New therapeutic strategies targeting transmembrane signal transduction in the immune system

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Single-chain receptors and multi-chain immune recognition receptors (SRs and MIRRs, respectively) represent families of structurally related but functionally different surface receptors expressed on different cells. In contrast to SRs, a distinctive and common structural characteristic of MIRR family members is that the extracellular recognition domains and intracellular signaling domains are located on separate subunits. How extracellular ligand binding triggers MIRRs and initiates intracellular signal transduction processes is not clear. A novel model of immune signaling, the Signaling Chain HOMoOLigomerization (SCHOOL) model, suggests that the homooligomerization of receptor intracellular signaling domains represents a necessary and sufficient condition for receptor triggering. In this review, I demonstrate striking similarities between a consensus model of SR signaling and the SCHOOL model of MIRR signaling and show how these models, together with the lessons learned from viral pathogenesis, provide a molecular basis for novel pharmacological approaches targeting inter- and intrareceptor transmembrane interactions as universal therapeutic targets for a diverse variety of immune and other disorders.

Single- and Multi-chain Receptors

Cells express at their surface a repertoire of receptors that recognize individual stimuli and transduce this information across the cell membrane, thus activating intracellular signaling pathways. The importance of receptors in health and disease makes the molecular understanding of transmembrane (TM) signal transduction critical in influencing and controlling this process for therapeutic purposes. Unrelated and functionally diverse receptors can be structurally classified as single- and multi-chain activating receptors. By definition, single-chain receptors (SRs) are receptors with binding and signaling domains located on the same protein chain (Fig. 1A). Examples include receptor tyrosine kinases (RTKs) that are TM glycoproteins consisting of a variable extracellular N-terminal domain, a single membrane spanning domain and a large cytoplasmic portion composed of a juxtamembrane domain, the highly conserved tyrosine kinase domain and a C-terminal regulatory region. In contrast, multi-chain receptors, key among which is the family of multi-chain immune recognition receptors (MIRRs) that are expressed on many different immune cells and mediate antigen recognition, are characterized by the most intriguing common structural feature: their extracellular recognition (binding) domains and intracellular signaling domains containing immunoreceptor tyrosine-based activation motifs (ITAMs) or the YxxM module are located on separate subunits (Fig. 2A). The association of the subunits in resting cells is mostly driven by the noncovalent TM interactions between recognition and signaling components (Fig. 2A) and plays a key role in receptor assembly and integrity. Typical examples of MIRRs include the T-cell receptor (TCR) complex, the B-cell receptor (BCR) complex, Fc receptors (e.g., FcγRI, FcγRII, FcγRIII), NK receptors (e.g., NKG2D, CD94/NKG2C, KIR2DS, NKp30, NKp44 and NKp46), immunoglobulin (Ig)-like transcripts and leukocyte Ig-like receptors (ILTs and LIRs, respectively), signal regulatory proteins (SIRPs), dendritic cell immunoactivating receptor (DCAR), myeloid DNAX adapter protein of 12 kD (DAP12)-associating lectin 1 (MDL-1), novel immune-type receptor (NITR), triggering receptors expressed on myeloid cells (TREM5), and the platelet collagen receptor, glycoprotein V1 (GPV1). Assuming that the similar architecture of the receptors within the single- and multi-chain receptor families dictates similar mechanisms of receptor triggering, I suggest that transmembrane signal transduction mediated by SRs and MIRRs is based on similar mechanistic principles which in turn provide the similarity of the revealed therapeutic targets. My hypothesis is that signaling chain homooligomerization in cytoplasmic milieu provides the necessary and sufficient event to trigger members of both receptor families. As a consequence of this hypothesis, receptor oligomerization induced or tuned upon ligand binding outside the cell is suggested to be translated across the membrane into protein oligomerization inside the cell, thus providing a general platform for receptor-mediated signaling. Together with the lessons learned from viral pathogenesis, this builds the structural basis for the development of novel pharmacological...
approaches targeting inter- and intra-receptor transmembrane interactions as universal therapeutic targets for a diverse variety of immune and other disorders. Importantly, this also allows transferring our current and future clinical knowledge, experience and therapeutic strategies between seemingly unrelated diseases.

**Transmembrane Signal Transduction: Basic Principles**

The molecular mechanisms underlying SR (i.e., receptor tyrosine kinases, RTKs) signaling have been fairly well delineated and suggest that intracellular formation of competent signaling oligomers plays a crucial role in receptor triggering (Fig. 1B). Ligand binding is believed to stimulate SR dimerization and trans-autophosphorylation at defined tyrosine residues through intrinsic kinase activity. Formation of competent signaling oligomers in cytoplasmic milieu has been shown to be a critical event in Fas receptor signaling. Some SRs, such, for example, as members of the tumor necrosis factor (TNF) receptor superfamily exist as pre-assembled oligomers on the cell surface. In these cases, binding to multivalent ligand results in reorientation of receptors in these oligomers to adopt an interunit geometry permissive for further receptor activation (Fig. 1B).

For multi-chain activating receptors that signal through ITAM/YxxM modules, the mechanism by which the MIRR transduces ordered information such as antigen recognition from outside the cell via receptor TM and juxtamembrane regions into intracellular biochemical events has been a long-standing open issue until recently, when, in the novel model of immune signaling, the Signaling Chain HOmoOLigomerization (SCHOOL)
rather than receptor clusters/oligomers per se, is necessary and sufficient to trigger the receptors and induce transmembrane signal transduction and the downstream signaling sequence (Fig. 2B). Similar to SRs, some MIRRs, such as TCR and major platelet collagen receptor GPVI, can exist as model, formation of ITAM-containing cytoplasmic signaling oligomers was for the first time suggested to play a critical role in TM signaling mediated by these receptors. The model suggests that formation of competent MIRR signaling subunit oligomers mediated by homotypic interactions in cytoplasmic milieu, rather than receptor clusters/oligomers per se, is necessary and sufficient to trigger the receptors and induce transmembrane signal transduction and the downstream signaling sequence (Fig. 2B). Similar to SRs, some MIRRs, such as TCR and major platelet collagen receptor GPVI, can exist as
pre-assembled oligomers on the cell surface.\textsuperscript{13,14} Within the SCHOOL model,\textsuperscript{4,12,15,16} binding to multivalent ligand results in reorientation of receptors in these oligomers to adopt an interunit geometry permissive for further receptor activation (Fig. 2B), again highlighting the mechanistic generality of receptor-mediated transmembrane signaling.

Intriguingly, in contrast to well-structured cytoplasmic signaling domains of SRs, cytoplasmic domains of MIRR signaling subunits belong to a novel class of intrinsically disordered proteins (IDPs; i.e., proteins that lack a well-defined ordered structure under physiological conditions in vitro). The recently discovered unusual biophysical phenomenon, the ability of these IDPs to homooligomerize,\textsuperscript{17,18} represents a missing key piece of the MIRR triggering puzzle (Fig. 2B). This raises an interesting question: Why for MIRRs, the receptors with extracellular recognition and intracellular signaling domains located on separate protein chains, nature selected to use an unusual and unique functional link between protein disorder and oligomericity? One can expect that further multidisciplinary studies will clarify this question of great interest and practical utility.

Thus, formation of competent signaling oligomers mediated by homointeractions between well-structured (in SRs) or intrinsically disordered (in MIRRs) cytoplasmic signaling domains is a necessary and sufficient to trigger receptor function. This dictates several important mechanistic principles of receptor-mediated transmembrane signaling:

- sufficient interreceptor proximity in receptor dimers/oligomers.
- correct (permissive) relative orientation of the receptors in receptor dimers/oligomers.
- long enough duration of the receptor-ligand interaction that generally correlates with the strength (affinity/avidity) of the ligand.
- sufficient lifetime of an individual receptor in receptor dimers/oligomers.

Interestingly, these general principles are common for SRs and MIRRs, thus linking mechanistically numerous structurally and functionally diverse receptors.

**Transmembrane Interactions as Therapeutic Targets**

Because of the ubiquitous nature of protein-protein interactions and the knowledge that inappropriate protein-protein binding can lead to disease, the specific and controlled inhibition and/or modulation of these interactions provides a promising novel approach for rational drug design, as revealed by recent progress in the design of inhibitory antibodies, peptides and small molecules.\textsuperscript{19-21} Suggesting important role of TM interactions that mediate ligand-induced SR dimerization (oligomerization) and homointeractions between cytoplasmic domains that result in formation of competent signaling oligomers (Fig. 1B), the consensus model of SR signaling reveals these interactions as important points for intervention by relevant targeted agents to modulate SR-mediated signaling. In this review, I focus mainly on TM interactions (Fig. 1C) as universal therapeutic targets for SR-related pathologies. Within the model, specific blockade of the ligand binding-induced TM interactions prevents oligomerization of these domains upon ligand stimulation (Fig. 1C). As a result, signaling oligomers are not formed, Tyr residues do not become phosphorylated and the signaling cascade is not initiated (Fig. 1C). Peptides and their derivatives, small-molecule disruptors of protein-protein interactions, site-specific mutations, and other similar agents/modifications can be used to affect these TM interactions.

Similarly, considering MIRR triggering as an outcome of the interplay between three crucially important interactions: (1) antigen/ligand-MIRR extracellular interactions, (2) intrareceptor TM interactions that stabilize and maintain receptor integrity in resting cells and (3) interreceptor cytoplasmic homointeractions that lead to the formation of competent signaling oligomers, the SCHOOL model reveals these specific protein-protein interactions as points of intervention to inhibit and/or modulate MIRR-mediated TM signaling, thus inhibiting and/or modulating the immune response. While antigen/ligand-receptor interactions are a well-known target for drug design and development,\textsuperscript{22-36} the last two protein-protein interactions that are critically involved in MIRR triggering/signaling, represent promising novel therapeutic targets as revealed by the model.\textsuperscript{4,12,15,37-39} In this review, I focus mainly on intra-MIRR TM interactions (Fig. 2C) as universal therapeutic targets for MIRR-related pathologies. As suggested by the model (Fig. 2C),\textsuperscript{4,12,15,37-39} specific blockade or disruption of the TM interactions between MIRR recognition and signaling subunits causes a physical and functional disconnection of the subunits. In this context, the term “physical disconnection” means “pre-dissociation” rather than full dissociation of the subunits because in the absence of stimulus, they can still remain together. Antigen/ligand stimulation of these “pre-dissociated” receptors leads to reorientation and clustering of the recognition but not signaling subunits. As a result, signaling oligomers are not formed, ITAM Tyr residues do not become phosphorylated and the signaling cascade is not initiated (Fig. 2C). These are common targets for all members of the MIRR family, which means that a general pharmaceutical strategy may be used to treat seemingly disparate disorders such, for example, as T cell-mediated skin diseases and platelet disorders.\textsuperscript{4,37,40-41} As with SRs, peptides and their derivatives, small molecule disruptors of protein-protein interactions, site-specific mutations, and other similar agents/modifications can be used to affect the MIRR TM interactions. Importantly, our current understanding of the MIRR structure and the nature and specificity of TM interactions between receptor recognition and signaling subunits allows us not only to block or disrupt these protein-protein interactions but to also modulate the interactions by sequence-based approach with using corresponding peptides and/or their derivatives. Strengthening/weakening and/or selective disruption of the association between particular recognition and signaling subunits might allow us not to inhibit, but rather to modulate the ligand-induced cell response. In addition, selective “disconnection” of particular signaling subunits from their recognition partner would be invaluable in studies of MIRR-mediated cell activation.
In summary, despite the difference in details of the molecular mechanisms of action, the use of the suggested SR- and MIRR-targeted TM agents represents a general therapeutic strategy for disorders mediated by members of both receptor families: SRs and MIRRs. By revealing specific protein-protein interactions critically involved in receptor-mediated signaling, current mechanistic models of transmembrane signal transduction (Figs. 1B and 2B) not only provide molecular explanations for many biological phenomena and processes and powerful tools for fundamental and applied research but also suggest novel avenues for drug discovery.16,37,40,42,43 Selected receptor-related therapeutic applications are illustrated in Figures 1D and 2D.

**Applications in Biology and Medicine: Single-Chain Receptors**

Studies with the epidermal growth factor (EGF) and ErbB2 receptors have shown that synthetic peptides encompassing the TM domains of these receptors inhibit the autophosphorylation and signaling pathway of their cognate receptor.44,45 These peptides are thought to block/disrupt specific TM interactions, thereby inhibiting receptor dimerization and activation.44,45 Using differential epitope tagging, it has been demonstrated that β2-adrenergic receptors form homodimers and that TM domain VI of the receptor may represent part of an interface for receptor dimerization.46 As shown, a peptide derived from this domain inhibits both dimerization and β-adrenergic agonist-promoted stimulation of adenyl cyclase activity.46 In contrast, a peptide based on the sequence of transmembrane domain 6 of the D1 dopamine receptor (D1DR) has been found to specifically inhibit D1DR binding and function without affecting receptor oligomerization.47 One possible explanation for this finding is that in addition to ligand-stimulated dimerization of receptors, the correct (permissive) relative orientation in the receptor dimers formed can also play an important role in D1DR signaling. The importance of the relative orientation has been shown for other SRs such as, for example, EGF receptors,48 Epo receptor,49-52 toll-like receptors (TLRs)53 and the integral membrane receptor LuxPQ.54 Thus, the presence of the TM peptide bound to the D1DR TM domain is likely to prevent ligand-induced formation of receptor dimers with correct intermolecular orientation, thus preventing generation of the activation signal.

Another example of SR-targeted TM inhibitory peptides, the short peptide sequences corresponding to the Neu RTK TM domain, have been also reported to independently fold in membranes, interact with the full length receptor and inhibit transformation of cells in vitro and in vivo.55

Thus, the sequence-based blockade of the interreceptor TM protein interactions as applied to SR signaling provides evidence for the importance and clinical significance of the SR-related TM-targeted strategy.

**Applications in Biology and Medicine: Multi-Chain Activating Receptors**

The vast majority of multi-chain activating receptors are immune receptors that recognize foreign antigens and initiate a variety of biological responses.5 For this reason, the most commonly used name for this family is multi-chain immune recognition receptors (MIRRs), the term that was first introduced in 1992 by Keegan and Paul.3

**T-cell antigen receptor.** TCR provides an intriguing ability of T cells to discern and differentially respond to major histocompatibility complex (MHC)-bound peptides that can differ by only a single amino acid. Structurally, TCR is a member of the MIRR family with the α and β antigen-binding subunits that are bound by electrostatic transmembrane interactions with three signaling homo- and heterodimers: ζζ, CD3εδ and CD3ζγ (Fig. 3), thus maintaining the integrity of TCR in resting T cells.56,57 Within the SCHOOL model of TCR-mediated TM signal transduction (Fig. 3), distinct TCR signaling is achieved through ζ and CD3 signaling oligomers (Fig. 3),12,15,16 and intra-receptor transmembrane interactions represent not only promising therapeutic targets but also an important point of viral attack (Fig. 4).4,16,37,40,58

Transmembrane peptides capable of inhibiting TCR-mediated cell activation, including the TCR core peptide (CP), a synthetic peptide corresponding to the sequence of the TCRα TM domain and known to interact with the TM domains of CD3εδ and ζ,56,57 were first reported in 1997.59 Interestingly, T-cell activation via anti-CD3 antibodies is not affected by this peptide. As shown, TCR CP might be a proper treatment for human T-cell-mediated dermatoses substituting for corticosteroids60 and a novel potential therapy for rheumatoid arthritis and other T-cell-mediated disorders.59,61,62 However, despite extensive studies,61,62 the mode of action of this clinically relevant peptide has not been elucidated until 2004 when the SCHOOL model of TCR signaling (Fig. 3) was first introduced.12

Briefly, as suggested by the SCHOOL model (Fig. 4),4,12,15,37,38 TCR CP competes with the TCRα chain for binding to CD3εδ and ζ hetero- and homodimers, respectively, thus resulting in disconnection/pre-dissociation of the signaling subunits from the remaining receptor complex (Fig. 4). This leads to inhibition of antigen- but not antibody-mediated TCR triggering and cell activation (Fig. 4). Interestingly, the proposed mechanism is the only mechanism consistent with all experimental and clinical data reported up to date for TCR and other MIRR TM peptides and their lipid and/or sugar conjugates.39,60,62-72

The SCHOOL model predicted that the same mechanisms of inhibitory action can be applied to MIRR TM peptides corresponding to the TM regions of not only the MIRR recognition subunits but to the corresponding signaling subunits as well.12,15 This was recently confirmed experimentally by showing
that the synthetic peptides corresponding not only to the TM sequence of the antigen recognition TCR α subunit (i.e., TCR CP) but also to the sequences of the TM regions of the signaling CD3 (δ, ε or γ) and ζ subunits are able to inhibit the immune response in vivo (CD3 TM peptides) and NK-cell cytolytic activity in vivo (ζ TM peptide).

Interestingly, the model suggests a molecular explanation for the apparent discrepancy in CD3 TM peptide activity between

Figure 3. For figure legend, see page 261.
in vitro and in vivo T-cell inhibition (Fig. 5). It has been shown that the CD3δ and CD3γ TM peptides do not impact T cell function in vitro (the CD3ε TM peptide has not been used in the reported in vitro experiments because of solubility issues) but that all three CD3 TM peptides decrease signs of inflammation in the adjuvant-induced arthritis rat model in vivo and inhibit an immune response. Within the SCHOOL model, the CD3δ and CD3γ TM peptides disconnect the corresponding signaling subunits (CD3δ and CD3γ, respectively) from the remaining receptor complex (Fig. 5). Thus, these subunits do not participate in further signaling processes upon antigen stimulation. On the other hand, the previously reported in vitro activation studies with T cells lacking CD3γ and/or CD3δ cytoplasmic domains indicate that antigen-stimulated induction of cytokine secretion and T-cell proliferation are intact, thus explaining the absence of inhibitory effect of the CD3δ and CD3γ TM peptides in the in vitro activation assays used. However, in vivo deficiency either of CD3δ or CD3γ results in severe immunodeficiency disorders. This could explain the inhibitory effect observed in the in vivo studies for all three CD3δ, γ and ε TM peptides (Fig. 5). These experimental data confirm that the ability to selectively “disconnect” specific signaling subunits using the MIRR TM peptides in line with the SCHOOL model can provide a powerful tool to study MIRR functions and immune cell signaling as well as to rationally design novel inhibitors and/or modulators of the immune response.

Glycoprotein VI receptor. Activation of circulating platelets by exposed vessel wall collagen is a primary step in the pathogenesis of thrombotic diseases such as heart attack and stroke. However, despite intensive research efforts in antithrombotic drug discovery and development, uncontrolled hemorrhage still remains the most common side effect associated with antithrombotic drugs that are currently in use. The selective inhibition of GPVI, the central platelet collagen receptor on platelets, and/or its signaling may inhibit thrombosis without affecting hemostatic plug formation, thus representing a magic bullet for platelet-mediated diseases. However, the mechanism of GPVI signaling has remained unknown till recently, hindering the further development of this promising antithrombotic strategy.

The GPVI-FcγR receptor complex is formed by the association of recognition GPVI subunit with signal-transducing FcγR subunit that contains the ITAM sequence in its intrinsically disordered cytoplasmic domain. Thus, structurally, GPVI belongs to the MIRR family and the SCHOOL model could be and was applied to explain the mechanisms of GPVI-mediated signaling. This resulted in the development of novel mechanistic concept of platelet inhibition and the invention of new platelet inhibitors.

NKG2D receptor. Inflammatory bowel diseases (IBDs) affect millions of people worldwide with 2.8 million currently diagnosed with ulcerative colitis (UC) or Crohn’s disease (CD) in the United States alone. There is still a great need for additional targets and agents for effectively reducing IBDs, despite advances in immune disorder research. In 2007, a unique subset of CD4+ T cells expressing the activating NKG2D receptor has been identified in both the mucosa and peripheral blood of IBD patients, thus suggesting an important role of this receptor in the pathogenesis of IBDs. Later, in two independent studies, regulation of the NKG2D signaling pathway has been proven to represent a new therapeutic target for IBDs and be of key importance in successful treatment of UC and CD. However, further development of this promising strategy has been hindered by the lack of knowledge about the mechanism of NKG2D signaling.

As a member of the MIRR family, the NKG2D activating receptor consists of recognition NKG2D subunit non-covalently associated in transmembrane milieu with signal-transducing DAP10 subunit that contains the YxxM signaling sequence in its cytoplasmic domain predicted to be intrinsically disordered. This suggests that the NKG2D receptor complex signals through the SCHOOL model-based mechanisms and the...
What Viral Pathogenesis Teaches Us

To successfully infect, replicate and persist in the host, viruses have evolved numerous strategies to take control of multiple cellular processes including those that target TM signal transduction mediated by immune receptors. Example is the inhibition of T-cell activation and immunosuppressive activity observed for the fusion peptide (FP) found in the N terminus of the HIV envelope glycoprotein 41 (gp41). These data are the first to demonstrate that FP not only functions to fuse the virion with the host cell membrane but also has immunomodulatory activity. This peptide has been shown to inhibit antigen-specific T-cell activation. As with TCR-CP, FP shows specific: T-cell activation via PMA/ionomycin or mitogenic antibodies to CD3 is not affected by FP.

**What Viral Pathogenesis Teaches Us**

**Figure 4.** SCHOOL model of T-cell receptor signaling in the presence of transmembrane peptides. A schematic representation of the SCHOOL-based mechanisms of action of T-cell receptor transmembrane inhibitors such as the T-cell receptor core peptide (CP) and HIV-1 gp41 fusion peptide (FP). Within the model, these peptides compete with the TCRα chain for binding to the CD3ε and ζ signaling subunits, thus disrupting the transmembrane (TM) interactions between these subunits and resulting in disconnection and predissociation of the relevant signaling subunits from the remaining receptor complex (also shown in the inset as a simplified axial view). This prevents formation of signaling oligomers upon multivalent antigen stimulation, thus inhibiting antigen-mediated T-cell activation. In contrast, stimulation of these "predissociated" MIRRs with cross-linking antibodies to signaling subunit(s) should still lead to receptor triggering and cell activation. The model predicts that the same mechanisms of inhibitory action can be applied to TCR TM peptides corresponding to the TM regions of not only the TCRαβ recognition subunits but the corresponding CD3ε, CD3δ, CD3γ and ζ signaling subunits as well.
However, stimulation with anti-CD3 antibodies of these “pre-dissociated” TCRs still results in receptor triggering and cell activation (Fig. 4). More recent studies have confirmed the predicted mechanism of action of the HIV FP. Charge distribution patterns for fusion protein regions are surprisingly conserved in many unrelated viruses and show similarities to those for TCR CP and HIV FP (Fig. 6). Thus, it is highly probable that these proteins would also target the TCR TM interactions using the SCHOOL model. Exploratory sequence investigation of FPs from SARS-CoV, Lassa virus immunosuppressive activity, inhibiting the activation of arthritogenic T cells in the autoimmune disease model of adjuvant arthritis and reducing the disease-associated IFNγ response. Similarly to TCR CP, HIV gp41 FP has been suggested for the treatment of T-cell-mediated pathologies. However, the mode of action of this peptide has remained unexplained until 2006 when the SCHOOL model was first applied to this area. Within the SCHOOL model, HIV gp41 FP prevents formation of signaling oligomers and thus inhibits antigen-dependent T-cell activation (Fig. 4), acting similarly in this respect to TCR CP. However, stimulation with anti-CD3 antibodies of these “pre-dissociated” TCRs still results in receptor triggering and cell activation (Fig. 4). More recent studies have confirmed the predicted mechanism of action of the HIV FP.

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disrupting receptor architecture and suppressing the immune system. The generality of the SCHOOL model suggests that TM interactions of other MIRRs can also represent a point of viral attack. As reported,94 the recognition of the human CMV tegument protein pp65 by NKp30, the natural killer (NK) cell activating receptor, does not lead to NK-cell activation but instead results in a general inhibition mediated by the dissociation of the NKp30-ζ complex and loss in the ability of cells to kill virus-infected cells. Within the context of the SCHOOL model, pp65 may target the TM interactions between NKp30 and ζ, leading to functional disconnection of ζ in a manner similar to the action of TCR CP and HIV FP (Fig. 4).

TM interactions can be targeted not only from outside but also from inside the cell. Recently, it has been shown that the HHV-6 U24 protein downregulates TCR surface expression and that U24-expressing T cells are resistant to activation by antigen-presenting cells.95 TCR downregulation activity has been also reported for the highly conserved membrane-proximal sequence of the tyrosine kinase-interacting protein (Tip) of HVS.96,97 Notably, primary sequences of HHV-6 U24, 28-60 and HIV FP exhibit a similar pattern with two Arg residues spaced apart by eight amino acids (Fig. 6). The positioning of the essential electropositive residues is remarkably conserved in HVS Tip, LASV, Lassa virus; LCMV, lymphocytic choriomeningitis virus; MOPV, Mopeia virus; SARS-CoV, severe acute respiratory syndrome coronavirus; SEBOV, Sudan Ebola virus; TACV, Tacaribe virus; Tip, tyrosine kinase interacting protein; Tio, two-in-one protein; TMD, transmembrane domain; ZEBOV, Zaire Ebola virus.

Figure 6. Similarities in the charge distribution patterns of different immunomodulatory viral sequences. Charge distribution patterns of different immunomodulatory viral sequences. Primary sequence analysis of proven and predicted immunomodulatory sequences of viral fusion protein regions and other domains shows a similarity in charge distribution pattern with two essential positively charged residues spaced apart by 4 (class I) or 8 (class III) amino acids or with three essential positively charged residues spaced apart by 3 amino acids (class II), suggesting a similarity of the SCHOOL-based mechanisms used by diverse viruses in their pathogenesis to modulate the host immune response. Abbreviations: TCR, T-cell receptor; CP, core peptide; HIV, human immunodeficiency virus; gp, glycoprotein; FP, fusion peptide/protein; TMD, transmembrane domain; CKS-17, a synthetic retroviral envelope heptadecapeptide; Fr-MLV, Friend murine leukemia virus; gp, glycoprotein; HHV-6 U24, human herpesvirus 6 U24 protein; HTLV-1, human T lymphotropic virus type 1; HVA, herpesvirus ateles; HVS, herpesvirus saimiri; ITAM, immunoreceptor tyrosine-based activation motif; LASV, Lassa virus; LCMV, lymphocytic choriomeningitis virus; MARV, Marburg virus; MOPV, Mopeia virus; SARS-CoV, severe acute respiratory syndrome coronavirus; SEBOV, Sudan Ebola virus; TACV, Tacaribe virus; Tip, tyrosine kinase interacting protein; Tio, two-in-one protein; TMD, transmembrane domain; ZEBOV, Zaire Ebola virus.
relevant domain of the two-in-one (Tio) protein of herpesvirus ates (HVA), and HTLV-1 gp21 (Fig. 6). Thus, the SCHOOL mechanisms similar to those applied for TCR CP and HIV gp41 FP (Fig. 4) can be used by HHV-6 and other viruses in their arsenal of immune evasion tactics. Importantly, as predicted, the viral agents prevent only antigen- but not antibody-specific T cell activation (Fig. 4). Indeed, anti-CD3 antibodies activate HHV-6-infected T cells, resulting to great increase of viral replication. Interestingly, increase of viral replication induced by clinically relevant antibody (OKT3)-mediated activation of HIV-infected T cells is currently used for purging of the latent HIV-1 reservoirs in vivo, thus suggesting a potential generality of the SCHOOL mechanism-based antiviral approaches.

There are several important lessons that we can learn from the molecular mechanisms of viral pathogenesis:

(1) using modern methodologies, it is possible to design and produce TM agents that are able to modulate the immune response as specific and effective as viruses do;

(2) as predicted, TCR CP and many different immunomodulatory viral sequences affect similar TCR TM interactions, suggesting that general principles of designing TM peptides might be readily used at this stage;

(3) antibodies to MIRR signaling subunits can be used to modulate the affected immune cell response during viral infection;

(4) considering our selective ability to functionally disconnect any particular TCR signaling subunits, we can use the relevant peptides as a powerful tool to dissect fine mechanisms of viral pathogenesis; and, finally,

(5) two unrelated viruses, HIV and human CMV, use a similar mode of action to modulate the host immune response mediated by two functionally different MIRRs-TCR and NKP30, thus suggesting that similar general mechanisms can be or are used by other viral and possibly non-viral pathogens.

In conclusion, 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 addition, as demonstrated by the similarity of natural HIV FP and synthetically derived clinically relevant 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 that we can use in our own attempts at immune evasion or immunomodulation.

Conclusions

Considering growing interest in targeting cell surface receptor signaling as a potential treatment strategy for different diseases, the development of novel pharmacological approaches critically depends on our improved understanding of the molecular mechanisms underlying receptor triggering and subsequent transmembrane signal transduction. Novel general platform for receptor-mediated signaling suggests that receptor oligomerization induced or tuned upon ligand binding outside the cell is translated across the membrane into protein oligomerization inside the cell. Within this platform, homooligomerization of receptor intracellular signaling domains is considered as a necessary and sufficient condition for receptor triggering. This reveals inter- and intracellular transmembrane interactions as universal therapeutic targets for a diverse variety of seemingly unrelated immune (and not only immune) disorders, and, together with the lessons learned from viral pathogenesis, opens new horizons in the development of novel therapeutic strategies targeting receptor-mediated transmembrane signal transduction.

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