[52,54,69-75] Consequently, membrane fusion is initiated by ternary complex-induced conformational changes in the gp120-gp41 complex that release gp41 from its metastable state and allow for the FP (aa512-535) to integrate into the target host membrane. Once the adjoining membranes are anchored by gp41, fusion events mediated by both gp41 and gp120 occur, allowing for viral entry.

Until recently,[40,76] the function attributed to gp41 and namely the FP has been limited to anchoring of the infecting HIV particle to the target T-cell. However, it is becoming increasingly evident that the FP contributes much more to the viral pathogenesis than simply viral entry. Investigation of the primary sequence of HIV FP yields the presence of two positively charged arginines (Fig. 2C) that lie on the same face of a predicted alpha helix. Interestingly, the TM domain (TMD) of the T-cell receptor alpha chain (TCRα) also contains two positively charged residues (R, K) that lie on the same face as well, being separated by 4 residues (Fig. 2). Because the TMDs of other components of the TCR, namely the CD3$\delta\varepsilon$ and $\zeta\zeta$ hetero- and homodimers, contain a negatively charged aspartate (D) residue, it is believed that electrostatic interactions drive TCR complex formation in the largely hydrophobic environment of the TM (Fig. 2A).[77] Therefore, by having similar electrostatic properties and distribution pattern of charged residues as the TCRα TMD, HIV FP may (1) specifically bind the electronegative components of the TCR complex in a transmembrane milieu and (2) physically and functionally disconnect the CD3$\delta\varepsilon$ and ζ signaling subunits from the remaining TCR complex by direct competition with the TCRα subunit (Fig. 2B).[40,41] This TCR-targeted functionality of the HIV FP adds a new dimension to the binding properties of the peptide and because of the adaptive immune function associated with the TCR, compounds an immunomodulatory role. These collective functions have been described in detail by the SCHOOL model[36,40,41,78,79] (see also Chapters 12 and 20) and are becoming increasingly substantiated by emerging experimental observation.

In in vitro coimmunoprecipitation and fluorescence resonance energy transfer (FRET) studies, HIV FP was demonstrated to specifically associate with TCR and the gp120 ligand, CD4 and to colocalize with TCR within 50Å.[80] Since neither gp120 nor the bulk of gp41 (aa535-684), which contains domains thought to also interfere with T-cell activation, were included in these experiments,[80] HIV FP must contain homing sequences that drive preferential localization and binding to the TCR without any extracellular contribution; the binding specificity is limited to the TM environment and is best explained by electrostatic interactions between the HIV FP and the TCR TMDs (Fig. 2B).[40]

Since gp120 is the primary HIV surface receptor that specifically binds CD4, HIV doesn't seemingly require gp41 or the FP particularly to serve as a binding partner for TCR or CD4. However, because the FP is heavily conserved amongst the divergent HIV subtypes, it must have other TCR-specific functions outside binding. In fact, FP was demonstrated to inhibit activation of primed lymph node cells and human T-cell lines in the presence of an activating antigen. However, in the presence of phorbol 12-myristate 13-acetate (PMA)/ionomycin or mitogenic antibodies to CD3, the inhibitory activity of HIV FP was abrogated.[80] These observations of FP closely mirror those of the recently studied TCR core peptide (CP) and are discussed in detail in Chapter 20. Briefly, TCR CP is a 9 amino acid peptide homologous to part of the TCRα TMD and contains the two electropositive residues (R, K) thought to be important for TCR complex formation (Fig. 2). TCR CP was also demonstrated to have immunosuppressive effects on T-cells in the presence of specific stimulating antigens, suggesting similar functionalities of TCR CP and

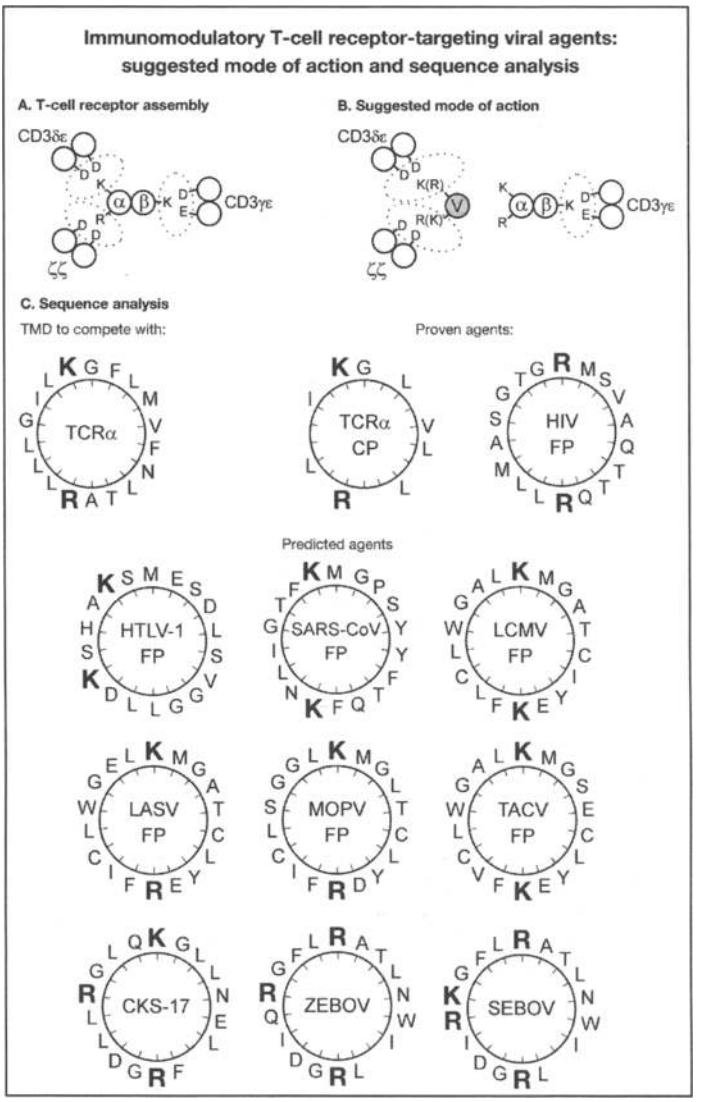

Figure 2. Suggested mode of action and sequence analysis of viral fusion protein regions and other domains proven and predicted to affect transmembrane interactions between T-cell receptor recognition and signaling subunits. A) Structural architecture of T-cell receptor is organized by three major assembly transmembrane forces, each involving one basic and two acidic amino acid residues. B) Within the SCHOOL model, viral agents (V) disrupt the transmembrane interactions between the ligand-binding TCRα chain and the CD3δε and ζζ signaling subunits which normally maintain the integrity of a functional T-cell receptor. This prevents formation of signaling oligomers upon multivalent antigen stimulation, thus inhibiting antigen-specific T-cell activation (see also Chapters 12 and 20). C) Helical wheel representations of proven and predicted immunomodulatory sequences of viral fusion protein regions and other domains. For illustrative purposes, the regions shown are restricted to 18 residues. As an ideal alpha helix consists of 3.6 residues per complete turn, the angle between two residues is chosen to be 100 degrees and thus there exists a periodicity after five turns and 18 residues. Positively charged residues are shown in bold. Legend continued on following page.

Figure 2, continued from previous page. Abbreviations: FP: fusion peptide; HIV: human immunodeficiency virus; HTLV-1: human T-cell lymphotropic virus type 1; LASV: Lassa virus; LCMV: lymphocytotic choriomeningitis virus; MOPV: Mopeia virus; SARS-CoV: severe acute respiratory syndrome coronavirus; SEBOV: Sudan Ebola virus; TACV: Tacaribe virus; TCR: T-cell receptor; V: viral agent; ZEBOV: Zaire Ebola virus.

HIV FP. However, those similarities were only described in retrospective analysis of the data, leading to assignments of novel functionality to the HIV FP.

As described by the SCHOOL model[36,41,78,79] (see also Chapters 12 and 20), both naturally-derived HIV FP and synthetically-designed TCR CP exploit their TM specificities for the CD3δε and ζζ components of the TCR to disrupt the TM interactions that hold the TCR complex together.[40,41] By disconnecting the recognition chains, TCRαβ, from the signaling chains, CD3δε and ζζ, HIV FP functionally disrupts the TCR complex and effectively disarms the MIRR. As a consequence, when TCRαβ recognizes and binds to its MHC-peptide partner on an APC, T-cell signaling is absent; the FP-associated signaling chains are unable to oligomerize and transduce the extracellular binding event (see Chapters 12 and 20).[40,41]

One of the defining features of the ability of HIV to replicate and proliferate is the low fidelity of HIV reverse transcriptase (RT) that leads to high mutability and sequence variability in HIV progeny during productive infection.[81] However, the HIV gp41 FP sequence is remarkably conserved among different HIV strains, suggesting a key role of not only the need for hydrophobic residues to embed in the target membrane and permit fusion but also the two electropositive residues that mediate binding to components of the TCR. As a result, HIV FP may not only serve as a fusogenic agent, but an immunosuppressive factor targeting the TCR as well, contributing to evasion of the adaptive immune response.

Human Cytomegalovirus

HCMV, a member of the betaherpesvirus subfamily of herpesviruses, is an enveloped virus characterized by a large genome (196 to 241 kbp) with the capacity to encode over 160 gene products. Existing as an opportunistic pathogen, HCMV proliferates during primary infection or reactivation of latent infection where an absence of effective immunity arises. Such conditions include modes where the immune system is compromised by other pathogenic agents (i.e., acquired immune deficiency syndrome, AIDS) or by prescribed immunosuppression (i.e., transplant recipients). However, the virus has also been demonstrated to replicate, reactivate and proliferate in environments where inflammation is markedly elevated.[82,83] Although the viral factors that mediate HCMV pathogenesis remain largely undetermined, three stages of HCMV pathogenesis have been described: (I) stimulation of a latently infected cell to differentiate and reactivate the latent virus to replicate by proinflammatory, cytokine-driven processes, (II) immunosuppression that allows amplification of productive viral replication, either systemically or locally and (III) direct or indirect viral or host immune-mediated damage that manifests as acute or chronic disease.[84] The immunosuppression or the ability of HCMV to evade and survive effector responses by innate and adaptive immune cells has been studied in great detail,[84] with novel mechanisms targeting disruption of MIRR signaling just now emerging.

Primarily infecting fibroblasts, HCMV has also been found to occupy professional APCs, namely macrophages and dendritic cells, following infection. Once inside the target host cell, HCMV prepares the cell for productive replication through two mechanisms: modulation of proinflammatory IFN cytokine production and reprogramming of cellular machinery. Immediately following entry, the tegument protein pp65, stored between the virion and surrounding envelope in the mature viral particle, is released and translocates to the nucleus, reducing the level of nuclear factor kappa B (NF-kB) production and blocking interferon regulatory factor-3 (IRF-3) activation.[85] Modulation of the IFN response is compounded by the activity of IE1-p72, a gene product expressed early after infection. By binding STAT1 and STAT2, IE1-p72 sequesters the signaling kinases and prevents their association with IRF-9, leading to the block of transcription of IFN-responsive genes.[86] HCMV also dramatically alters cellular gene expression and cell cycle

progression immediately following infection, allowing for productive replication; the cell cycle is dysregulated and kept in a mitosis-like state, permitting early viral gene expression and productive replication of viral progeny before apoptosis occurs.

In addition to modulation of IFN signaling pathways in the infected cell, HCMV has been described extensively to have developed mechanisms of evading the natural killer cell (NK cell) arm of the innate immune system.[84] NK cells surveil the host environment and are able to discriminate normal cells from those under duress or infection by monitoring the differential surface expression of MHC molecules on cells through the killer cell immunoglobulin-like receptors (KIRs). Once downregulation of MHC expression is detected, ligation of the natural cytotoxic receptors (NCR) NKp46, NKp44 by viral hemagglutinin or NKp30 by unidentified ligands results in NK cell-mediated cytotoxicity and lysis of the affected cell. While production of MHC analogs by HCMV in an infected cell to conceal the infectious process has been described in great detail,[87] mechanisms of viral evasion targeting the NCR have not garnered much attention until recently.

NKp30 exists on the surface of NK cells as an NKp30-ζ receptor complex, comprised of the recognition subunit NKp30 associated with the immunoreceptor tyrosine activation motif (ITAM)-containing ζ signaling subunit homodimer to form a canonical MIRR. Supported by experimental evidence[88] and described by the SCHOOL model,[36,79] ligation of the recognition subunit NKp30 and subsequent oligomerization of the ζ signaling subunit results in full activation of the MIRR. Although natural ligands for NKp30 have yet to be extensively identified, recent studies have demonstrated that the tegument protein pp65 interacts specifically and directly with the NKp30 complex, thus representing one of the first molecules to be classified as a NKp30 ligand.[88] However, rather than induce activation of the targeted NK cell, pp65 exhibits deleterious effects and inhibits NK cell activation, resulting in the inability of the NK cell to kill normal, tumor and virus-infected cells. This inhibitory effect of pp65 is explained and described to be the consequence of dissociation of the signaling ζ chains from the recognition NKp30 receptor, which renders the MIRR nonfunctional.[88] However, until recent application of the SCHOOL model (see also Chapter 20),[36,41] the mechanism for how binding of the NKp30-ζ complex and dissociation of NKp30 from ζ results in the inhibition of NKp30 signaling was unknown.

Investigation of the primary sequence of the N-terminal domain of pp65 reveals the presence of several electronegative and more importantly, electropositive amino acid residues (see Chapter 20, Table 6) that may disrupt the TMD interactions between NKp30 and ζ and result in the inhibition of NK cell activation observed. By taking advantage of the presence of a negatively charged aspartate (D) in the TMD of ζ, the highly positively charged pp65 N-terminus may preferentially bind ζ through a TM interaction, effectively releasing NKp30 from its binding partner, similar to the described actions of HIV FP and TCR CP (Fig. 2). Experimental evidence substantiating this mechanism of defusion will need to be demonstrated, however it is evident that HCMV has developed specific mechanisms to target MIRRs redundant with other viral strategies, such as those previously described for HIV FP in this chapter.

While the primary function of pp65 has been attributed to immediate inhibition of NF-kB production and IRF-3 promoter-driven gene expression inside the infected cell,[85] pp65's effects on NK cell activity have been described as a result of extracellular exposure of pp65 to the NKp30-ζ complex[88]—a quandary that needs further investigation. Whether exogenous pp65's origins come from secretion of the protein or more likely release from apoptotic cells, the membrane targeting activity of pp65 may not be as disparate from HIV FP as one would imagine, despite the nonfusogenic activity of pp65 or the major classification differences between HIV and HCMV (Table 1). Demonstrated to specifically target the NKp30-ζ complex, pp65 may act identically to HIV FP in targeting an MIRR and disengaging the receptor to suppress the immune cell and permit viral persistence.

Prediction of MIRR-Targeting Viral Agents: HTLV-1 and Other Viruses

Like other retroviruses, HTLV-1 enters permissive cells by binding to cellular surface molecules such as heparin sulfate proteoglycans[89] and the ubiquitous glucose transporter GLUT1 that serves

as a receptor for both HTLV-1 and HTLV-2 viruses,[90-92] followed by subsequent fusion of the viral and target cell membranes, thus releasing the viral core into the host cell cytoplasm.[90,93-96] This fusion is mediated by several viral envelope (Env) glycoproteins that are presented on the surface of virus or infected cell as a trimer of surface (SU) glycoprotein subunits anchored to a trimer of TM glycoproteins. Remarkably, infection with cell-free HTLV-1 virions remains inefficient because naturally infected lymphocytes produce very few cell-free virions and because, of the HTLV-1 virions that are released, only 1 in 10^5 to 10^6 is infectious.[91,94,95] The most efficient mode of HTLV-1 infection is cell-to-cell transmission that likely represents the sole mode of in vivo transmission for all retroviruses. Using confocal microscopy, the transfer of different HTLV-1 virion components from lymphocytes of infected patients to non-infected recipient lymphocytes has been directly visualized.[95]

Viral fusion results from a conformational change in the TM subunit of the Env protein, triggered by the SU/receptor interaction. This engagement exposes a FP located at the N terminus of the HTLV-1 TM protein gp21.[96,97] Similar to HIV gp41 FP, this sequence inserts into target cellular membranes and is well-known to be critical for membrane fusion activity.[98,99] However, in contrast to the HIV FP, there has been no report to date of an immunomodulatory activity of the HTLV-1 FP.

Because T-lymphocytes represent the major target cells for HTLV-1, it can be easily suggested that the TCR is a favorable target for inhibition at the viral entry stage. For these purposes, a TM-targeted strategy intended to physically and functionally disconnect TCR recognition and signaling subunits (Fig. 1A) might be effectively used by HTLV-1 as was described for HIV. The SCHOOL model[36,41,79] (see also Chapters 12 and 20) suggests that this "secret weapon" of HTLV-1 can be represented by the viral sequence that mimics the TMD of the TCR recognition subunit (for example, the TMD of TCRα chain) and is able to insert into the cell membrane where it competes with TCRα for binding to the CD3$\delta\varepsilon$ and ζ signaling chains in the TM milieu (Fig. 2B), thereby resulting in inhibition of antigen-induced T-cell activation as with HIV FP. Through helical wheel prediction (Fig. 2C) of the HTLV-1 FP, similarities in the location of electropositive residues previously described to be essential for the action of HIV FP, TCR CP and HCMV pp65 are revealed. Positioning of the charged lysine (K) residues in HTLV-1 FP is almost identical to those for the TCR CP and closely resemble those of the MIRR-disrupting viral agents HIV FP and HCMV pp65. Therefore, it is highly likely that HTLV-1 FP targets the TCR complex in a manner identical to HIV FP, TCR CP and HCMV pp65 and disrupts the TM interactions that hold the complex together, resulting in a defused TCR (Fig. 2B).

Intriguingly, analysis of other seemingly unrelated viruses has yielded similar correlations in primary structure and function. Earlier studies have reported an inhibitory effect of the CKS-17 peptide on lymphocyte proliferation, a synthetic 17-mer peptide with sequence corresponding to a highly conserved region of retroviral TM proteins of human and animal retroviruses including HTLV-1.[100] Later, the reported immunosuppression was further confirmed and further localized to a sequence essentially identical to the sequence present in the TM protein gp21 of HTLV-1,[101] supporting the hypothesis that this protein participates in the mechanism of immunosuppression previously reported for the TM proteins of feline leukemia virus and other animal retroviruses.

Interestingly, peptides corresponding to regions of HIV TM protein gp41 homologous to the highly conserved and immunosuppressive sequence contained within the TM proteins p15E and gp21 of animal and human retroviruses, respectively, have been also reported to inhibit lymphoproliferation.[101] Recently, filoviral 17-mer peptides corresponding to a 17 amino acid domain in filoviral glycoproteins that resembles an immunosuppressive motif in retroviral envelope proteins have been demonstrated to inhibit TCR-mediated T-cell activation and cell proliferation, providing new insights in the immunopathogenesis of Ebola and Marburg viruses.[102] In all these peptides (CKS-17; Zaire Ebola virus, ZEBOV and Sudan Ebola virus, SEBOV; Table 1), a striking similarity is observed between these peptides in charged or polar residue distribution patterns with positioning of the charged lysine (K) and/or arginine (R) residues almost identical

to those for the HIV FP (Fig. 2C), suggesting again a similarity in the molecular mechanisms of their immunosuppressive action.

Based on the surprising conservation in positioning of the essential electropositive residues in the helical wheel predictions of HIV-1 FP and HTLV-1 FP and its similarity to those for the TCR CP, it is highly probable that proteins from other unrelated viruses that also participate in viral fusion would also target MIRRs on the surface of their target cell. Exploratory sequence investigation of FPs from severe acute respiratory syndrome coronavirus (SARS-CoV), Lassa virus (LASV), lymphocytic choriomeningitis virus (LCMV), Mopeia virus (MOPV) and Tacaribe virus (TACV) reveal evidence of such a hypothesis. As shown in Figure 2, there is striking similarity in the positioning of the electropositive residues on one face of the helix, despite the fact that the amino acid residues aren't necessarily conserved; for example, MOPV FP contains an arginine and lysine whereas TACV contains only lysine residues. This clearly demonstrates that viruses, despite their differences in virion structure, genomic composition or classification, have adopted similar mechanisms of specifically targeting MIRRs, disrupting their architecture and suppressing the immune system. Importantly, by virtue of the acquired insight into this conserved structural motif, expanded predictions, hypotheses and conclusions can be derived to begin answering the question of if shared MIRR-targeted strategies represent a conserved function or if they represent a convergent tactic of divergent viruses.

Viral Replication

Similar to viral entry, viruses have developed subprocesses targeting MIRRs that underlie other viral stages, namely viral replication, for enhancement of viral production and persistence. Following entry and uncoating in the target cell, viruses undergo an efficient and economical process of replication where copies of the viral genome are abundantly produced, viral genes are expressed and viral protein translations begin to assemble into competent viral particles. Due to the diversity in genomic structure found among the different viruses, there is also great diversity in the replication strategies they employ. Contrary to cellular genomes that are comprised uniformly of dsDNA, viral genomes span all possible structural organizations: dsDNA, dsRNA, positive-sense ssDNA, negative-sense dsDNA, positive-sense ssRNA, negative-sense ssRNA and mixed (ambisense) ssDNA or ssRNA. Consequently, viruses have developed unique replication strategies, used by the Baltimore classification method to group viruses, that require different host proteins as well as inclusion of different virally encoded proteins in their genomes. For example, group I dsDNA viruses, such as members of the adenoviral family, require host cell DNA polymerases to replicate their viral genomes and are therefore highly dependent on the replicative state of the cell; the target cell must be undergoing active replication and cell division where the cell's polymerases are most active. In contrast, group VI positive-sense ssRNA viruses, such as members of the retrovirus family, replicate their genomes by RNA-dependent DNA synthesis not by any host polymerases but by virus-encoded RT; the transcribed DNA is then used as the viral template for integration into the host genome and transcription. Because RT is not supplied by the target cell, it must be packaged with the viral progeny for further replication. Regardless of the structure and replication strategy of their genomes, all viruses express their genes as functional mRNAs early in infection and direct the cell's translational machinery to make viral proteins for eventual viral packaging.

Efficiency is essential to every viral stage but particularly to replication as it represents a pivotal point in virus production. Viruses have therefore optimized their replication strategies to exploit naturally occurring biological and cellular processes of their hosts, effectively hijacking the replication, transcription and translational machinery. However, replicative efficiency has its drawbacks; viruses are consequently dependent largely on the replicative capacity of their target cells and what functional state they are in during the infection. To overcome these limitations, several viruses have developed mechanisms of activating the infected target cell from within the cytoplasmic environment to enhance viral replication (Fig. 1B). In this section, we describe subprocesses within the realm of viral replication that two members of the retrovirus family enact by targeting a specific MIRR, namely the TCR, from the cytoplasmic environment. Coupled with the TM-targeted

strategy employed by HIV-1 FP and HTLV-1 FP (Fig. 1A), the cytoplasmic-targeted strategy represents an interesting dichotomy of site of action and function that converge on the identity of the specific target.

Human Immunodeficiency Virus

Characterized by its positive polarity ssRNA genome and group VI classification, HIV shares a unique replicative process with other members of the retrovirus family that differs significantly from other viruses. Prior to replication, HIV virions attach to and enter T-lymphocytes following formation of the ternary HIV gp120-CD4-chemokine receptor CCR4/CXCR5 complex and direct membrane fusion mediated by HIV gp41, respectively.[52,54,69-75] Once inside the cell, the virion partially uncoats in the cytoplasm, releasing viral accessory proteins and the two copies of the positive-sense ssRNA genome housed inside the viral particle. HIV RT then initiates transcription of the viral genome, producing double-stranded cDNA transcription products that immediately associate with a number of viral (integrase, RT, matrix, Vpr)[103-105] and cellular (IN1, HMGA1, BAF, EED, LEDGF/p75)[106] proteins to form the preintegration complex (PIC). Due to the low fidelity of HIV RT[81] that results in 3×10^{-5} mutations per replication cycle in vivo,[107] HIV enjoys incredible genetic diversity during virus production that closely resembles evolution but in a rapid timescale. Viral particles that introduce mutations in their genomes that exhibit increased replicative capacity will propagate and dominate the infection whereas replication-deficient variants will cease to exist.

Once formed, the PIC migrates to the nucleus by the host nuclear import machinery that only actively translocates the PIC when the cell is arrested in the G1 phase of the cell cycle and nondividing. Following import into the nucleus, RT-transcribed viral cDNA is integrated into the host chromosome via HIV integrase, a hallmark event that is unique to HIV. Once integrated, HIV DNA is left untranscribed in a latent stage of infection until the infected T-lymphocyte is activated and coordinated interactions between HIV-encoded Tat protein, host NF-kB, Sp1 transcriptional transactivating proteins and the RNA polymerase II transcriptional complex facilitate production of high levels of viral RNA.[108] Newly transcribed mRNAs are exported from the nucleus to the cytoplasm by HIV Rev and then translated by host ER-associated and cytoplasmic ribosomes to yield gp120 Env and Gag/Gag-Pol polyproteins, respectively. Each viral protein species translocates to the cytoplasmic face of the plasma membrane where they associate with dimeric viral positive-sense ssRNA to form the premature viral bud that subsequently undergoes further processing, entering the final stages of viral assembly and release.

While much of the work investigating HIV replication has focused on the role of the viral regulatory protein Tat on HIV RNA transcription,[109-112] reports have suggested a key role of cellular activating factors in enhancing replication.[108] In order for HIV to emerge from latent infection where the HIV genome is transcriptionally silent, the infected T-lymphocyte must become activated and initiate a signaling cascade that ultimately results in the release of NF-kB from sequestration by IkB. Therefore, any mechanism that induces a state of activation within the infected cell would effectively enhance NF-kB activity and downstream replication of HIV. Recently, the viral accessory protein Nef has been described to affect the activation profile of CD4+ T-lymphocytes by reducing the threshold of T-cell activation[113,114] and also initiating a transcriptional program in Jurkat T-cells similar to that of a T-lymphocyte exogenously activated through the TCR.[115] Localization to the cytoplasmic face of the plasma membrane seems to be required for Nef-induced activation or augmentation of activation[116] and association with lipid rafts and cytoplasmic signaling proteins has been proposed to play a key role.[117] However, details of the specific mechanisms underlying Nef-mediated augmentation of activation or reduction in threshold for activation remain largely unknown.

Originally coined "negative factor" under reports that HIV Nef reduced replication by suppressing transcription of integrated HIV genes,[118] it is now evident that Nef mediates several processes that collectively enhance viral replication: (1) downmodulation of surface receptors, namely CD4,[119,120] MHC Class I proteins (HLA-A, B but not C or E),[121-123] CD28[124] and TCR

in the context of simian immunodeficiency virus (SIV),[125] (2) enhancement of viral infectivity[126] and (3) modulation of signaling pathways. Among all of Nef's functions, downmodulation of the TCR remains the most controversial and intriguing. Because of its role in initiation of the signaling cascade in T-lymphocytes, TCR fills a strong potential role in Nef's reported effects on increasing the activation state of the cell. Interestingly, HIV-2 and SIV Nef have been reported to specifically interact with the ζ signaling chain of the TCR complex but additionally induce downregulation of surface TCR from the cell surface.[127-129] Functional mapping of SIV Nef has revealed that the C-terminal core domain, conserved among the different HIV-1 clades and strains, is responsible for specific ζ binding whereas the nonconserved N-terminal domain cooperatively binds AP-2 from the host thereby inducing downregulation of the bound TCR. Extrapolation of these results explains the lack of TCR downmodulation observed for several HIV-1 Nef variants,[130] considering the genetic variability in the N-terminal domain and strengthens the observed binding data surrounding the HIV-1 Nef-ζ interaction that has been previously disputed.[127,128]

Armed with the ability to form homooligomers on the one hand and specifically bind the signaling ζ chain of the TCR, on the other, HIV Nef can exert activating or augmenting effects on TCR-mediated stimulation, as described recently by the SCHOOL model (see also Chapter 20).[36,78,79] In contrast to extracellular targeting of the TCR by HIV FP as described earlier in this chapter, HIV Nef targets the TCR from the cytoplasmic environment and rather than inhibit TCR activation, enhances it. In the case of HIV FP, the signaling subunits of the TCR are physically and functionally disconnected from the recognition subunits through TMD interactions formed with HIV FP that effectively results in inhibition of antigen-mediated TCR signaling. HIV Nef may crosslink with the ζ signaling subunits through cytoplasmic interactions[36] (Fig. 1B; see also Chapters 12 and 20), cluster TCRs and instead of disengaging the receptor, activate it or prime it for activation. While a large component of the SCHOOL model requires the ability of the signaling chains of the TCR to homooligomerize in receptor clusters, HIV Nef has been reported to self-oligomerize, a property already described to be vital for function.[131-135] Therefore, through the combination of an interaction with ζ and self-oligomerization, HIV Nef may induce the formation of higher order receptor oligomers that directly activate the cell[115] or effectively reduce the threshold of stimulus required for full activation.[113,114] Recent studies have indeed demonstrated clustering of HIV Nef at the immunological synapse,[136] the interface between the infect T-lymphocyte and an APC, furthering supporting the notion that Nef interacts with cytoplasmic components of the TCR and likely participates in higher order oligomerization conducive to T-cell activation.

Interestingly, SIV seems to have developed additional methods of further exploiting the TCR-targeted augmentation of cellular activation Nef enacts. Characterized by rapid viral kinetics and the novel ability to replicate and proliferate in non-exogenously-stimulated macaque peripheral blood mononuclear cells (PBMC),[137] SIVsmPBj, a highly pathogenic strain of SIV, induces acute, destructive disease while exhibiting an augmented replicative state.[137,138] Underlying this disease is the presence of an ITAM sequence in SIVsmPBj Nef similar to that found in the signaling domains of the CD3δ, CD3ε, CD3γ and ζ components of the TCR. Therefore, upon localization to the inner leaflet of the plasma membrane and association with ζ during acute infection, SIVsmPBj Nef forms high order heterooligomeric Nef-ζ complexes with significantly increased numbers of ITAM domains as compared to nonSIVsmPBj variants. Consequently, the infected cell will be prone to not only clustering of the signaling chains of the TCR by binding Nef but additional induced activation by virtue of the supplied ITAM sequences present in the viral protein. By including ITAM sequences, SIVsmPBj effectively clusters viral ITAMs with host ITAMs to induce acute activation and replication.

Despite targeting the same receptor as HIV FP, HIV Nef has the complete opposite effect on its function; rather than inactivate the receptor as observed with HIV FP, HIV Nef activates it. Explained to be the result of a cytoplasmic-targeted strategy (Fig. 1B), it is intriguing that HIV developed two mechanisms of acting on the same receptor, but eliciting different outcomes depending on the viral stage and site of action. However, through those developed viral strategies, details on how MIRRs function and initiate the intracellular cascade are revealed and provide

methods of studying immune regulation but also new avenues for development of novel immunomodulatory therapeutics.

Human T-Cell Lymphotropic Virus

There is a growing line of evidence that the accessory proteins of HTLV-1 are critically involved in viral transmission and propagation and may in fact be multifunctional proteins. Key among them is the p12 protein of HTLV-1, a small oncoprotein that is produced during the course of the natural infection in vivo and has been shown to have multiple functions. Analogous to the accessory HIV-1 Nef protein,[139,140] p12 is required for optimal viral infectivity in nondividing primary lymphocytes.[141-143] HTLV-1 viral infection of T-lymphocytes is known to induce T-cell activation.[138] As suggested, one mechanism involves activation of T-cells harboring the virus and is exemplified in vivo by infected, non-immortalized T-cell clones that display prolonged states of activation, whereas with a separate mechanism, virus-infected cells can induce activation of uninfected T-cells via T-cell-T-cell interactions.[138] In non-immortalized, HTLV-1-infected T-cells, spontaneous clonal proliferation is resistant to immunosuppression by transforming growth factor-β (TGF-β), a cytokine implicated in terminating T-cell activation, suggesting a potential role of HTLV-1 in a defense against TGF-β-induced immune suppression of the host cell.[144]

Spontaneous proliferation and virus production have been reported to increase in the presence of anti-CD3 and anti-TCR antibodies while addition of HLA class I antibodies, but not HLA class II or viral proteins, shut down virus production and cell proliferation.[145] These findings suggest that both virus and cell activation may occur through the TCR on the infected cell. Expression of p12 has been shown to induce nuclear factor of activation of T-cells (NFAT), enhance the production of interleukin-2 (IL-2), decrease MHC-I expression, increase cytoplasmic calcium and signal transducer and activator of transcription 5 (Stat 5) activation in T-cells further supporting the hypothesis that p12 may alter T-cell signaling.[143,146-150] Interestingly, p12 is important for viral infectivity in quiescent human peripheral blood lymphocytes (PBLs) and PBMCs and the establishment of persistent infection in vivo, suggesting a role for p12 in the activation of quiescent lymphocytes, a prerequisite for effective viral replication in vivo.[141,151] In this context, function of p12 in conditions where the majority of viral target cells are in quiescent states has been predicted to be similar to that of Nef.[141] HTLV-1 p12-expressing cells were reported to display a decreased requirement for IL-2 to induce proliferation during suboptimal stimulation with anti-CD3 and anti-CD28 antibodies.[149] HTLV-1 replication in infected lymphocytes has been also been reported to increase upon CD2 cross-linking.[152] This receptor is known to signal primarily through the associated CD3ε and ζ chains.[153,154] Studies have shown that the mitogenic activity of HTLV-1 viral particles is restricted to virus-producing T-cells, requires cell-to-cell contact and may be mediated through the lymphocyte-associated antigen 3 (LFA-3)/CD2 activation pathway and that HTLV-1 virions interfere mainly with activation of peripheral T-cells via CD2/ζ but not via the CD3/TCR complex.[155]

Overall, p12 seems to augment T-cell activation and facilitate viral replication. Thus, despite the distinct structures, both retroviral accessory proteins HTLV-1 p12 and HIV Nef are able to modulate TCR-mediated signaling and play a critical role in enhancing viral infectivity in primary lymphocytes and infected animals. Interestingly, it has been recently reported that p12 could complement for effects of Nef on HIV-1 infection of Magi-CCR5 cells, which express CD4, CXCR4 and CCR5 on the surface, or macrophages.[156] Also, Jurkat cell clones that express high levels of p12 have been found to exhibit a more rapid rate of cell proliferation than the parental cells.[156] Similarly to HIV Nef, the p12 protein, upon engagement of the TCR, localizes to the interface between T-cells and antigen-presenting cells, namely the immunological synapse.[157]

Intriguingly, similarly to HIV-1 Nef protein,[133] HTLV-1 p12 has also been shown to form dimers.[149] It can be suggested that homooligomerization of p12 contributes to p12-mediated augmentation of T-cell activation and that molecular mechanisms of this phenomenon are similar to those that have been suggested previously for Nef through application of the SCHOOL model

of TCR signaling (see also Chapters 12 and 20).[36] If true, the homooligomerization interface(-s) of p12 represent potential therapeutic targets for antiviral treatment.

Translation of Redundant Viral Strategies into Disease Care

As depicted by members of the retroviridae and herpesviridae, namely HIV, HTLV and HCMV, a wide range of viruses has developed methods of targeting members of the MIRR family of surface receptors. However, depending on the needs of the virus and at which stage of viral replication the virus is in, MIRR-induced signaling is either disrupted or enhanced. More specifically, when HIV undergoes viral entry, MIRR-triggered activation is abrogated through disruption of TM interactions in TCR by HIV FP in order to evade immune activation. Similar function is required during persistence of HCMV infection where signaling through NKp30 is abrogated so as to inactivate the NK cell response and accompanying immune activation. However, where MIRR-triggered activation is needed for enhanced replication, exemplified by HIV and HTLV, viral proteins once again specifically target MIRRs, but in a concerted effort to induce triggering and subsequent cellular activation mechanisms conducive to viral production. Therefore, although viruses may be structurally different, contain different types of genomes and exhibit different replication strategies, many converge in their immune modulation strategies.

The combination of retrospective analysis of previous experiments investigating details of HIV, HTLV and HCMV pathogenesis and application of a novel model of MIRR triggering,[36,79] has revealed a couple of key features of MIRR triggering that viruses redundantly interfere to modulate the immune response: TM interactions between the recognition and signaling subunits of MIRRs and oligomeric clustering of signaling domains. Described as TM and cytoplasmic targets (Fig. 1), respectively, these two classes of interactions represent the foundations of MIRR triggering and provide avenues for novel but universal antiviral therapies and importantly, immunomodulatory treatment as well.

Current small molecule, antiviral research has focused on exploiting the differences between virus and host and selectively targeting a viral enzyme or process. However, due to the high mutation rate many viruses enjoy, therapies against protease or reverse transcriptase in HIV are being selected, resulting in drug resistant viral strains that exhibit even increased pathogenicity and necessitating the discovery of novel therapeutic targets. Our discussion of the specific targeting of MIRR signaling subunits, namely ζ, by HIV and HTLV provides that opportunity. Targeting of TCR-mediated signaling seems to be a shared feature of both HIV and HTLV-1 viruses and reflects a similar evolutionary pathway towards their adaptation to the host immune response that may also be shared with other unrelated viruses. Instead of inhibiting a specific enzymatic function, Nef and p12 functional targeting strategy would involve disrupting the protein-protein interface between the viral protein and the partner signaling chain to abrogate its activating function. In addition, the homointeractions between viral proteins may also emerge as a functional target since homooligomerization of viral proteins has also been shown to be essential for function. Careful investigation of the interacting surfaces on both the viral and MIRR may reveal unique features essential for binding, highlighting more rationalized drug targeting. Finally, extension of this protein-protein interaction disruption strategy should also be applied to other viruses to determine if there is increased redundancy in the processes outlined by Nef and p12. If so, MIRR-targeted antiviral research may provide a new line of generic but universal antiviral therapies.

An intriguing extension of the revealed strategies viruses redundantly use to target MIRRs is the application of them towards development of immunomodulatory agents. Viruses have evolved over thousands to millions of years and have optimized methods of disarming and evading the immune response for self-preservation. Therefore, investigation of how viruses have adapted to disarm the innate and adaptive immune system will prove invaluable in rational drug design efforts aiming to reduce immune activation or inflammation. One viral strategy, namely the disruption of TMD interactions between the signaling and recognition subunits in MIRRs suggested for HIV, HTLV, HCMV and other viruses here (Fig. 1A; see also Chapter 20), provides such an avenue for exquisite drug development that has the potential for rapid development.

Retrospective analysis of the primary sequence of HIV FP and HCMV pp65 revealed the presence of specific electropositive residues that mirror those found in the TMD of the TCRα signaling subunit (Fig. 2C; see also Chapter 20, Table 6). Combining that observation with functional data describing the inhibitory effect they have on TCR and NKp30 signaling, it is highly probable that they compete with TCRα for binding with its signaling binding partners, effectively disrupting the TCR complex and rendering it useless (Fig. 2B; see also Chapter 20). Therefore, membrane-targeted strategies mimicking those of HIV FP and pp65 and exploiting the binding contribution of electropositive amino acid residues will likely have similar effects and provide useful as therapies for immune disorders characterized by chronic inflammation. Coincidentally, one such avenue of TCR-targeting research has already undergone development with promising results. Derived from the primary sequence of the TCRα TMD region, synthetic hydrophobic peptides, coined the TCR core peptides or TCR CPs, were produced and exhibit inhibitory function in not only T-cells, but B cells and NK cells as well.[158] Further studies with a D-amino acid variant also show strong efficacy, suggesting that chirality plays little role in the function of the peptide, leaving sequence pattern and electrostatics as the only mediators of function.[159]

Although the TM-targeting strategy employed by TCR CP was not a prospective application based on learned viral strategies, it displays the intellectual and rational research power that can be attained by investigating what viruses and nature have already employed and optimized. Hence, we have begun to investigate the primary sequences (Fig. 2C; see also Chapter 20)[40,41] of several unrelated viruses and see a remarkable homology in primary sequence and sequence pattern of a number of viral proteins, highlighting the presence of electropositive residues that may also target MIRRs. Future collaborations in bioinformatics, biochemistry and virology will undoubtedly reveal new details of the viral immune evasion strategies that are shared amongst a number of viruses that may prove useful in developing rational approaches to immune therapy.

Conclusions and Perspectives

Viral infection and the resultant immune response form a violent interplay where host homeostasis is interrupted by a propagating virus seeking to proliferate and the immune system working to quell the infection. In many cases, the virus and human host have coevolved to exist symbiotically where the virus resides in a latent phase nonpathogenic to the host. However, as new viruses emerge or crossover from other species, they will need to replicate rapidly and efficiently so as to proliferate as quickly as possible. This poses the largest pathogenic threat to humans and incurs disease that defeats the immune system and results in death of the human host. Therefore, we are forced to develop novel strategies to target the infecting virus. However, rather than targeting virus-specific proteins or processes, it would be advantageous to transfer therapeutic strategies that target redundant processes found among a number of viruses. In this chapter, we have described the universal targeting of members of the MIRR family by a number of seemingly unrelated viruses that function through similar mechanisms. Therefore, it is possible to take advantage of these general processes in drug development; the tedious work of developing virus-specific therapies would be eliminated and powerful far-reaching agents could be conceived.

In addition to the antiviral lessons learned from investigating the role of MIRRs in viral pathogenesis, several details regarding normal MIRR structure-function relationships and therapeutic intervention can be extrapolated. As demonstrated by the similar function of natural HIV FP and synthetically derived TCR CP, viral immune evasion strategies can be transferred to therapeutic strategies that require similar functionalities. Viruses represent years of evolution and the efficiency and optimization that come along with it. Therefore, viral functions should not only be studied as foreign processes but as efficient strategies we can use in our own attempts at immune evasion or immunomodulation.

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