CHAPTER 20

SCHOOL Model and New Targeting Strategies

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Abstract

Protein-protein interactions play a central role in biological processes and thus are an appealing target for innovative drug design and development. They can be targeted by small molecule inhibitors, peptides and peptidomimetics, which represent an alternative to protein therapeutics that carry many disadvantages.

In this chapter, I describe specific protein-protein interactions suggested by a novel model of immune signaling, the Signaling Chain HOmoOLigomerization (SCHOOL) model, to be critical for cell activation mediated by multichain immune recognition receptors (MIRRs) expressed on different cells of the hematopoietic system. Unraveling a long-standing mystery of MIRR triggering and transmembrane signaling, the SCHOOL model reveals the intrareceptor transmembrane interactions and interreceptor cytoplasmic homointeractions as universal therapeutic targets for a diverse variety of disorders mediated by immune cells. Further, assuming that the general principles underlying MIRR-mediated transmembrane signaling mechanisms are similar, the SCHOOL model can be applied to any particular receptor of the MIRR family. Thus, an important application of the SCHOOL model is that global therapeutic strategies targeting key protein-protein interactions involved in MIRR triggering and transmembrane signal transduction may be used to treat a diverse set of immune-mediated diseases. This assumes that clinical knowledge and therapeutic strategies can be transferred between seemingly disparate disorders, such as T-cell-mediated skin diseases and platelet disorders, or combined to develop novel pharmacological approaches. Intriguingly, the SCHOOL model unravels the molecular mechanisms underlying ability of different human viruses such as human immunodeficiency virus, cytomegalovirus and severe acute respiratory syndrome coronavirus to modulate and/or escape the host immune response. It also demonstrates how the lessons learned from viral pathogenesis can be used practically for rational drug design.

Application of this model to platelet collagen receptor signaling has already led to the development of a novel concept of platelet inhibition and the invention of new platelet inhibitors, thus proving the suggested hypothesis and highlighting the importance and broad perspectives of the SCHOOL model in the development of new targeting strategies.

Introduction

Specific protein-protein interactions are responsible for the function of numerous processes in the cell and constitute the foundation for the majority of cell recognition, proliferation, growth,

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differentiation, programmed cell death and signal transduction in health and disease.[1-4] It seems that almost every important pathway includes and is critically influenced by protein-protein interactions.[1] Because of the ubiquitous nature of these 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. A number of recent reviews have addressed this topic.[5-12] Thus, revealing information about specific protein-protein interactions in any particular pathway (i.e., transmembrane signaling) can provide targets for a generation of new drugs.

Long-Standing Mystery of MIRR Triggering and Transmembrane Signaling

Multichain immune recognition receptors (MIRRs) recognize foreign antigens and initiate a variety of biological responses. Examples of MIRRs include the T-cell receptor (TCR) complex, the B-cell receptor (BCR) complex, Fc receptors (e.g., FcεRI, FcαRI, FcγRI and 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 (TREMs) and the platelet collagen receptor, glycoprotein VI (GPVI). MIRR-mediated transmembrane (TM) signal transduction plays an important role in health and disease[13-21] making these receptors attractive targets for rational intervention in a variety of immune disorders. Thus, future therapeutic strategies depend on our detailed understanding of the molecular mechanisms underlying the MIRR triggering and subsequent TM signal transduction.

All members of the MIRR family are multisubunit complexes formed by the association of recognition subunits with signal-transducing subunits that contain in their cytoplasmic (CYTO) domains the immunoreceptor tyrosine-based activation motif (ITAM) or the YxxM motif, found in the DAP-10 CYTO domain (see Chapter 12). This association in resting cells is mostly driven by the noncovalent TM interactions between recognition and signaling components and plays a key role in receptor assembly and integrity (see also Chapters 1-5).[18,21-26] Crosslinking of the receptors after ligand binding results in phosphorylation of the ITAM/YxxM tyrosines, which triggers the elaborate intracellular signaling cascade. The extracellular (EC) recognition of an antigen/ligand and the sequence of biochemical events that ensues after the phosphorylation of ITAMs/YxxM are understood in significant detail. However, the molecular mechanism linking EC antigen/ligand-induced clustering of MIRR ligand-binding subunits to intracellular phosphorylation of signaling subunits has been a long-standing unsolved mystery. It was also unknown how this putative mechanism can explain the intriguing ability of immune cells to discern and differentially respond to slightly different ligands. This impeded our advance understanding of the immune response, the development of novel pharmacological approaches and even more important, the potential transfer of clinical knowledge, experience and therapeutic strategies between seemingly disparate immune disorders.

Despite numerous models of MIRR-mediated TM signal transduction suggested for particular MIRRs (e.g., TCR, BCR, FcRs, NK receptors, etc.), no current model fully explains how ligand-induced TM signal transduction commences at the molecular level. As a consequence, these models are mostly descriptive and do not reveal clinically important potential points of therapeutic intervention. In addition, no general model of MIRR-mediated immune cell activation has been suggested, thus preventing the potential transfer of therapeutic strategies between seemingly disparate immune disorders.

A recently developed novel mechanistic model, the SCHOOL model,[27-30] describes the crucial protein-protein interactions underlying the molecular mechanism of MIRR triggering and TM signaling (Fig. 1, see also Chapter 12). In this chapter, I describe these specific interactions as new

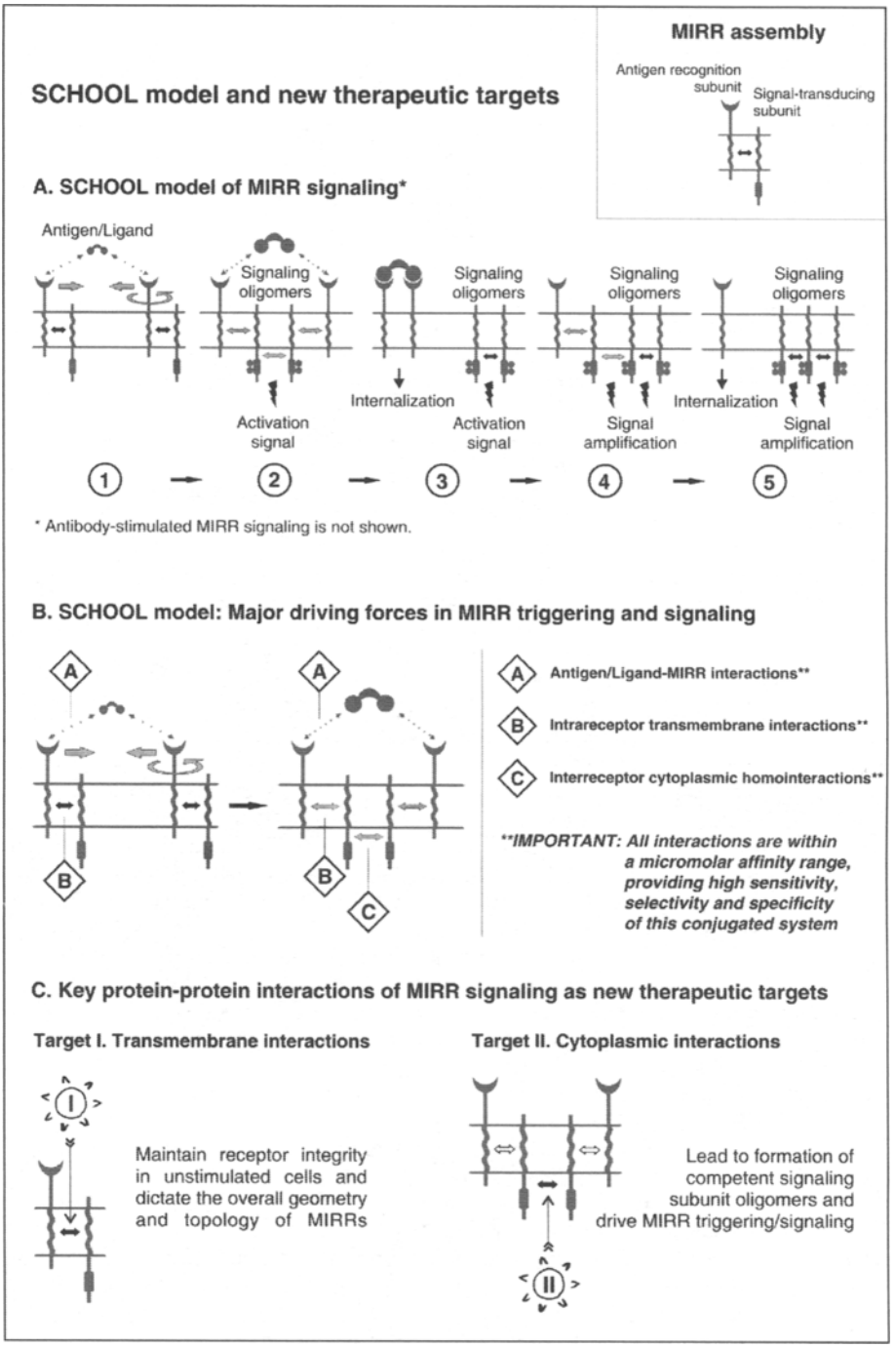

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Figure 1, viewed on previous page. Structural assembly of MIRRs (the inset), the signaling chain homooligomerization (SCHOOL) model of MIRR signaling (A,B) and new therapeutic targets revealed by the model (C). The model proposes that formation of competent MIRR signaling subunit oligomers driven by the homooligomerization of signaling subunits is necessary and sufficient to trigger the receptors and induce transmembrane (TM) signal transduction and downstream sequence (see Chapter 12 for detail). All interchain interactions in this intermediate are shown by light gray arrows reflecting their transition state. Immunoreceptor tyrosine-based activation motifs (ITAMs) are shown as gray rectangles. Circular arrow indicates ligand-induced receptor reorientation. Phosphate groups are shown as gray circles. Small solid black arrows indicate specific intersubunit hetero- and homointeractions between TM and cytoplasmic (CYTO) domains, respectively. Within the model, MIRR triggering and signaling is an outcome of the ligand-induced interplay between three key protein-protein interactions: antigen/ligand-MIRR interactions, intrareceptor TM interactions and interreceptor CYTO homointeractions (B). Two of these interactions can be considered as new therapeutic targets (C): 1) TM interactions between MIRR antigen-recognizing and signal-transducing subunits (target I) that play an important role in receptor assembly and integrity on resting cells; and 2) CYTO homointeractions between MIRR signaling subunits (target II) that represent a main driving force of MIRR triggering/signaling.

therapeutic targets revealed by the model for the treatment of diverse immune and other disorders mediated by MIRRs. Assuming that the similar structural architecture of the MIRRs dictates similar mechanisms of MIRR triggering and subsequent TM signal transduction, the model suggests that these targets are similar in seemingly unrelated diseases. This builds the structural basis for the development of novel pharmacological approaches as well as the transfer of clinical knowledge, experience and therapeutic strategies between various immune disorders. In addition, it significantly improves our understanding of the immunomodulatory activity of human viruses such as human immunodeficiency virus (HIV), human cytomegalovirus (CMV) and severe acute respiratory syndrome coronavirus (SARS-CoV), human T-cell leukemia type 1 virus (HTLV-1) and assumes that the lessons learned from viral pathogenesis can be used for the development of new therapeutic approaches. An important application of this hypothesis is that a general pharmaceutical approach may be used to treat diverse immune-mediated diseases.

SCHOOL Model of MIRR Triggering and Signaling: Basic Concept, Major Driving Forces, Restraints and Advantages

Basic Concept

Recently, a novel biophysical phenomenon, the homointeractions of intrinsically disordered CYTO domains of ITAM-containing MIRR signaling subunits, has been discovered.[31] It demonstrates that intrinsically disordered proteins do not necessarily undergo a transition between disordered and ordered states upon interaction,[32,33] a finding that opposes the generally accepted view on the behavior of natively unfolded proteins. Interestingly, this homooligomerization is best described by a two-step monomer-dimer-tetramer fast dynamic equilibrium with dissociation constants in the micromolar affinity range.[31,33] The overall binding affinity between proteins is known to depend on the function of the protein complex. For example, obligate homodimers have been reported to associate strongly with nano- or picomolar binding affinity[34] while, in contrast, proteins that associate and dissociate in response to changes in their environment, such as the majority of signal transduction mediators, tend to bind more weakly. In this context, micromolar binding affinities, in combination with a rapid association and dissociation kinetics,[31] make the homotypic CYTO interactions between MIRR signaling subunits a valid candidate for involvement in MIRR-mediated signal transduction.

Hypothesizing a crucial physiological role of these unique homointeractions, the SCHOOL model suggests that formation of competent MIRR signaling subunit oligomers is necessary and sufficient to trigger the receptors and induce TM signal transduction and the downstream signaling sequence (Fig. 1A, see also Chapter 12).[27-29] Within the model, MIRR engagement by multivalent

antigen or anti-MIRR antibodies (e.g., anti-CD3ε and anti-TCRβ for TCR or anti-Igβ antibodies for BCR) leads to receptor clustering coupled with a multi-step structural reorganization driven by the homooligomerization of MIRR signaling subunits (Fig. 1A). Ligand-induced MIRR clustering leads to receptor reorientation and formation of a dimeric/oligomeric intermediate in which signaling chains from different receptor units start to trans-homointeract and form signaling oligomers (Fig. 1A, stages 1 and 2). Upon formation of signaling oligomers, protein tyrosine kinases phosphorylate the tyrosine residues in the ITAMs located on the CYTO tails of MIRR signaling subunits, leading to the generation of intracellular activation signal(s), dissociation of signaling oligomers and internalization of the engaged MIRR ligand-binding subunits (Fig. 1A, stages 2 and 3). Then, signaling oligomers interact with the signaling subunits of nonengaged receptors resulting in formation of higher-order signaling oligomers, thus propagating and amplifying the activation signal and resulting in internalization of the non-engaged MIRR recognition subunits (Fig. 1A, stages 4 and 5).

Major Driving Forces

Introducing the homotypic interactions between MIRR signaling subunits as one of the key interactions involved in MIRR triggering and TM signaling, the plausible and easily testable SCHOOL model defines this process as an outcome of the interplay between three major driving forces (Table 1, Fig. 1B):

1) Antigen/ligand-MIRR interactions. These interactions cluster two or more MIRRs in sufficient proximity and correct (permissive) relative orientation to initiate homointeractions between particular MIRR signaling subunits.
2) Intrareceptor TM interactions. These interactions stabilize and maintain receptor integrity in resting cells and balance opposing interactions, the interreceptor CYTO homointeractions, in stimulated cells, thus helping to discriminate ligands/antigens in their functional ability to trigger MIRRs and induce a cellular activation signal.
3) Interreceptor homointeractions. These interactions between the CYTO domains of MIRR signaling subunits lead to the formation of oligomeric signaling structures, thus triggering phosphorylation of ITAMs and initiating the signaling cascade.

Thus, the SCHOOL model reveals the last two key interactions of MIRR triggering/signaling as new therapeutic targets (Fig. 1C).

Antigen/ligand-MIRR interactions are generally of low affinity (micromolar range) and have rapid association and dissociation kinetics (reviewed, for example, for TCR in 35). This low affinity binding in combination with fast kinetics allows immune cells to recognize and discriminate a variety of antigens/ligands with high specificity, selectivity and sensitivity in order to respond with a variety of biological responses. Considering that EC and TM regions of MIRRs are well-ordered receptor segments while MIRR signaling CYTO domains have been recently shown to represent a novel class of intrinsically disordered proteins,[31-33] an important and intriguing question is raised: how do MIRRs transduce highly ordered information about antigen recognition/discrimination from outside the cell through the cell membrane into intracellular biochemical events, thus triggering specific pathways and resulting in a specific functional outcome?

Despite intensive studies of MIRR-mediated TM signal transduction, the only model that can answer this question and even more important, mechanistically explain how this signaling starts, is the SCHOOL model (see also Chapter 12).[27-29] Intriguingly, all three protein-protein interactions, namely antigen/ligand-MIRR EC interactions as well as intrareceptor TM heterointeractions and interreceptor CYTO homointeractions (Fig. 1B, Table 1), fall within the similar micromolar affinity range and are characterized by relatively rapid kinetics.[31,35-41] This conjugated and well-balanced system of interprotein interactions provides the ideal basis to explain the molecular mechanisms of the ability of MIRRs to transduce the extracellular information about recognition of different ligands/antigens through the cell membrane and translate it into different activation signals, thus triggering different intracellular pathways and resulting in different cell responses. Within the model, the MIRR-generated intracellular activation signals are combinatorial in nature and

Table 1. Major driving forces in MIRR triggering and transmembrane signaling as revealed by the SCHOOL model

Protein-Protein Interactions	Interaction Milieu	Role in MIRR Triggering/Signaling	Affinity Range
Between antigen/ligand and MIRR recognition subunit(s)	EC	Cluster MIRRs in sufficient interreceptor proximity and correct (permissive) orientation relative to each other to promote the interreceptor CYTO homointeractions between MIRR signaling subunits, resulting in formation of competent signaling oligomers and thus initiating the downstream signaling cascade	μM
Between MIRR recognition and MIRR signaling subunits*	TM	Define the overall rigid geometry and topology of the MIRR. Maintain the integrity of a functional receptor in resting cells. Balance opposing interactions, the CYTO homointeractions, thus helping to discriminate ligands/antigens in their functional ability to cluster MIRRs in sufficient interreceptor proximity and correct (permissive) orientation relative to each other to promote formation of competent signaling subunit oligomers	μM
Homointeractions between MIRR signaling subunit(s)*	CYTO	Lead to formation of competent signaling subunit oligomers, thus initiating the downstream signaling cascade	μM

*Within the SCHOOL model, these TM and CYTO interactions represent the opposing forces that balance resting and differently triggered patterns of MIRR receptor triggering and signaling. Abbreviations: CYTO, cytoplasmic; EC, extracellular; MIRR, multichain immune recognition receptor; SCHOOL model, signaling chain homooligomerization model; TM, transmembrane.

involve multiple components such as formation of different competent MIRR signaling subunit oligomers (see also Chapter 12)[27-30] and different ITAM Tyr phosphorylation patterns.[42-54] This system also explains mechanistically high specificity, selectivity and sensitivity of immune cells in recognition and discrimination of different antigens/ligands and how this recognition/discrimination results in different functional outcomes. This is particularly important for the TCR[55] that has four different signaling subunits, namely ζ and CD3ε, CD3δ and CD3γ, known to play different roles in T-cell biology (see Chapters 1 and 12). In addition, in contrast to other MIRR signaling subunits, ζ has three ITAMs that can provide differential tyrosine phosphorylation patterns in response to different ligands, initiating different intracellular signaling pathways. Thus, within the model, TCR-mediated signaling and cell activation has the highest combinatorial potential as compared to other MIRRs, explaining a high variability of distinct TCR-triggered intracellular signaling pathways and therefore distinct T-cell functional responses depending on the nature of the stimulus (see also Chapter 12).[27-30]

Restraints

Interactions between TM helices of recognition and signaling MIRR subunits maintain receptor integrity in unstimulated cells and determine the relative positions of these subunits in the recep-

tor complex (angles, distances, etc.), thus dictating the overall geometry and topology of MIRRs. Within the SCHOOL model, the overall structural architecture (i.e., geometry and topology) of MIRRs that is dictated and maintained by TM interactions between MIRR recognition and signaling subunits (Fig. 1, see also Chapter 12),[27-30] in combination with the requirement to initiate interreceptor homointeractions between MIRR signaling subunits (Fig. 1), impose several restraints for multivalent antigen/ligand-induced MIRR triggering (Table 2, see also Chapter 12):[27-30]

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

The importance of these factors for productive MIRR triggering and TM signaling is strongly supported by a growing body of evidence and described in detail in Chapter 12 of this book. Briefly, it should be noted that the restraints imposed by the model play an especially important role during the first stage of MIRR triggering (Fig. 1). At this point, these spatial, structural and temporal requirements (correct relative orientation, sufficient proximity, long enough duration of the MIRR-ligand interaction and lifetime of MIRR dimers/oligomers) should be fulfilled to favor initiation of trans-homointeractions between MIRR signaling subunits and formation of competent signaling subunit oligomers. If these requirements are not fulfilled at this "final decision-making" point, the formed MIRR dimers/oligomers may dissociate from the ligand and remain signaling-incompetent and/or break apart to its initial monomeric receptor complexes. Also, at this stage, slightly different ligands may bring two or more MIRRs in different relative orientations that favor homointeractions between different signaling subunits and result in formation of different signaling oligomers or their combinations, thus initiating distinct signaling pathways. This mechanism can explain the ability of MIRRs to differentially activate a variety of signaling pathways depending on the nature of the stimulus.

Advantages

The SCHOOL model is fundamentally different from those numerous models that have been previously suggested for particular MIRRs and has several important advantages (see also Chapter 12):[27-30]

- This is the first general mechanistic model for all MIRRs known to date, including TCR, BCR, Fc receptors, NK receptors, ILTs, LIRs, SIRPs, DCAR, MDL-1, NITR, TREMs, GPVI and others and for those that will be discovered in the future. Assuming the general principles underlying MIRR triggering and TM signaling mechanisms are similar for all MIRRs, the SCHOOL model can easily be applied to any particular receptor of the MIRR family,
- This is the first model that is based on specific protein-protein interactions—biochemical processes that can be influenced and controlled[2,10-12,56]—and specific 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.[1,3-8,12]
- Introducing the CYTO homointeractions between MIRR signaling subunits as one of the key elements of MIRR triggering and signaling, the SCHOOL model imposes functionally important restraints (Table 2, see also Chapter 12) and suggests molecular mechanisms for the vast majority of unexplained immunological observations accumulated to date (see also Chapter 12).[27-30]
- Unraveling the molecular mechanisms underlying MIRR triggering and subsequent TM signaling, the model suggests unique and powerful tools to study the immune response and a means to control and/or modulate it (see also Chapter 12).[27-30,57]

Table 2. Selected main restraints for MIRR signaling imposed within the SCHOOL model by the overall structural architecture and topology of MIRRs in combination with the major driving forces in MIRR triggering and transmembrane signaling

Restraints	Functional Significance
Sufficient interreceptor proximity in MIRR dimers/oligomers	Two or more antigen/ligand-clustered MIRRs should be in sufficient proximity to each other to initiate CYTO homointeractions between signaling subunits with subsequent formation of competent signaling subunit oligomers
Correct (permissive) relative orientation of the receptors in MIRR dimers/oligomers	Within two or more antigen/ligand-clustered MIRRs, particular MIRR signaling subunit(s) should be in correct orientation relative to each other to initiate CYTO homointeractions between these signaling subunits with subsequent formation of competent signaling subunit oligomers
Long enough duration of the MIRR-ligand interaction that generally correlates with the strength (affinity/avidity) of the ligand	Main protein-protein interactions involved in MIRR triggering and TM signaling (Table 1) fall into a similar low/moderate (micromolar) affinity range. For this reason, the multivalent antigen/ligand-receptor contact should last long enough to bring two or more MIRRs in sufficient proximity and correct relative orientation toward each other and hold them together to promote the interreceptor CYTO homointeractions between MIRR signaling subunits, resulting in formation of competent signaling subunit oligomers and thus initiating the downstream signaling cascade
Sufficient lifetime of an individual receptor in MIRR dimers/oligomers	Similarly to a restraint on duration of antigen/ligand-MIRR contact, in order to initiate the downstream signaling cascade, a lifetime of an individual receptor in antigen/ligand-clustered MIRRs should be sufficient to promote the interreceptor CYTO homointeractions between MIRR signaling subunits

Abbreviations: CYTO, cytoplasmic; MIRR, multichain immune recognition receptor; SCHOOL model, signaling chain homooligomerization model; TM, transmembrane.

- Based on specific protein-protein interactions, the SCHOOL model reveals new therapeutic targets (Fig. 1) for the treatment of a variety of disorders mediated by immune cells.[27-30,57,58]
- An important application of the SCHOOL model is that similar therapeutic strategies targeting key protein-protein interactions involved in MIRR triggering and TM signal transduction may be used to treat diverse immune-mediated diseases. This assumes that clinical knowledge, experience and therapeutic strategies can be transferred between seemingly disparate immune disorders or used to develop novel pharmacological approaches and that a general pharmaceutical approach may be used to treat diverse immune disorders.

SCHOOL Model: New Intervention Points for MIRR-Mediated Immune Disorders

As mentioned previously (Table 1, Fig. 1B), the SCHOOL model defines MIRR triggering and subsequent TM signaling as an outcome of the interplay between three crucially important interactions (Table 1, Fig. 1B): (1) antigen/ligand-MIRR EC interactions, (2) intrareceptor TM interactions and (3) interreceptor CYTO homointeractions. 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 (see also Chapters 15, 17-19),[59-73] the last two protein-protein interactions that are critically involved in MIRR triggering/signaling, represent promising novel therapeutic targets as revealed by the model (Fig. 1C).[27-30,57,58] As suggested by the model, controlled inhibition/modulation of these particular interactions represents a means to inhibit/modulate MIRR-mediated TM signaling and specific downstream signaling pathways, thus inhibiting/modulating the immune response. This can be used in rational drug design and the development of novel strategies for the treatment of a variety of diseases and medical conditions that involve MIRR-mediated signaling. Importantly, unraveling the molecular basis of MIRR triggering and signaling and revealing specific protein-protein interactions that play a critical role in MIRR-mediated TM signal transduction and cell activation, the SCHOOL model suggests invaluable and unique powerful tools to dissect mechanisms of the related cell functional outcomes in response to antigen/ligand and to study many important aspects of viral pathogenesis (see also Chapter 22).[27-30,57,58]

In this Chapter, I demonstrate how the SCHOOL model, together with the lessons learned from viral pathogenesis, can be used practically for rational drug design and the development of new therapeutic approaches to treat a variety of seemingly unrelated disorders, such as T-cell-mediated skin diseases and platelet disorders.

Transmembrane Interactions as Immunotherapeutic Targets

Main Concept

Since it was first published in 2004,[28] the SCHOOL model has revealed intra-MIRR TM interactions as important therapeutic targets as well as points of great interest to study the molecular mechanisms underlying the MIRR-mediated cell response in health and disease (Figs. 1 and 2).[27-30,57,58] Notably, the model has provided a mechanistic explanation at the molecular level for specific processes behind "outside-in" MIRR signaling that were unclear (see also Chapter 12).[27-30,57,58] Examples include molecular mechanisms of action of the therapeutically important TCR TM peptides[74-77] first introduced by Manolios et al in 1997[78] and the mechanism underlying HIV-1 fusion peptide (FP)-induced inhibition of antigen-dependent T-cell activation.[79] The relevance of the latter mechanism has since been confirmed experimentally.[80]

Within the SCHOOL model, upon antigen/ligand stimulation, the intra-MIRR TM interactions balance opposing interactions, the inter-MIRR CYTO homointeractions and represent one of three major driving forces of MIRR triggering that helps to discriminate ligands/antigens in their functional ability to trigger MIRRs and induce a cellular activation signal (Table 1, Fig.

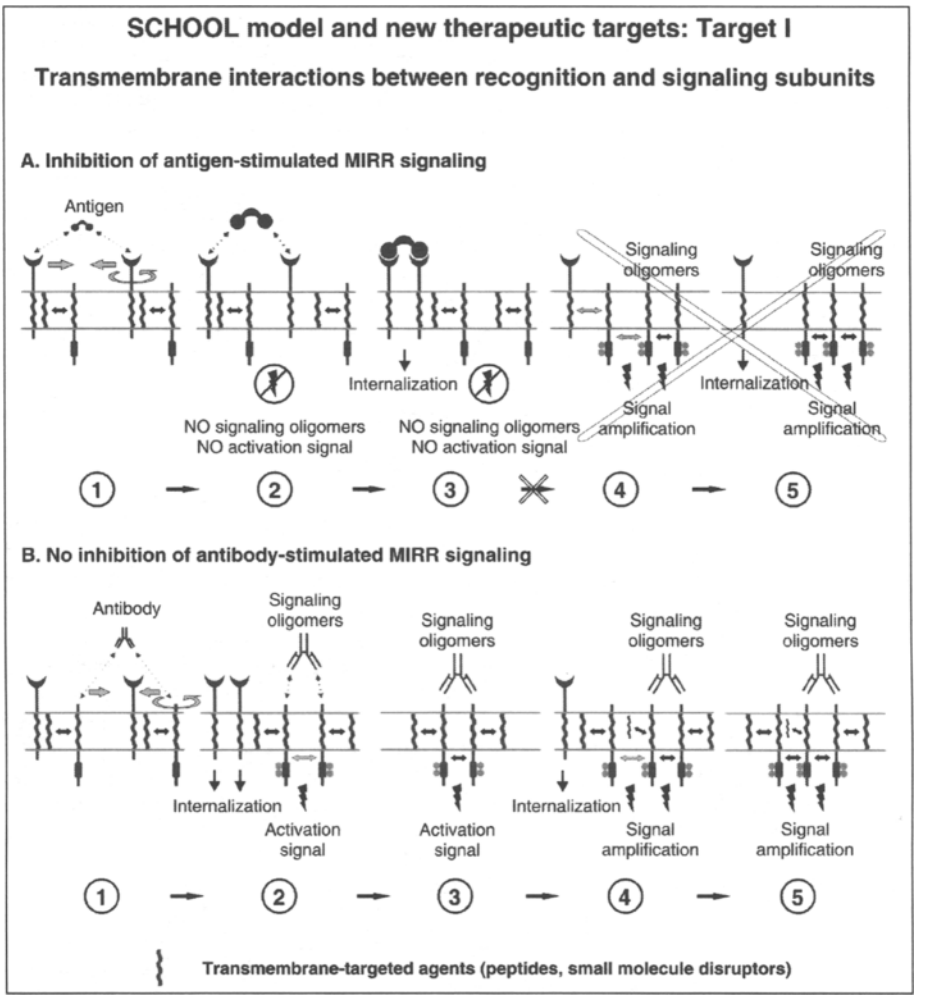

Figure 2. Target I. Transmembrane interactions between MIRR recognition and signaling subunits. This is a simplified graphical illustration of the molecular mechanisms underlying proposed intervention by transmembrane-targeted agents (peptides and their derivatives, small molecule disruptors of protein-protein interactions, etc). Within the SCHOOL model, specific blockade of transmembrane interactions between recognition and signaling subunits is proposed to result in "predissociation" of the receptor complex, thus preventing formation of competent signaling oligomers and inhibiting antigen-dependent immune cell activation (A). In contrast, stimulation of these "predissociated" MIRRs with cross-linking antibodies to signaling subunit should not effect, according to the model, on receptor triggering and cell activation (B). It is noteworthy that the proposed strategies can be used not only to inhibit but also to modulate MIRR-mediated transmembrane signal transduction, thus modulating the immune response (see main text for details). Abbreviations and symbols as in Figure 1. Reprinte from Trends Pharmacol Sci, 27, Sigalov AB, Immune cell signaling: a novel mechanistic model reveals new therapeutic targets, 518-524, copyright 2006 with permission from Elsevier.

1B). As suggested by the model (Figs. 1C and 2),[27-30,57,58] specific blockade or disruption of the TM interactions between MIRR recognition and signaling subunits causes a physical and functional disconnection of the subunits. 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. It should be noted that in this context, a physical disconnection means "predissociation" rather than full dissociation of the subunits because in the absence of stimulus, they can still remain together. Antigen/ligand stimulation of these "predissociated" 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. 2A). In contrast, this "predissociation" does not prevent the formation of signaling oligomers when signaling subunits are clustered by specific antibodies that trigger cell activation, e.g., anti-MIRR signaling antibodies (Fig. 2B) such as anti-CD3 for TCR and anti-Igβ antibodies for BCR, or anti-TCRβ antibodies for TCR (not illustrated).

Our current understanding of the MIRR structure and the nature and specificity of TM interactions between receptor recognition and signaling subunits not only allows us to block or disrupt these protein-protein interactions but also to 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 functional disconnection of particular signaling subunits from their recognition partner represents an invaluable tool in studies of MIRR-mediated TM signaling and cell activation. It should also be noted that methods of computational design, synthesis and optimization of TM peptides and peptidomimetics, as well as high-throughput screening techniques to search for the relevant TM mutations or small molecule disruptors, are currently developed and well-established,[1-11,34,76,81-90] making the proposed powerful approach both feasible and of great fundamental and clinical value.

I suggest that the TM interactions between recognition and signaling MIRR subunits represent extremely important points of control in MIRR triggering and cell activation. Since we can now use the SCHOOL model to design the TM-targeted agents effective in inhibition and/or modulation of MIRR-mediated TM signaling (Figs. 1 and 2, see also Chapter 12),[27-30,57,58] we have a powerful and well-controlled influence upon MIRR-mediated cell activation and control the immune response. The relevant TM-targeted agents for any particular member of MIRR family can be readily designed using the SCHOOL model and our knowledge about structural organization of this receptor. Examples include the TM peptides of TCR,[74-76,78,81] NK receptors[91] and GPVI[58] tested to inhibit/modulate the relevant receptor-mediated cell response. Importantly, the SCHOOL model unravels the TM-targeted molecular mechanisms underlying ability of different human viruses such as human immunodeficiency virus, cytomegalovirus and severe acute respiratory syndrome coronavirus to modulate and/or escape the host immune response.[29,30,57] It also demonstrates how the lessons learned from viral pathogenesis can be used practically for rational drug design.[30,58] These and other examples that successfully prove the main concept of the SCHOOL model-driven TM strategy are considered in detail below.

Obviously, allowing us to effectively control MIRR signaling and therefore the immune response, the MIRR intrareceptor TM interactions represent an important target of pharmacological intervention as first revealed and suggested by the SCHOOL model in 2004.[28] It further assumes that a general therapeutic strategy aiming to disrupt/modulate these interactions in the MIRRs may be used in the existing and future treatment of seemingly unrelated immune diseases. In other words, according to the main concept of the SCHOOL model, specific therapeutic agent(s) that target particular MIRR(s) involved in pathogenesis of the relevant immune disorder can be readily designed using basic principles of structural assembly of this receptor and the SCHOOL model as applied to this particular member of MIRR family.

Table 3. Selected agents reported to modulate the immune cell response and suggested or predicted by the SCHOOL model to affect MIRR transmembrane interactions

Agent	MIRR	Action	Mechanism as Suggested by the SCHOOL Model	Potential Clinical Use
TCR CP	TCR	Selectively inhibits antigen-stimulated TM signal transduction[75,76,78,81] Efficiently abrogates T-cell-mediated immune responses in mice and man in vitro and in vivo[74,77]	Disrupts TCRα-CD3δε and TCRα-ζ TM interactions resulting in disconnection/predissociation of these signaling subunits from the remaining complex and thus preventing the formation of signaling oligomers upon antigen stimulation and, consequently, inhibiting T-cell activation (Figs. 2 and 3, see also Chapter 12)[27-30]	T-cell-mediated immune disorders: dermatoses, arthritis, etc. Anti-tumor therapy
CD3δ-CP CD3ε-CP CD3γ-CP	TCR	CD3δ-CP and CD3γ-CP do not inhibit antigen-stimulated T-cell proliferation and IL-2 secretion.[77]CD3δ-CP, CD3ε-CP and CD3γ-CP prevent disease development and progression in rats with adjuvant-induced arthritis[77]	Disrupt TCRα-CD3δ (CD3δ-CP), TCRα-CD3ε (CD3ε-CP), TCRβ-CD3ε (CD3ε-CP) and TCRβ-CD3γ (CD3γ-CP) TM interactions, resulting in selective disconnection/ predissociation of the particular signaling subunits from the remaining receptor complex, thus modulating the cell response[27-30]	T-cell-mediated immune disorders: dermatoses, arthritis, etc. Anti-tumor therapy
NK-CP ζ-CP	NKp44 NKp46 NKp30 NKG2D NKG2C KIR2DS	Inhibit NK cell cytolytic activity[91]	Disrupt the TM interactions between NK receptor ligand-binding subunits and associated homodimeric signaling subunits, such as ζ–ζ, γ–γ or DAP-12 (Fig. 2)[27-30]	NK cell-mediated diseases
NK-CP	NKG2D	Predicted to inhibit NKG2D signaling pathway critically involved in CD4+ T-cell-mediated colitis progression*	Predicted to disrupt the TM interactions between NK2D receptor ligand-binding subunits and associated homodimeric DAP-10 signaling subunit (Fig. 2)[27-30]	Inflammatory bowel diseases[210]
GPVI-CP	Platelet GPVI	Inhibits collagen-induced platelet activation and aggregation[58]	Disrupts the TM interactions between collagen-binding GPVI subunit and associated homodimeric γ-chain (Fig. 4)[58,108]	Platelet-mediated diseases and conditions

*To be proved in the future.

Abbreviations: CP, core peptide; DAP-12, DNAX activation protein 12; GPVI, glycoprotein VI; MIRR, multichain immune recognition receptor; NK cells, natural killer cells; TCR, T-cell antigen receptor; TM, transmembrane.

An exciting and promising example of using the SCHOOL model-driven TM approach for both fundamental and clinical applications has been recently demonstrated by Collier et al,[77] as covered in more detail below.

Direct and Indirect Evidence: Transmembrane Peptides and Immune Cell Activation

Direct Evidence

The SCHOOL model is the first model to clearly explain molecular mechanisms of action of TCR TM peptides (see also Chapter 16) and extend the concept of their action through these mechanisms to any other TM peptides of MIRRs and to the MIRR-mediated processes involved in viral pathogenesis.[27-30,57,58] Selected agents suggested or predicted by the SCHOOL model to affect MIRR TM interactions, thus inhibiting or modulating MIRR-mediated immune cell activation, are listed in Table 3.

TM peptides capable of inhibiting MIRR-mediated cell activation were first reported in 1997 for antigen-stimulated TCR-mediated T-cell activation by Manolios et al.[78] Since that time, despite extensive basic and clinical studies of these and several other TM peptides (see also Chapter 16),[74,75,77,92-101] the molecular mechanisms of action of these clinically relevant peptides have not been elucidated until 2004 when the SCHOOL model was first introduced.[28]

The vast majority of basic and clinical findings were reported for the TCR TM core peptide (TCR CP), or TCR mimic peptide, which represents a synthetic peptide corresponding to the sequence of the TM region of the ligand-binding TCRα chain critical for TCR assembly and function. This TM region has been shown to interact with the TM domains of the signaling CD3δε and ζ subunits,[22,23] thus maintaining the integrity of the TCR in resting T-cells.

Briefly, as suggested by the SCHOOL model (Figs. 2 and 3, Table 3, see also Chapter 12),[27-30,57] the TCR CP competes with the TCRα chain for binding to CD3δε and ζ hetero- and homodimers, respectively, thus resulting in disconnection/predissociation of the signaling subunits from the remaining receptor complex (Fig. 3). This leads to inhibition of antigen- but not antibody-mediated TCR triggering and cell activation (Figs. 2 and 3). It should be highlighted that 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.[58,74,75,77,92-100]

Recently, new experimental evidence supporting the proposed mechanism of inhibitory action of TCRα CP has been reported.[100] This study has clearly shown that this peptide does not affect TCR assembly and cell surface expression.[100] Most strikingly, Kurosaka et al[100] have demonstrated that TCRα CP coprecipitates with CD3δε. This finding perfectly fits the molecular explanation of its inhibitory action suggested for the first time in 2004 by applying the SCHOOL model[28] and later developed further.[27-30,57] Again, within the model, competing with the TCRα chain, TCRα CP binds to CD3δε and ζ signaling subunits, preventing an antigen-induced formation of the relevant competent signaling oligomers and thus inhibiting an antigen-dependent T-cell response (Figs. 2 and 3, Table 3, see also Chapter 12).

The SCHOOL model predicts 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.[27-30] This was recently confirmed experimentally[77,91] by showing that the synthetic peptides corresponding 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) (Table 3).

Interestingly, the model suggests a molecular explanation for the intriguing phenomenon recently reported by Collier et al[77] and interpreted by the authors as a discrepancy in CD3 TM peptide activity between in vitro and in vivo T-cell inhibition. 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

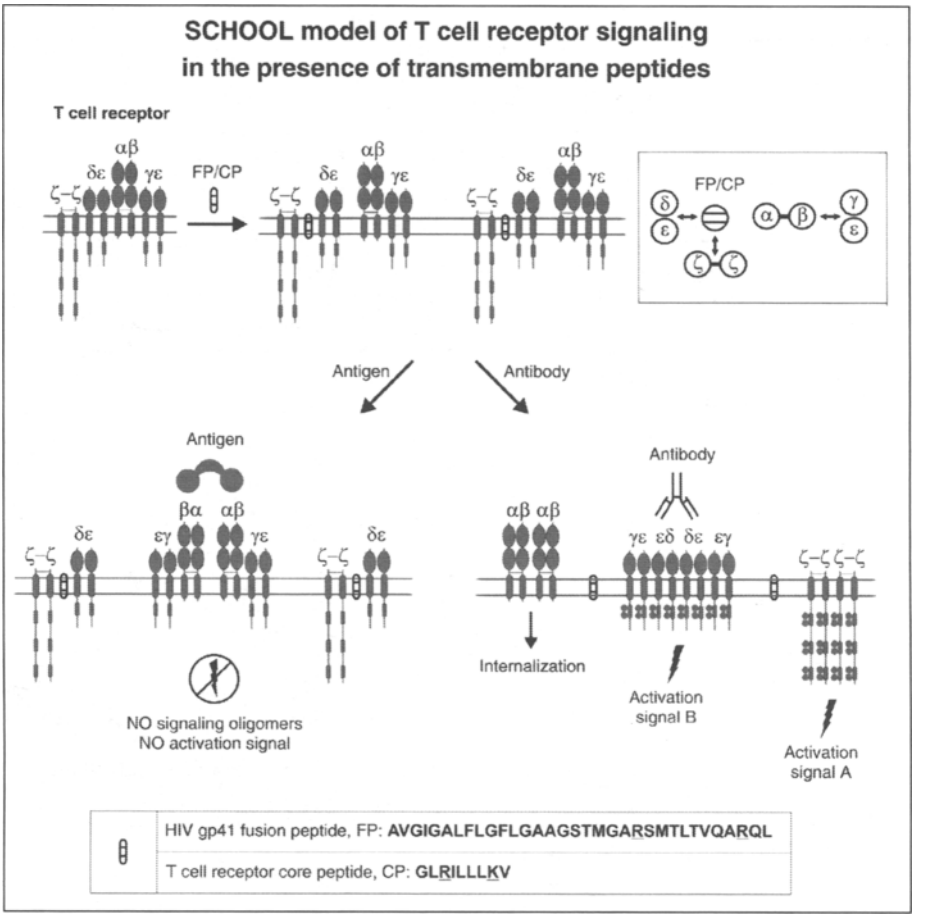

Figure 3. A proposed molecular mechanism of action of the T-cell receptor core peptide (CP) and HIV-1 gp41 fusion peptide (FP). Considering the close similarity in patterns of inhibition of T-cell activation and immunosuppressive activity observed for CP and FP, the SCHOOL model reasonably suggests a similar molecular mechanism of action for both peptides. Within the SCHOOL model, these peptides compete with the TCRα chain for binding to the CD3δε 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 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. In addition, similar mechanisms are proposed to be used by other viruses, such as cytomegalovirus and severe acute respiratory syndrome-associated coronavirus, in their pathogenesis to modulate the host immune response.

immune response.[77] Within the SCHOOL model, these data do not reveal any discrepancy between in vivo and in vitro experiments. Instead, they can be considered, in fact, as the first direct experimental evidence of our ability to selectively modulate the MIRR-mediated TM signaling

and the immune response, as predicted by the model.[27-30] In this context, the CD3δ and CD3γ TM peptides disconnect the corresponding signaling subunits (CD3δ and CD3γ, respectively) from the remaining receptor complex (Table 3). Therefore, antigen stimulation does not result in formation of the relevant competent CD3δ or CD3γ signaling oligomers and phosphorylation of their ITAM tyrosine residues, preventing initiation of the corresponding signaling pathways and cell responses. Further, in their in vitro experiments, the authors[77] used an interleukin 2 (IL-2) production assay and T-cell proliferation as markers of T-cell activation. However, the previously reported in vitro activation studies with T-cells lacking CD3γ and/or CD3δ cytoplasmic domains clearly indicate that antigen-stimulated induction of cytokine secretion and T-cell proliferation in these cells are intact,[102-105] explaining the absence of inhibitory effect of the CD3δ and CD3γTM peptides in the in vitro activation assays used.[77] However, in vivo deficiency either of CD3δ or CD3γ results in severe immunodeficiency disorders.[106,107] This can explain the inhibitory effect observed in the in vivo studies for all three CD3 TM peptides.[77]

These experimental data[77] successfully proved that our ability to selectively physically disconnect specific signaling subunits using the MIRR TM peptides in line with the SCHOOL model can result in their selective functional disconnection and thus provide a powerful tool to study MIRR functions and immune cell signaling.[27-30] Even more importantly, it also confirms that as predicted using the SCHOOL model,[27-30] agents targeted specific intra-MIRR TM interactions can be designed not only to inhibit but also specifically modulate the immune response and therefore result in the development of novel therapeutic strategies for a variety of immune disorders.

Similar molecular mechanisms of action are suggested by the SCHOOL model for other MIRR TM peptides and describe and/or predict their inhibitory/modulatory effect on MIRR-mediated cell activation (Table 3). Recently, the SCHOOL model-driven TM-targeted strategy has been successfully applied to develop a novel concept of platelet inhibition and resulted in the invention of a new class of platelet inhibitors (Fig. 4, Table 3, see also Chapter 12).[58,108] This issue will be covered in more detail below.

In summary, considering the high therapeutic potential of the MIRR TM peptides illustrated by the clinical results for the TCR CP (Table 4) and the promising results for other peptides (Table 3),[58,108] the SCHOOL model represents an invaluable tool in further development of this novel pharmacological approach targeting MIRR TM interactions.

Indirect Evidence

In contrast to MIRRs, single-chain receptors (SRs) can be characterized in the structural context as receptors with extracellular recognition domains and intracellular signaling domains located on the same protein chain. 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. Ligand binding is believed to stimulate monomeric receptor dimerization and trans-autophosphorylation at defined tyrosine residues through intrinsic kinase activity.[109-111] Further, the basic principles of SR signaling, namely the ligand-induced receptor dimerization/oligomerization and trans-autophosphorylation

Table 4. Effect of TCR core peptide on T-cell-mediated dermatoses in man*

Diagnosis	Number of Patients	Cure	Improvement	No Effect
Atopic dermatitis	5	3	2	-
Lichen planus	2	1	1	-
Psoriasis	2	-	1	1

*Adapted from [Gollner GP, Muller G, Alt R et al. Therapeutic application of T-cell receptor mimic peptides or cDNA in the treatment of T-cell-mediated skin diseases. Gene Ther 2000; 7:1000-1004].

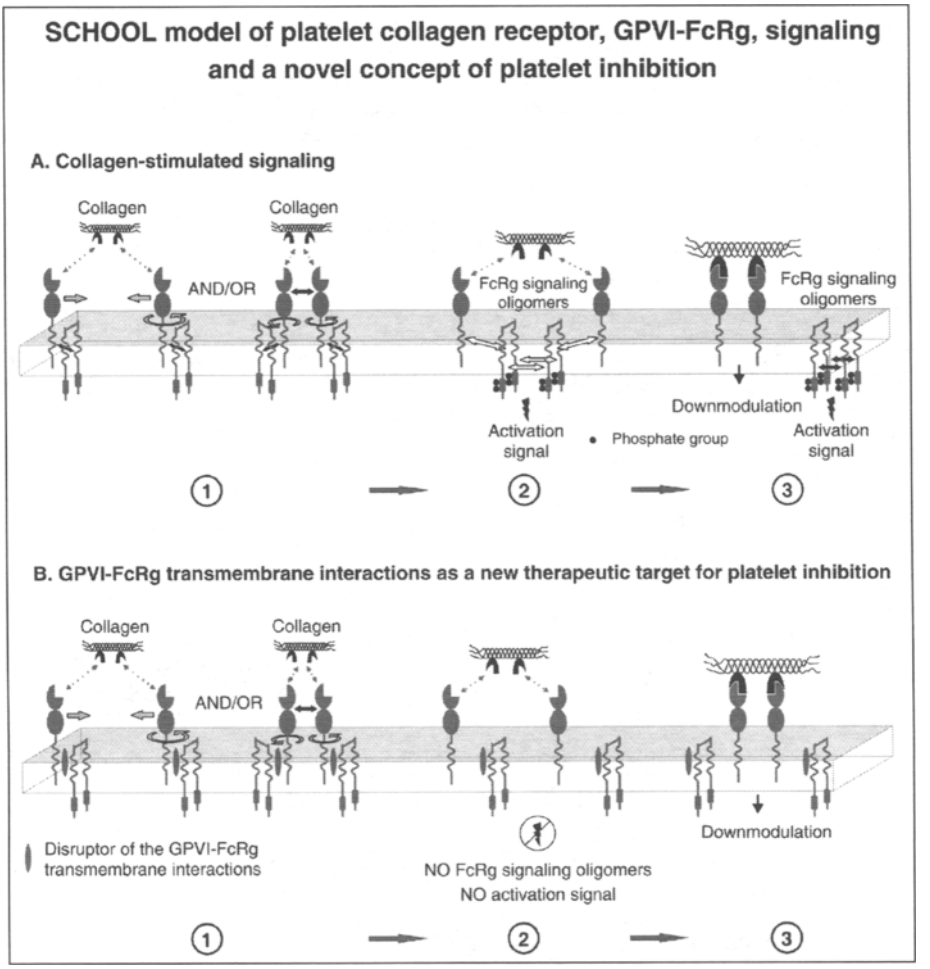

Figure 4. Novel concept of platelet inhibition. A) The signaling chain homooligomerization (SCHOOL) model of collagen-stimulated GPVI-FcRγ transmembrane (TM) signaling, proposing that the homooligomerization of the FcRγ signaling subunit plays a central role in triggering the GPVI-FcRγ receptor complex. The model also assumes that not only is sufficient proximity of the receptor units in formed and/or preformed receptor dimers/oligomers required to trigger MIRRs but also a correct interunit relative orientation and geometry. Small solid black arrows indicate specific intersubunit hetero- and homointeractions between TM and cytoplasmic domains, respectively. Circular arrows indicate collagen-induced receptor reorientation. All interchain interactions in a dimeric intermediate are shown by large white arrows reflecting their transition state. Immunoreceptor tyrosine-based activation motifs are shown as dark gray rectangles. Phosphate groups are shown as black circles. B) Specific disruption of the GPVI-FcRγ TM interactions results in "predissociation" of the GPVI-FcRγ receptor complex, thus preventing formation of FcRγ signaling oligomers and inhibiting collagen-dependent platelet activation and aggregation. Reproduced with permission from Wiley-Blackwell Publishing Ltd. Sigalov AB. More on: glycoprotein VI oligomerization: a novel concept of platelet inhibition. J Thromb Haemost 2007; 5:2310-2312.

of Tyr residues in CYTO signaling domains, are considered to represent common mechanisms of triggering and TM signal transduction for the vast majority of various receptors.[12,83,112-123]

Dimerization of SRs is known to be mostly driven by the homointeractions between receptor TM domains.[83,113,117,119-122,124] These findings reveal the interreceptor TM protein-protein interactions as an attractive point of pharmacological intervention. At present, there is a growing line of experimental evidence indicating that an application of TM-targeted strategy to inhibit/modulate SR signaling might represent a promising therapeutic strategy.[83,117,121,124-130]

It should be noted that despite apparent similarities in using TM peptides in both SR- and MIRR-targeted TM strategies, the basic principles of the molecular mechanism underlying inhibition and/or modulation of SR and MIRR signaling by using the TM agents are totally different. As established in the field of SR signaling, the SR-targeted TM peptides/agents block/disrupt/modulate interreceptor TM interactions crucial for antigen/ligand-induced receptor oligomerization. This prevents the formation of competent receptor oligomers, whereas MIRR-targeted TM peptides/agents, as suggested by the SCHOOL model (see also Chapter 12)[27-30,57,58] affect intra-MIRR TM interactions between recognition and signaling subunits, thus preventing upon antigen/ligand stimulation, formation of competent MIRR signaling oligomers but not MIRR oligomers/clusters in terms of MIRR recognition subunits and those signaling subunits that are not affected by the TM agents. Because of this fundamental difference in molecular mechanisms of action of SR- and MIRR-targeted TM peptides/agents, I consider the SR-related findings as indirect evidence for the fundamental and clinical relevance of the MIRR TM-targeted strategy suggested by the SCHOOL model. Several examples of using TM peptides to inhibit SR signaling are described in more detail below.

Ligand binding-induced association of the TM domains has been proposed to favor productive dimerization of intracellular kinase domains to promote trans-autophosphorylation.[124] 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.[124,129] These peptides are thought to block/disrupt specific TM interactions, thereby inhibiting receptor dimerization and activation.[124,129]

Using differential epitope tagging, it has been demonstrated that β2-adrenergic receptors form homodimers and TM domain VI of the receptor may represent part of an interface for receptor dimerization.[125] As shown, a peptide derived from this domain inhibits both dimerization and β-adrenergic agonist-promoted stimulation of adenylyl cyclase activity.[125] 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.[126] 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,[131] Epo receptor,[114,132-134] toll-like receptors (TLRs)[135] and the integral membrane receptor LuxPQ.[136] 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.[137]

G-protein-coupled receptors (GPCR) are characterized by the presence of seven TM domains and represent a superfamily of proteins that mediate the function of neurotransmitters and peptide hormones and are involved in viral entry and perception of light, smell and taste. Structural analogs of individual TM domains of GPCRs have been reported to serve as potent and specific receptor inhibitors.[128] Peptide sequences corresponding to the TM domains of chemokine receptors, CXCR4, also called fusin, an alpha-chemokine receptor specific for stromal-derived-factor-1 and CCR5, the chemokine receptor which HIV uses as a coreceptor to gain entry into macrophages,

have been demonstrated to specifically inhibit receptor signaling and the in vitro replication of HIV-1.[128] Similarly, peptides mimicking the TM domains of cholecystokinin receptor A, have been found to abolish ligand binding and signaling through the receptor.[128]

Thus, the sequence-based blockade of the interreceptor TM protein interactions as applied to SR signaling provides indirect evidence for the importance and clinical significance of the intra-MIRR TM-targeted strategy suggested by the SCHOOL model.

Transmembrane Interactions and Viral Pathogenesis

In general terms, viral pathogenesis is the process by which viral infection leads to disease. The consequences of a viral infection depend on a number of viral and host factors that affect pathogenesis. Infection of host cells by enveloped viruses requires fusion of the viral membrane with the host cell membrane. This fusion is mediated by viral glycoproteins (gp), the proteins that are anchored to the viral membrane. The fusion glycoproteins of enveloped viruses, typically type-I integral membrane proteins, are known to contain in their sequences a short region called the "fusion peptide" (FP), which is required for mediating membrane fusion.[138,139] This region interacts with the host cell membrane at an early stage of the membrane fusion process. Despite advances in our understanding of the major principles of viral fusion mediated by the fusion glycoproteins,[138-143] little is known about their role in functional modulation of MIRR-mediated TM signal transduction.

In this section, I focus on MIRR signaling-related immunomodulatory activity recently reported for HIV and CMV. As suggested by the SCHOOL model, the molecular mechanisms underlying this activity affect MIRR TM interactions and can be also used by other viruses. To illustrate this point, I describe an application of the model in the pathogeneses of two other viruses, SARS-CoV and HTLV-1. I also demonstrate how the SCHOOL model-driven TM strategy, together with the lessons learned from viral pathogenesis, can be used practically for rational drug design and the development of new therapeutic approaches.

HIV Pathogenesis

CD4+ T-cells are the main targets of HIV-1 in the host. The magnitude of viral replication in these cells is closely linked to their activation state. In activated memory CD4+ T-cells, HIV-1 readily undergoes multiple rounds of replication, whereas resting helper T-cells are largely refractory to productive infection.[144,145] Indeed, several steps in the life cycle of HIV-1 have been identified where potent blocks in virus propagation occur when ample T-cell activation is lacking.

Fusion Peptide

The FP found in the N terminus of the HIV envelope glycoprotein gp41 functions together with other gp41 domains to fuse the virion with the host cell membrane.[146,147] Surprisingly, this peptide has been recently shown to have not only a fusogenic activity but also a T-cell-targeted immunomodulatory activity: it colocalizes with CD4 and TCR molecules, coprecipitates with the TCR and inhibits antigen-stimulated T-cell proliferation and proinflammatory cytokine secretion in vitro.[79] These effects are specific, T-cell activation via PMA/ionomycin or mitogenic antibodies to CD3 is not affected by FP and FP does not interfere with antigen-presenting cell function.[79] In mice, HIV FP shows immunosuppressive activity, inhibiting the activation of arthritogenic T-cells in the autoimmune disease model of adjuvant arthritis and reducing the disease-associated interferon-γ (IFN-γ) response.[79] The close match between these findings[79] and the experimental data generated for TCR CP[75,78,98] suggests a mechanistic similarity underlying the TCR-targeted HIV FP and TCR CP activities.

However, as with TCR CP, despite ongoing studies of HIV gp41 FP,[80] the molecular mechanisms of immunomodulatory action of this peptide have not been elucidated until 2006 when the SCHOOL model was first applied to this area.[29] Considering the close similarity in patterns of inhibition of T-cell activation and immunosuppressive activity observed for FP[79] and CP,[75,78,98] the SCHOOL model reasonably suggests a similar molecular mechanism of action for TCR TM peptides and HIV gp41 FP (Fig. 3, Tables 3 and 5).[27-30,57] Primary sequence analysis of these two

peptides (Table 6) shows different primary sequences but a similarity in charged or polar residue distribution patterns with two positively charged residues spaced apart by 4 (CP) or 8 (FP) amino acids. For CP, Arg and Lys residues are known to mediate the interaction between recognition TCRα subunit and signaling CD3δε and ζ subunits.[23] Importantly, for FP, both arginines are located in the C-terminal half, suggesting that this sequence could be important for the interaction with the TCR. Figure 3 shows a potential mode of action of CP and FP as proposed by the SCHOOL model (see also Tables 3 and 5). Briefly, CP and FP compete with the TCRα chain for binding to CD3δε and ζ hetero- and homodimers, respectively, thus resulting in TM disconnection/predissociation of the signaling subunits from the remaining receptor complex (Fig. 3). This mechanism of FP action suggests the existence of an interaction interface in the C-terminal half of the peptide. Within the model,[29,30,57] the peptide prevents formation of CD3δε and ζ signaling oligomers and thus inhibits antigen-dependent T-cell activation (Fig. 3, Table 5), acting similarly in this respect to TCR CP (Fig. 3, Tables 3 and 4).[27-30,75] However, stimulation with anti-CD3 antibodies of these "predissociated" TCRs still should result[27-30,57] and results[79,80] in receptor triggering and cell activation. The model suggests that clinically relevant antibodies (OKT3) could be used to modulate the affected T-cell response during HIV infection. Recently, OKT3 antibodies have been used successfully in HIV therapy to augment immune activation.[148] More recent studies[80] have confirmed the predicted molecular mechanism of immunomodulatory activity of the HIV FP. Finally, it should be noted that the proposed mechanism is the only mechanism consistent with all experimental data on immunomodulatory action of HIV gp41 FP reported up to date.[79,80]

A highly specific natural inhibitor of HIV-1 gp41 FP has been recently reported to block HIV-1 entry.[149,150] This agent that has been isolated from human hemofiltrate and designated VIRus Inhibitory Peptide (VIRIP),[150] represents a 20-residue peptide, corresponding to the C-proximal region of α1-antitrypsin. Importantly, it has been shown that VIRIP directly interacts with the gp41 FP and a few amino acid changes increase its antiretroviral activity potency by two orders of magnitude, thus demonstrating the usability and efficiency of rational peptide design approaches.[150]

According to the SCHOOL model, the TCR TM interactions represent not only important therapeutic targets for immune-mediated diseases but also a point of HIV intervention. The molecular mechanisms revealed by the model can be used in rational antiviral drug design and the development of novel antiviral therapies.

HIV Nef Protein

Another application of the SCHOOL model to HIV pathogenesis is related to the molecular mechanisms of action of the HIV pathogenicity factor Nef, a key protein in viral replication and progression of disease. Several studies have shown that this protein interacts with the TCR ζ chain and mediates downmodulation of TCR—CD3 complex.[151-153] Notably, Nef lowers the threshold of CD4+ T-cell activation.[154,155] Other study showed that Nef induces transcription of an array of genes almost identical to that triggered upon exogenous stimulation of TCR.[156] Nef has been also reported to affect T-cell activation events through its interactions within the lipid raft microenvironment,[157] induce signal transduction via the recruitment of a signaling machinery, thereby mimicking a physiological cellular mechanism to initiate the TCR cascade[158] and, finally, to form a signaling complex with the TCR, which bypasses the requirement of antigen to initiate T-cell activation.[159] Thus, the extent of T-cell activation imprinted by expression of Nef is a matter of controversy. In addition, although we know that Nef binds the TCR ζ chain,[152,160] the role of this interaction and the mechanism used by Nef to modulate T-cell activation remain unknown.

Importantly, similar to ζ,[31,33] Nef exists in several discrete oligomeric species, namely monomers, dimers and trimers.[161] Within the model,[27-29] natively oligomeric Nef may crosslink homodimeric ζ chains, leading to the formation of multivalent TCR complexes that have been shown to be responsible for sensing low concentrations of antigen.[162] This mechanism could explain the observed activation sensitization in T-cells by Nef.[154,155] On the other hand, Nef dimers may crosslink ζ homodimers in the "permissive" relative orientation and promote formation of competent signaling ζ oligomers, generating an activation signal A (Fig. 3, see also Chapter 12)[27-29] and resulting in

Table 5. Selected viral agents reported to modulate the immune cell response and suggested or predicted by the SCHOOL model to affect MIRR transmembrane interactions

Agent	MIRR	Action	Mechanism as Suggested by the SCHOOL Model
HIV gp41 FP	TCR	Colocalizes with CD4 and TCR molecules, coprecipitates with the TCR and inhibits antigen-specific T-cell proliferation and proinflammatory cytokine secretion in vitro[79] Blocks the TCR/CD3 TM interactions needed for antigen-triggered T-cell activation[80]	Similarly to the TCR CP, disrupts TCRα-CD3δε and TCRα-ζ TM interactions resulting in dissociation of these signaling subunits from the remaining complex and thus preventing the formation of signaling oligomers upon antigen stimulation and, consequently, inhibiting T-cell activation[29,30,57]
CMV pp65	NKp30	Interacts directly with NKp30, leading to dissociation of the linked ζ subunit and, consequently, to reduced killing[193]	Affects the NKp30-ζ TM interactions resulting in dissociation of the ζ signaling subunit from the remaining complex and thus preventing the formation of ζ signaling oligomers upon antigen stimulation and, consequently, inhibiting NK cell cytolytic activity[29,30]
SARS-CoV FP	TCR*	Not reported yet	Similarly to the TCR CP and HIV gp41 FP, disrupts TCRα-CD3δε and TCRα-ζ TM interactions resulting in dissociation of these signaling subunits from the remaining complex and thus preventing the formation of signaling oligomers upon antigen stimulation and, consequently, inhibiting T-cell activation*

*As predicted by the SCHOOL model.
Abbreviations: CMV, human cytomegalovirus; CP, core peptide; FP, fusion peptide; gp, glycoprotein; MIRR, multichain immune recognition receptor; NK cells, natural killer cells; pp65, 65 kDa phosphoprotein; SARS-CoV, severe acute respiratory syndrome coronavirus; TCR, T-cell antigen receptor; TM, transmembrane.

Table 6. Primary sequences of MIRR transmembrane domains involved in viral pathogenesis and viral fusion proteins and peptides suggested or predicted by the SCHOOL model to affect MIRR transmembrane interactions

MIRR	Description	Sequence*
TCR	TCRα TMD	VIGF**R**ILLL**K**VAGFNLLMTL
	TCRα CP	GL**R**ILLL**K**V**
	SARS-CoV FP	MY**K**TPTL**K**YFGGFNFSQIL
	HIV gp41 FP	AVGIGALFLGFLGAAGSTMGA**R**SMTLTVQA**R**QL
	HTLV-1 gp21***	AVPVAVWLVSALAMGAGVAGGITGSMSLASG**K**SLLHEVD**K**D
MIRR?	LASV FP	GTFTWTLSDSEG**K**DTPGGYCLT**R**WMLIEAEL**K**CFGNTAV
	LCMV FP	GTFTWTLSDSSGVENPGGYCLT**K**WMILAAEL**K**CFGNTAV
	MOPV FP	GLFTWTLSDSEGNDMPGGYCLT**R**SMLIGLDL**K**CFGNTAI
	TACV FP	AFFSWSLTDPLGNEAPGGYCLE**K**WMLVASEL**K**CFGNTAI
NKp30	NKp30 TMD	GTVLLL**R**AGFYAVSFLSVAVG
	ζ subunit TMD	LCYLL<u>D</u>GILFIYGVILTALFL****
	CMV pp65***	M<u>E</u>S**R**G**RR**CP<u>E</u>MISVLGPISGHV**L**K**A**VFS**R**G<u>D</u>TPVLPH<u>E</u>T**R**LLQTGIHV**R**VSQPSLILVSQYTP<u>D</u>STPCH**R******

*Basic amino acid residues are indicated in bold.
**Corresponds to the predetermined assembly TM sequence of murine TCRα.[78]
***N-terminal end.
****Acidic amino acid residues are underlined.
Abbreviations: CMV, human cytomegalovirus; CP, core peptide; FP, fusion peptide; gp, glycoprotein; HTLV-1, human T-lymphotropic virus type 1; LASV, Lassa virus; LCMV, lymphocytic choriomeningitis virus; MOPV, Mopeia virus; MIRR, multichain immune recognition receptor; NK cells, natural killer cells; pp65, 65 kDa phosphoprotein; SARS-CoV, severe acute respiratory syndrome coronavirus; TACV, Tacaribe virus; TCR, T-cell antigen receptor; TCRα, TCR alpha chain; TM, transmembrane; TMD, transmembrane domain.

dissociation of the ζ signaling oligomers from the remaining receptor complex with its subsequent internalization. The SCHOOL model suggests that the oligomer interfaces of ζ and/or Nef are involved in the molecular mechanisms underlying the immunomodulatory effects of Nef.[27-29] As recently shown,[163] a Nef mutant carrying a mutation targeted to the conserved residue D123, in addition to losing the ability to oligomerize, is defective for major histocompatibility complex class I (MHC-I) downmodulation and enhancement of viral infectivity, suggesting that the oligomerization of Nef may be critical for its multiple functions.

In this regard, I suggest that both proposed mechanisms may take place in vivo and selection between these two alternative pathways may possibly depend on the type of cells infected and/or on the cell membrane lipid content. Thus, CYTO heterointeractions at the Nef-ζ interface and CYTO homointeractions in Nef and ζ oligomers may represent attractive targets for the design of antiviral agents.

The usability and efficiency of this SCHOOL model-driven CYTO approach have been later demonstrated for Nef-mediated internalization of surface CD80 or CD86 that is dependent on the binding of Nef to the CYTO domain of the target CD80 or CD86 molecule, respectively.[164] This issue will be covered in more detail below.

SARS-CoV Pathogenesis

The coronavirus SARS CoV is the etiological agent of SARS that represents the life-threatening disease associated with a mortality of about 10%.[165] In recent studies, in which a total of 38 patients with SARS were enrolled, have shown that CD4+ and CD8+ T-lymphocyte levels were reduced in 100% and 87% of patients, respectively.[166] Thus, one can suggest that the virus can have an immunomodulatory activity and this activity is TCR-targeted.

In the traditional view of HIV disease course, acute HIV infection is characterized by massive and rapid CD4+ T-cell loss, whereas chronic infection is characterized by persistent immune activation that drives viral replication and further CD4+ T-cell depletion.[167,168] Thus, HIV infection has been thought of as a relatively indolent disruption of CD4+ T-cells eventually leading to collapse of immune function. As with SARS CoV,[166] this notion has been largely based on measurements of CD4+ T-cell counts in peripheral blood.[167,168]

Despite the lack of direct evidence, it is reasonable to suggest that SARS-CoV has a TCR-targeted immunomodulatory activity. More specifically, as with HIV, this activity might be especially important during virus entry to suppress the host response to virus infection. Like other enveloped viruses encoding class I viral fusion proteins such as HIV[169] and Ebola and avian sarcoma viruses,[170] SARS-CoV is presumed to use membrane fusion mechanisms for viral entry.[171-173] It has been shown that the SARS-CoV viral spike (S) protein 2 (S2) is a class I viral fusion protein and is responsible for driving viral and targeT-cell membrane fusion.[174] Recently, inhibitory peptides derived from the membrane-proximal heptad repeat region (HR2) of the S2 protein have been suggested as an attractive basis for the development of therapeutics for SARS.[175] The putative SARS-CoV FP has also been identified at the N terminus of the SARS-CoV S2 subunit.[176] As shown by using synthetic peptides,[176] the fusogenic activity of the SARS-CoV FP appears to be dependent on its amino acid sequence, as scrambling the peptide renders it unable to partition into large unilamellar vesicles (LUVs), assume a defined secondary structure, or induce both fusion and leakage of LUV.

Primary sequence analysis of the SARC-CoV FP and TCRα TM domain (or TCR CP) shows different primary sequences but reveals a similarity in charged or polar residue distribution patterns with two positively charged residues spaced apart by 4 amino acids (Table 6). These two positively charged residues are critical for TCR assembly and function. Within the SCHOOL model,[27-30,57] the RILLLK and RSMTLTVQAR motifs in TCR CP and HIV FP (Table 6), respectively, play an important role in mimicking the TCRα TM region and therefore in an inhibitory activity of these peptides (Fig. 3, Tables 3 and 5). Intriguingly, SARS CoV FP has a structural motif KTPTLK that is strikingly similar to that of TCR CP (Table 6). Considering the common structural features of three peptides (TCR CP, SARS-CoV FP and HIV FP) as well as functional similarities between HIV FP and SARS FP in the context of their T-cell-targeted activities, I suggest that like TCR

CP and HIV FP, SARS-CoV FP should mimic the TCRα TM domain and therefore exhibit an inhibitory effect on the antigen-mediated TCR TM signaling (Table 5). In the context of the SCHOOL model, molecular mechanisms of this inhibitory action of SARS-CoV FP are similar to those suggested for TCR CP and HIV FP (Fig. 3, Tables 3 and 5).[27-30,57]

As hypothesized, the TCR TM interactions might represent a point of SARS-CoV intervention. If true, the molecular mechanisms revealed can be used in rational antiviral drug design and the development of novel antiviral therapies. I believe that future studies will experimentally prove this hypothesis.

HTLV-1 Pathogenesis

HTLV-1 is a type C complex retrovirus. It infects and immortalizes human CD4+ T-cells in vitro and is associated with the development of adult T-cell leukemia/lymphoma (ATL).[177-180] Recent observations demonstrate an immunomodulatory ability of the HTLV-1 regulatory protein p12[181,182] and suggest roles that T-cell activation may play in the pathogenesis of HTLV-1-induced disease.[177,181-183]

Below, I consider similarities between the HIV gp41 and HTLV-1 gp21 FPs and the Nef and HTLV-1 p12 proteins, respectively and describe my structural and functional predictions related to potential TCR-targeted activities of HTLV-1 FP and p12. Currently, there is no experimental evidence for these predicted activities. Despite this, I believe that future studies will prove the hypotheses made by using the SCHOOL model.

Fusion Peptide

Similarly to HIV gp41 protein,[146,147] the ectodomain of HTLV-1 TM protein (gp21) contains an N-terminally located fusion peptide, a sequence that inserts into target cellular membranes and is well-known to be critical for membrane fusion activity.[184,185] However, in contrast to the HIV FP, there has been no report to date of an immunomodulatory activity of the HTLV-1 FP.

Primary sequence analysis of these two FPs (Table 6) indicates different sequences but reveals an interesting similarity in charged or polar residue distribution patterns with two positively charged residues spaced apart by 8 (HIV FP) or 7 (HTLV-1 FP) amino acids. Considering the structural similarities of both FPs and the fact that T-cells are main target for both viruses, it is reasonable to suggest a TCR-targeted immunomodulatory activity for the HTLV-1 FP. As proposed by the SCHOOL for HIV FP and TCR CP (Fig. 3, Tables 3 and 5),[27-30,57] a potential mode of action of HTLV-1 can involve the TM competition with the TCRα subunit for binding to CD3δε and ζ subunits, thus resulting in TM disconnection/predissociation of the signaling subunits from the remaining receptor complex (Fig. 3, Table 3 and 5). As with the HIV FP, this mechanism of HTLV-1 FP action suggests the existence of an interaction interface in the C-terminal half of the peptide. Within the model, the peptide should prevent formation of signaling-competent CD3δε and ζ oligomers and thus inhibit antigen-dependent T-cell activation, acting similarly in this respect to both TCR CP and HIV FP (Fig. 3, Tables 3, 4 and 5).[27-30,57,75] However, stimulation with anti-CD3 antibodies of these "predissociated" TCRs should still result (Fig. 3)[27-30,57] in receptor triggering and cell activation. As with HIV infection, the model suggests that clinically relevant antibodies (i.e., OKT3) could be used to modulate the affected T-cell response during HTLV-1 infection.

In summary, I propose a new hypothesis that considers the largely unexplored immunomodulatory role of the FP in the HTLV-1 infection and pathogenesis of ATL. If true, this hypothesis will generate new therapeutic targets and opportunities. I also suggest that our current and future clinical knowledge, experience and therapeutic strategies can be potentially transferred in this respect between the HIV- and HTLV-1-related medical conditions.

HTLV-1 p12 Protein

The p12 protein of HTLV-1 is a small oncoprotein that has been shown to have multiple functions. Expression of p12 has been demonstrated to induce nuclear factor of activation of T-cells (NF-AT), increase calcium release and transcriptional factor Stat 5 activation in T-cells suggest-

ing that p12 may alter T-cell signaling.[186-188] Interestingly, p12 is important for viral infectivity in quiescent human peripheral blood lymphocytes (PBLs) and the establishment of persistent infection in rabbits.[189,190] 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. 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.[182] Also, the clones of Jurkat cells expressing the highest levels of p12 have been found to exhibit a more rapid rate of cell proliferation than the parental cells.[182] Similarly to HIV Nef, the p12 protein, upon engagement of the TCR, relocalizes to the interface between T-cells and antigen-presenting cells, defined as the immunological synapse (IS).[181] Both Nef and p12 are recruited to the IS, but Nef potentiates TCR signaling[191] while p12 dampens it.[181]

In summary, targeting TCR-mediated signaling seems to be a shared feature of both HIV and HTLV-1 viruses, reflecting probably their similar evolutionary pathway towards their adaptation to the host immune response. Thus, it is possible that similar molecular mechanisms may be involved in TCR-targeting strategies used by Nef and p12 to modulate TCR-mediated signaling pathways. If true, this hypothesis will generate new therapeutic targets (i.e., protein-protein interactions at the interface of p12 and its potential TCR-related partners) and opportunities, similar to those suggested for HIV Nef.

CMV Pathogenesis

To escape from NK cell-mediated surveillance, human CMV interferes with the expression of NKG2D ligands in infected cells. In addition, the virus may keep NK inhibitory receptors engaged by preserving human leukocyte antigen (HLA) class I molecules that have a limited role in antigen presentation.[192] Despite considerable progress in the field, a number of issues regarding the involvement of NK receptors in the innate immune response to human CMV remain unresolved.

Recently, a direct interaction between the human CMV tegument protein pp65 and the NK cell activating receptor NKp30 has been reported.[193] It has been shown that the binding of pp65 to NKp30 is specific and functional. Surprisingly, the recognition of pp65 by NKp30 does not lead to NK cell activation but instead results in a general inhibition mediated by the dissociation of the signaling ζ subunit from the NKp30-ζ receptor complex.[193] This results in the diminishing of activating signals and loss in the ability of NK cells to kill normal, tumor and virus-infected cells.[193]

Within the context of SCHOOL model,[27-30] the reported action of the human CMV pp65 protein may be due to its potential impact on the TM interactions between NKp30 and ζ, leading to disconnection and dissociation of the ζ subunit.[30] This would prevent the formation of signaling-competent ζ oligomers upon ligand stimulation and consequently, inhibit NK cell cytolytic activity (Fig. 2, Table 5) in a manner similar in this respect to the inhibitory action of TCR CP (Fig. 3, Table 3). Primary sequence analysis of the N-terminal end of pp65 shows the existence of multiple positively and negatively charged amino acid residues (Table 6). This pp65 region possibly contains the sequence that mimics the NKp30 or ζ TM domain with the Arg or Asp residues, respectively, that are known to mediate the interaction between recognition NKp30 chain and signaling ζ subunit (see also Chapter 4).[18] However, further experimental studies are needed to confirm the proposed mechanism.

Lessons from Viral Pathogenesis

General issues related to viral pathogenesis in the context of MIRR TM signaling are covered in more detail in Chapter 22. In this section, I briefly describe several important lessons that we can learn from the SCHOOL model-revealed similarity of the molecular mechanisms underlying viral pathogenesis and MIRR signaling-targeted immunomodulatory viral activity important for viral immune escape. I also consider the striking similarities of the molecular mechanisms and basic structural principles that are suggested by the model to explain immunomodulatory effects of viral

fusion and accessory proteins and synthetic agents affecting intra- or inter-MIRR protein-protein interactions in the TM or CYTO milieus, respectively.

It seems that in general, viruses use TM-targeted immunomodulatory activity of their fusion proteins mostly during virus entry to suppress the host immune response, whereas modulation of CYTO interactions by using accessory proteins such as HIV Nef and HTLV-1 p12 plays a role in viral replication and enhancing viral infectivity in the host. Thus, our improved understanding of MIRR signaling-targeted immunomodulatory viral activity might allow us to reveal novel targets at these stages of viral pathogenesis.

I believe that lessons that we can learn from viral pathogenesis in the context of the SCHOOL model of immune signaling are very important for our further understanding of the molecular mechanisms used by viruses to infect the host and escape its immune response. I also believe that these lessons are of both fundamental and clinical value. Why?

1. Now we know the molecular mechanisms of inhibitory action of MIRR TM peptides such as TCR TM peptides,[74,77,78,81,93,95-97] NK TM peptides[91] and GPVI TM peptide,[58] as suggested by the SCHOOL model.[27-30,58] We also know that the same mechanisms are very likely to be used in vivo by HIV gp41 FP and also, as predicted by the SCHOOL model, by fusion proteins of other viruses, such as SARS-CoV and HTLV-1 FPs, to suppress the host immune response.[29,30,57] Considering the high specificity and efficiency of viral agents in inhibition of immune receptors in combination with our current knowledge of the protein-protein interactions underlying this process, we can now use modern well-established computational, bioinformatic and synthetic methodologies[82-90] to design and produce highly specific and effective TM-targeted agents that are able to affect specific TM interactions of a targeted MIRR and suppress and/or modulate the MIRR-mediated immune response. These agents would be of great fundamental and clinical value.
   Similar conclusions can be drawn for our ability to use the SCHOOL model of immune signaling and the lessons learned from our current knowledge of the CYTO-targeted viral strategies to design and produce efficient and specific CYTO-targeted agents.
2. According to the SCHOOL model, TCR CP, HIV gp41 FP and, as predicted, SARS-CoV FP and HTLV-1 FP, affect similar TCR TM interactions (Fig. 3, Tables 3 and 5).[27-30,57] Primary sequence analysis of these peptides (Table 6) shows different primary sequences but a striking similarity in charged or polar residue distribution patterns, suggesting that a computational approach combined with the molecular mechanisms of action of these peptides revealed by the SCHOOL model, can and should be used in the rational design of effective immunomodulatory TM-targeted peptides. General well-known principles of designing TM peptides with an ability to insert into the membrane might be readily used at this stage.[86]
3. As suggested by the SCHOOL model (Fig. 2), TM-targeted agents should inhibit MIRR-mediated cell activation induced only by antigen/ligand but not antibodies to MIRR signaling subunits. Indeed, it has been shown for TCR CP[75] and HIV FP[79] that these TM peptides inhibit only antigen-mediated T-cell activation, whereas stimulation with anti-CD3 antibodies in the presence of the peptides still results in functional cell response. Entirely similar considerations can be applied for other viral FPs such as SARS-CoV FP and HTLV-1 FP. Thus, the SCHOOL model suggests that antibodies to MIRR signaling subunits can be used as immunotherapeutics to modulate the affected immune cell response during viral infection.
4. For TCR, considering our selective ability to physically and more importantly, functionally disconnect any particular CD3 and/or ζ signaling subunits from the remaining receptor by using the relevant TM peptides and basic principles of the SCHOOL model of TCR signaling (Fig. 3), we can design, synthesize and use these peptides as a powerful tool to dissect fine molecular mechanisms of viral pathogenesis in the context of TCR signaling.

5. Two unrelated enveloped viruses, HIV and human CMV, use a similar mechanism to modulate the host immune response mediated by two functionally different MIRRs—TCR and NKp30. As predicted by the SCHOOL model, SARS-CoV and HTLV-1 can also use similar mechanisms during virus infection. Intriguingly, as shown in Table 6, similar positively charged residue distribution pattern with two Arg and/or Lys residues spaced apart by 8 amino acids is observed for the FPs of seemingly unrelated viruses such as HIV, Lassa virus (LASV),[194] lymphocytic choriomeningitis virus (LCMV),[194] Mopeia virus (MOPV)[194] and Tacaribe virus (TACV).[194] Thus, it is very likely that similar general immunomodulatory mechanisms can be or are used by other viral and possibly nonviral pathogens (see also Chapter 22). In addition, as with HIV gp41 FP,[149,150] it is promising to apply a similar strategy to block viral entry by using the agents able to interact directly with FPs of other viruses.

Novel Concept of Platelet Inhibition

Damage to the integrity of the vessel wall results in exposure of the subendothelial extracellular matrix, which triggers platelet adhesion and aggregation.[195,196] The consequence of this process is the formation of a thrombus, which prevents blood loss at sites of injury or leads to occlusion and irreversible tissue damage or infarction in diseased vessels.[196] 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 major physiological function of platelets is hemostasis, prevention of bleeding and the effect of aspirin has established that they are also involved in its pathological variant, thrombosis.[20] Platelets also play a critical role in coronary artery disease and stroke, as evidenced by the well-documented benefits of antiplatelet therapy.[197]

Platelet adhesion, aggregation and activation induced by collagen is critically dependent upon the engagement and clustering of GPVI, a type I transmembrane platelet glycoprotein of about 62 kDa and the major signalling receptor for collagen on platelets (see also Chapter 5).[21,196,198-200] GPVI has no intrinsic signaling capacity and signaling is achieved through the association with its signaling partner, the FcRγ chain.[21] The selective inhibition of GPVI and/or its signaling is thought by most experts in the field to inhibit thrombosis without affecting hemostatic plug formation, thus providing new therapeutical strategies to fight platelet-mediated diseases (see also Chapter 5).[21,201-204] In contrast to antithrombotic drugs that are currently in use, GPVI receptor-specific inhibitors represent an ideal class of clinically suitable antithrombotics. However, despite intensive studies of the GPVI-FcRγ receptor complex,[21,199,205,206] the mechanism of GPVI signaling was not known until very recently when the SCHOOL model was introduced and applied to GPVI triggering and TM signal transduction.[27-30,58] This resulted in the development of a novel concept of platelet inhibition and the invention of new platelet inhibitors within this promising antithrombotic strategy.[58,108] The invented inhibitors are proposed to be useful in the prevention/treatment of thrombosis and other medical conditions involving collagen-induced platelet activation and aggregation as well as in the production of drug-coated medical devices.[58,108]

Within the SCHOOL model, GPVI-mediated platelet activation is a result of the interplay between GPVI-FcRγ TM interactions, the association of two TM Asp residues in the FcRγ homodimer with the TM Arg residue of GPVI,[207] that maintain receptor integrity in platelets under basal conditions and homointeractions between FcRγ subunits, leading to initiation of a signaling response (Table 3, Fig. 4A). Binding of the multivalent collagen ligand to two or more GPVI-FcRγ receptor complexes pushes the receptors to cluster, rotate and adopt an appropriate orientation relative to each other (Fig. 4A, step 1), at which point the trans-homointeractions between FcRγ molecules are initiated. Upon formation of FcRγ signaling oligomers, the Src-family kinases Fyn or Lyn phosphorylate the tyrosine residues in the FcRγ ITAM that leads to TM transduction of the activation signal (Fig. 4A, step 2) and dissociation of FcRγ oligomers and downmodulation of the engaged GPVI subunits (Fig. 4A, step 3). Later, the dissociated oligomeric FcRγ chains can interact with FcRγ subunits of the non-engaged GPVI-FcRγ complexes, resulting in formation

of higher-order signaling oligomers and their subsequent phosphorylation, thus providing lateral signal propagation and amplification (not shown).

For the preformed oligomeric receptor complexes described by Berlanga et al,[208] this model suggests that under basal conditions, the overall geometry of the receptor dimer keeps FcRγ chains apart, whereas stimulation by collagen results in breakage of GPVI-GPVI extracellular interactions and reorientation of signaling FcRγ homodimers, thus bringing them into a close proximity and an appropriate relative orientation permissive of initiating the FcRγ homointeractions (Fig. 4A). Thus, the SCHOOL model highlights a striking similarity between the data on the coexistence of mono- and multivalent TCRs[162] or GPVIs[208] in resting T-cells or nonstimulated platelets, respectively and suggests a similar molecular explanation to answer an important and intriguing question raised in these studies: why does the observed basal TCR or GPVI oligomerization not lead to receptor triggering and subsequent T-cell or platelet activation, respectively, whereas agonist-induced receptor crosslinking/clustering does? See also Chapter 12.[27-30,58]

Suggesting how binding to collagen triggers the GPVI-mediated signal cascade at the molecular level, the SCHOOL model of collagen-induced GPVI signaling reveals GPVI-FcRγ TM interactions as a novel therapeutic target for the prevention and treatment of platelet-mediated thrombotic events.[29,30,58] Specific blockade or disruption of these interactions causes a physical and functional disconnection of the subunits (Fig. 4B, Table 3). Antigen stimulation of these "predissociated" receptor complexes leads to clustering of GPVI but not FcRγ subunits. As a result, FcRγ signaling oligomers are not formed, ITAM Tyr residues do not become phosphorylated and the signaling cascade is not initiated. Agents that target GPVI-FcRγ TM interactions may thus represent a novel class of platelet inhibitors. These include, but are not limited to, peptides, peptide derivatives and compositions and nonpeptide small molecule inhibitors. Preliminary experimental results[58,108] provided support for this novel concept of platelet inhibition and demonstrated that incubation of whole blood samples with a peptide corresponding to the TM domain of GPVI (Gly-Asn-Leu-Val-Arg-Ile-Cys-Leu-Gly-Ala-Val) at a final concentration of 100 μM prior to addition of collagen (10 and 20 μg/ml) or convulxin (10 ng/ml) leads to a 30-60% reduction in both the percentage of P-selectin-positive platelets and the expression of the platelet activation markers, P-selectin and PAC-1 (Sigalov AB, Barnard, MR, Frelinger AL, Michelson AD, unpublished results). This effect is specific: platelet activation via ADP (20 μM) is not affected by the peptide. As assumed by the SCHOOL model, this peptide penetrates the platelet membrane and competitively binds to the FcRγ TM domain, thus replacing GPVI receptor from its interaction with the signaling FcRγ subunit and resulting in "predissociation" of the GPVI—FcRγ receptor complex (Fig. 4B). Notably, a control peptide containing a single amino acid substitution (Arg to Ala) does not display inhibitory activity, a phenomenon predicted by the SCHOOL model since this peptide cannot compete with GPVI for binding with FcRγ in the TM milieu.

In conclusion, a combination of basic principles of the SCHOOL model with a recently reported computational design of peptides that target TM helixes in a sequence-specific manner[85] and other well-established techniques to search for the relevant TM mutations or small molecule disruptors as well as to synthesize and optimize TM peptides and peptidomimetics[1-12,34,76,81-90] opens up a new avenue for designing novel platelet inhibitors, making the proposed strategy both feasible and of great fundamental and clinical value. Combining breakthrough scientific ideas and advances in different fields[28-31,33,57,58] and the high market potential,[209] the suggested technology opens new perspectives in innovative antithrombotic drug discovery and development.

Inflammatory Bowel Diseases: a Novel Treatment Strategy

As another interesting application of the SCHOOL model that challenges its predictive power, I describe my prognosis related to the use of TM- and possibly CYTO-targeted agents as therapeutics to treat inflammatory bowel diseases (IBDs). Briefly, intestinal inflammation in colitic severe combined immunodeficiency (SCID) mice has been recently shown to be characterized by significant increase of CD4+NKG2D+ T-cells.[210] As also demonstrated,[210] neutralizing anti-NKG2D mAb treatment prevents or ameliorates the development of colitis primarily by inhibiting the expansion and/or infiltration of pathogenic T-cells in the colon and secondarily

by inhibiting the development of pathogenic Th1 cells. The authors concluded that targeting of NKG2D signaling in NKG2D-expressing pathogenic CD4+ T-cells may be a useful strategy for the treatment of Th1-mediated chronic intestinal inflammation such as Crohn's disease.[210]

I suggest that TM-targeted agents such as the TM peptides designed by using the SCHOOL model-driven TM-targeted strategy should have specific NKG2D-inhibitory activity and therefore can be used as promising therapeutics to prevent and/or treat IBDs (Table 4). Future studies will prove or disprove this hypothesis.

Cytoplasmic Homointeractions as Immunotherapeutic Targets

Main Concept

As mentioned above, the CYTO domains of the vast majority of MIRR signaling subunits, namely, CD3e, CD3d, CD3g, ζ, Igα, Igβ and FcRγ, have been recently shown to represent a new class of intrinsically disordered proteins (IDPs, see also Chapter 12).[31-33] By definition, IDPs (or natively unfolded, or intrinsically unstructured) are proteins that lack a well-defined ordered structure under physiological conditions in vitro, i.e., neutral pH and room temperature.[211] A highly flexible, random coil-like conformation is the native and functional state for many proteins known to be involved in cell signaling.[212-214]

In addition, intrinsically disordered regions of human plasma membrane proteins have been very recently demonstrated to preferentially occur in the cytoplasmic segment.[215] Finally, it has been suggested that protein phosphorylation, one of the critical and obligatory events in cell signaling, occurs predominantly within intrinsically disordered protein regions.[216] My major assumption is that a flexible, random-coil conformation of the MIRR signaling subunit CYTO domains plays an important role in MIRR triggering and TM signaling.[27-29,31-33] I also suggest that the CYTO domains of those MIRR signaling subunits that have not been studied so far (e.g., DAP12, DAP10 and FcεRIβ), are IDPs as well. Future studies will prove or disprove this hypothesis.

Surprisingly, all intrinsically disordered CYTO domains studied exist under physiological conditions as specific oligomers (mostly, dimers), as I discovered in 2001 and published in 2004[31] and even more interestingly, these IDPs do not undergo a transition between disordered and ordered states upon dimerization.[31-33] This specific dimerization is distinct from nonspecific aggregation behavior seen in many systems. These findings oppose the generally accepted view on the behavior of IDPs, providing first evidence for the existence of specific dimerization interactions for IDP species and thus opening a new line of research in this new and quickly developing field of IDPs. The unusualness and uniqueness of the discovered biophysical phenomenon that was found to be a general phenomenon with all CYTO domains studied in this work,[31] led me to hypothesize that the homointeractions between MIRR signaling subunits represent the missing piece in the puzzle of MIRR triggering and TM signal transduction and to develop the SCHOOL model (see also Chapter 12).[28-30,57,58]

Since it was first published in 2004,[28] the SCHOOL model has revealed inter-MIRR CYTO homointeractions as important therapeutic targets as well as points of great interest to study molecular mechanisms underlying the MIRR-mediated cell response in health and disease (Figs. 1 and 5).[27-30,57,58] Within the model, upon antigen/ligand stimulation, these interactions represent one of three major driving forces of MIRR triggering signal (Table 1, Fig. 1B, see also Chapter 12). As suggested by the SCHOOL model, specific blockade of the interreceptor CYTO homointeractions between MIRR signaling subunits by CYTO-targeted agents or site-specific point mutations within the dimerization/oligomerization interfaces prevents formation of competent signaling oligomers (Figs. 1 and 5) and initiation of a MIRR-mediated cell response. Similar to Target I, the intra-MIRR TM interactions, modulation of the inter-MIRR homointeractions between particular signaling cytoplasmic domains might allow us to modulate the ligand-induced cell response. In addition, our ability to selectively prevent the formation of signaling oligomers of particular subunit(s) might also prove to be an important tool in functional studies of MIRRs. 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 CYTO interactions. As mentioned above,

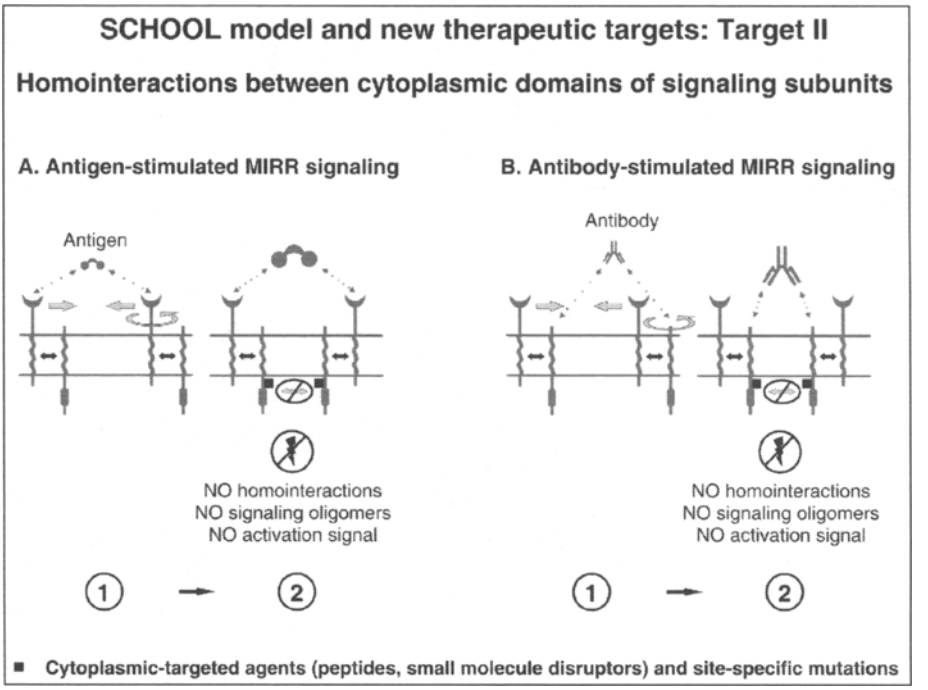

Figure 5. Target II. Cytoplasmic homointeractions between MIRR signaling subunits. This is a simplified graphical illustration of the molecular mechanisms underlying proposed intervention by cytoplasmic-targeted agents (peptides and their derivatives, small molecule disruptors of protein-protein interactions, etc) and site-specific mutations. Specific blockade of homointeractions between signaling subunits is proposed to prevent formation of signaling oligomers, thus inhibiting antigen-dependent immune cell activation (A). Within the model, in contrast to transmembrane-targeted agents (Target I, Fig. 2), stimulation of MIRRs with cross-linking antibodies to signaling subunit in the presence of cytoplasmic-targeted agents should not result in receptor triggering and cell activation (B). The proposed cytoplasmic-targeted strategies can be used not only to inhibit but also to modulate MIRR-mediated transmembrane signal transduction, thus modulating the immune response (see main text for details). Abbreviations and symbols as in Figure 1.

methods of computational design, synthesis and optimization of peptides and peptidomimetics as well as high-throughput screening techniques to search for the relevant mutations or small molecule disruptors are currently developed and well-established,[1-11,56,217-221] thus making the proposed CYTO-targeted approach both feasible and of great fundamental and clinical value.

Importantly, in contrast to TM-targeted agent-affected MIRRs that can be still activated by specific antibodies (Fig. 2B), stimulation of CYTO-targeted agent-affected MIRRs with specific antibodies that trigger cell activation should not result in MIRR triggering and generation of the activation signal (Fig. 5).

Thus, I suggest that like intra-MIRR TM interactions, the interreceptor CYTO homointeractions between MIRR signaling subunits represent extremely important points of control in MIRR triggering and cell activation. Since now we can use the SCHOOL model to design the CYTO-targeted agents effective in inhibition and/or modulation of MIRR-mediated TM signaling (Figs. 1 and 5, see also Chapter 12)[27-29] and have a powerful and well-controlled influence upon MIRR-mediated cell activation, thus controlling the immune response. The relevant

CYTO-targeted agents for any particular member of MIRR family can be readily designed using the SCHOOL model and our knowledge about structural organization of this receptor.

Evidence: Cytoplasmic Agents and Immune Cell Activation

Since homooligomerization of the MIRR signaling subunit CYTO domains was discovered[31] and these CYTO homointeractions were suggested to represent an important therapeutic target,[27-29] no direct experimental evidence has been reported to support this hypothesis. However, there is a growing line of indirect evidences indicating the importance of CYTO domains in functionally relevant homooligomerization of other receptors in vivo and demonstrating that the SCHOOL model-driven MIRR CYTO-targeted strategy using a variety of CYTO-targeted agents and/or mutations (Fig. 5) is technologically feasible and can be readily applied in both fundamental and clinical applications. These findings are mostly related to the field of SR triggering and TM signaling and will be described below.

Mutations

Fas (CD95, APO-1, TNFRSF6) is a tumor necrosis factor (TNF) receptor superfamily member that directly triggers apoptosis and contributes to the maintenance of lymphocyte homeostasis and prevention of autoimmunity.[222] Although Fas-associated death domain (FADD) and caspase-8 have been identified as key intracellular mediators of Fas signaling, it is not clear how recruitment of these proteins to the Fas death domain (DD) leads to activation of caspase-8 in the receptor signaling complex.[222,223] Recently, ligand-induced formation of surface receptor oligomers has been reported for Fas receptor.[224] A cytoplasmic DD of this SR, upon ligand stimulation, binds to the homologous DD of the adaptor protein FADD and homooligomerizes, thus initiating the caspase signaling cascade (Fig. 6A). Interestingly, an autoimmune lymphoproliferative syndrome-linked mutation in Fas cytoplasmic domain (T225K) impairs receptor oligomerization and inhibits Fas-mediated signaling but retains the ability to interact with FADD (Fig. 6A).[224] This suggests that homointeractions between signaling cytoplasmic tails themselves play an important role in ligand-induced surface receptor oligomerization and subsequent signaling.

This interesting finding supports the proposed MIRR CYTO-targeted strategy and provides a promising direction for future research. One can also hypothesize that similar mutations located in the CYTO domains of MIRR signaling subunits might occur naturally in MIRR-mediated disorders and disturb the homooligomerization interface(s), thus preventing formation of competent signaling subunit oligomers and MIRR triggering.

Cytoplasmic Peptides and Peptidomimetics

There is growing line of evidence indicating that CYTO peptides and peptidomimetics can be successfully used to target CYTO hetero- or homointeractions between entire protein molecules or the CYTO domains of TM proteins.[164,225-230] This means that once we can identify a new promising therapeutic CYTO target, it is technologically feasible to design, synthesize and use the relevant peptide-based agents, peptidomimetics and small molecules (or screen for the appropriate agents by using high throughput screening assays). Selected examples of CYTO-targeted agents used to inhibit CYTO protein-protein interactions, thus modifying the functional response, are considered in more detail below.

Myeloid differentiation factor 88 (MyD88) is a critical adaptor protein that recruits signaling proteins to TLR/IL-1 receptor (IL-1R) superfamily and thus plays a crucial role in the signaling pathways triggered by these receptors in innate host defense.[231,232] A critical event in MyD88-trgiggered signaling pathway is homodimerization of MyD88 mediated by its TLR/IL-1R translation initiation domain (TIR) that is able to heterodimerize with the receptor and homodimerize with another MyD88 molecule (Fig. 6B).[228,229,232] Dimerization of MyD88 favors the recruitment of downstream signaling molecules such as two IL-1R-associated kinases (IRAKs): IRAK1 and IRAK4 (Fig. 6B). Recently, eptapeptides that mimic the BB-loop region of the conserved TIR domain of MyD88, have been shown to effectively inhibit homodimerization with either the isolated TIR or full-length MyD88 (Fig. 6B).[229] The authors also demonstrated

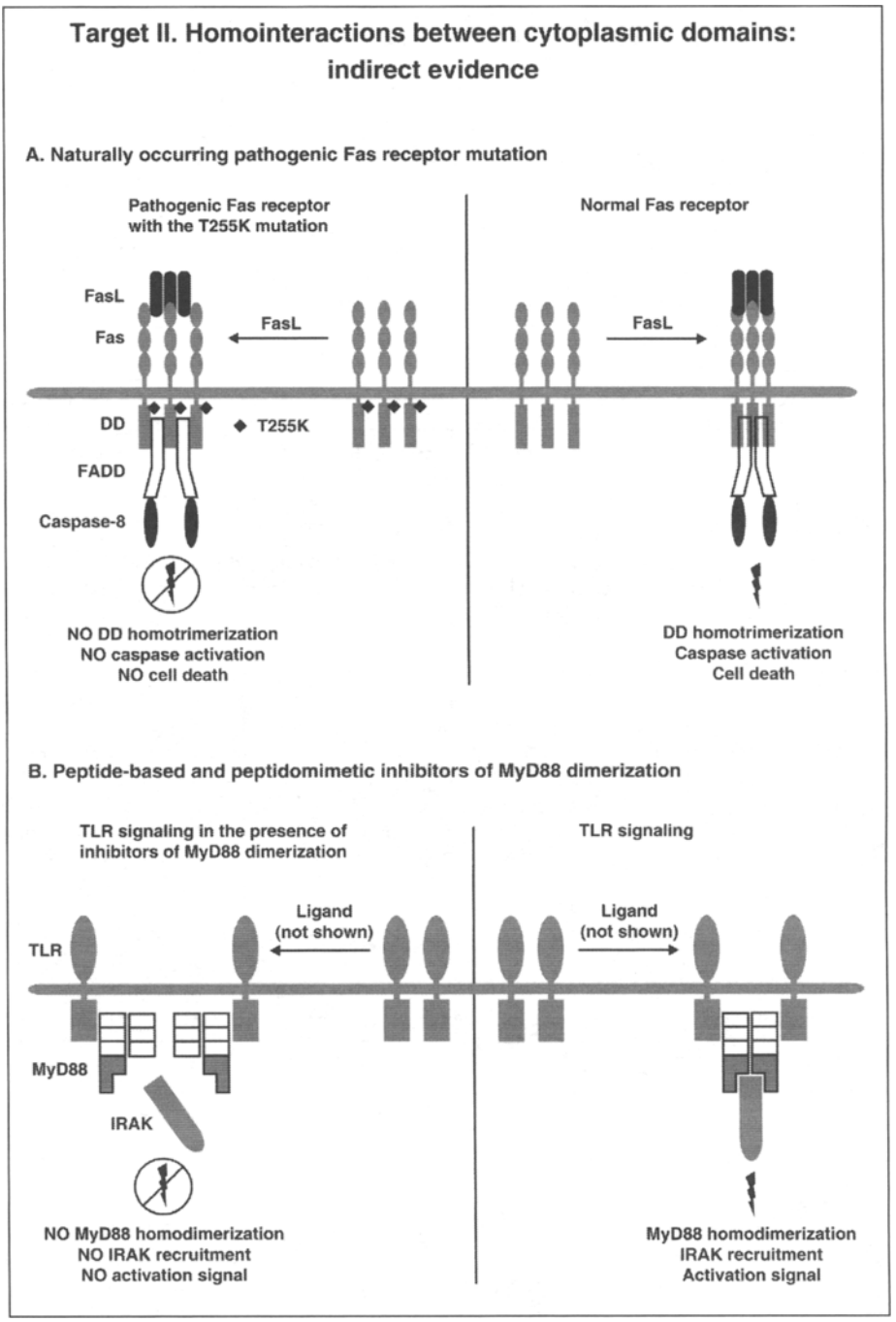

Figure 6, legend viewed on following page.

Figure 6, viewed on previous page. Indirect evidence for importance of the proposed cytoplasmic-targeted strategy. A) Fas apoptosis signaling by a normal Fas receptor and the receptor with the Fas T225K mutation that naturally occurs in patients with the autoimmune lymphoproliferative syndrome (ALPS). In contrast to all other ALPS-associated Fas DD mutations, this pathogenic mutation specifically disrupts homooligomerization of the cytoplasmic tails of the receptor but retains the ability to interact with FADD [Siegel RM, Muppidi JR, Sarker M et al. SPOTS: signaling protein oligomeric transduction structures are early mediators of death receptor-induced apoptosis at the plasma membrane. J Cell Biol 2004; 167:735-744]. As shown, the blockade of the cytoplasmic homointeractions does not allow full caspase-8 activation and apoptosis induction, thus revealing these protein-protein interactions as a therapeutic target. Abbreviations: FasL, Fas Ligand; DD, Death Domain; FADD, Fas-associated Death Domain protein. B) TLR signaling in the absence or presence of peptide-based and peptidomimetics inhibitors of MyD88 dimerization. As reported [Loiarro M, Capolunghi F, Fanto N et al. Pivotal Advance: Inhibition of MyD88 dimerization and recruitment of IRAK1 and IRAK4 by a novel peptidomimetic compound. J Leukoc Biol 2007; 82:801-810; Loiarro M, Sette C, Gallo G et al. Peptide-mediated interference of TIR domain dimerization in MyD88 inhibits interleukin-1-dependent activation of NF-κB. J Biol Chem 2005; 280:15809-15814], cell-permeable analogs of MyD88 peptides derived from the TIR domain of MyD88 as well as a synthetic peptidomimetic compound effectively inhibit homodimerization of MyD88 TIR domains, significantly reducing IL-1 signaling in vitro and dose-dependently inhibiting IL-1β-induced production of IL-6 in treated mice. This suggests that inhibition of MyD88 homodimerization in the cytoplasmic milieu may have therapeutic potential.
Abbreviations: TLR, toll-like receptor; MyD88, myeloid differentiation factor 88; IRAK, interleukin-1 (IL-1) receptor-associated kinase; TIR, toll/IL-1 receptor domain.

that a cell permeable analog of MyD88 eptapeptide inhibits homodimerization of MyD88 TIR domains in an in vitro cell system and significantly reduces IL-1 signaling, indicating that the MyD88 homodimerization interface is a good target for specific inhibition of MyD88-mediated signaling in vivo.[229]

Importantly, a synthetic peptidomimetic compound modeled after the structure of a heptapeptide in the BB-loop of the MyD88-TIR domain has been shown very recently to inhibit MyD88 dimerization in coimmunoprecipitation experiments.[228] This effect is specific for homodimerization of the TIR domains and does not affect homodimerization of the DDs. The agent causes inhibition of IL-1β-mediated activation of NF-κB transcriptional activity.[228] After oral administration, the compound results in dose-dependent inhibition of IL-1β-induced production of IL-6 in treated mice.[228] In addition, it suppresses B-cell proliferation and differentiation into plasma cells in response to CpG-induced activation of TLR9, a receptor that requires MyD88 for intracellular signaling.[228] These data indicate that the peptidomimetic compound studied blocks IL-1R/TLR signaling by interfering with MyD88 homodimerization. This suggests that inhibition of MyD88 homodimerization in the CYTO milieu by peptide-based agents or peptidomimetics may have therapeutic potential in treatment of chronic inflammatory diseases.[228]

These findings strongly support basic principles of the SCHOOL model-suggested strategy with its new point of intervention to inhibit/modulate MIRR triggering and the immune response, the MIRR signaling subunit CYTO homointeractions. As with TM-targeted strategy, our current understanding of MIRR structure and the nature and specificity of antigen/ligand-induced homointeractions between receptor signaling subunits not only allows us to inhibit these protein-protein interactions but also to modulate the interactions by a sequence-based approach using corresponding peptides and/or their derivatives. Peptidomimetics and small molecules can be used for these purposes, as well. Strengthening/weakening and/or selective inhibition of the association between particular signaling subunits might allow us not to inhibit, but rather to modulate the ligand-induced cell response. In addition, selective functional inhibition of particular signaling subunits represents an invaluable tool in studies of MIRR-mediated TM signaling and cell activation.

As another example, the processes by which Nef mediates the redistribution of CD80 and CD86 in human monocytic cells can be considered.[164] The endocytic mechanism used to trigger

internalization of CD80 and CD86 is known to involve Nef binding to the CYTO tails of these target proteins.[164] In an inhibition assay, synthetic peptides corresponding to the CYTO domains of CD80 or CD86 have been demonstrated to inhibit Nef binding to the same peptides immobilized on polystyrene plates.[164] Introduction of these CYTO peptides into Nef-expressing U937 cells using the Chariot reagent at 4°C causes substantial reduction in the loss of CD80 or CD86, respectively, from the cell surface of Nef-expressing cells,[164] thus proving the principal feasibility and the utility of the CYTO-targeted strategy suggested by the SCHOOL model.

Interestingly, unlike wild-type Nef, the Nef D123G mutant has been shown to lose its ability to mediate efficient internalization of cell-surface CD80 or CD86, or bind to the CYTO peptides of CD80 or CD86.[164] As mentioned before, mutation of a conserved D123 residue affects the ability of Nef to form dimers and results in impairment of Nef biological functions such as MHC class I downmodulation and enhancement of viral infectivity, indicating that the oligomerization of Nef may be critical for its multiple functions.[163] In this regard, I suggest that the impaired function of the Nef D123G mutant with regard to downmodulation of CD80/CD86 can be explained by its inability to form oligomers. If true, this means that the rational design of antiviral agents that are able to target CYTO homointeractions in Nef oligomers may represent an attractive target in the CYTO milieu, not only with regard to Nef-mediated modulation of TCR triggering and TM signaling, but also with respect to other Nef biological functions.

Peptide-based CYTO-targeted strategy has been also successfully applied to modulate outside-in TM signaling mediated by the platelet receptors such as GPIb/IX/V,[225] GPIIb[226] and the megakaryocyte- and platelet-specific integrin αIIbβ3.[227]

The platelet GPIb/IX/V receptor plays a key role in platelet adhesion at sites of vascular damage through its interaction with subendothelial-bound von Willebrand factor (VWF).[233,234] However, despite the crucial role that the GPIb/IX/V receptor complex plays in hemostasis, the molecular mechanisms of its signaling are not completely understood. The GPIb/IX/V complex consists of four subunits, namely, GPIbα, GPIbβ, GPIX and GPV. An amino acid sequence in the CYTO domain of the GPIbβ subunit between residues R151 and A161 has been shown to be highly conserved across species and plays an important physiological role.[225] It has been also reported[225] that a synthetic CYTO-targeted agent, the cell-permeable palmitylated peptide corresponding to this sequence, completely inhibits low-dose thrombin- and ristocetin-induced aggregation in washed platelets, significantly reduces thromboxane (TXA) production in platelets stimulated by thrombin compared with collagen, substantially decreases activation of the integrin αIIbβ3 in response to thrombin and significantly reduces the adhesion of washed platelets to VWF under static conditions and the velocity of platelets rolling on VWF. This demonstrates an effective impact of this peptide-based CYTO-targeted agent on platelet function in terms of rolling velocity, adhesion, spreading, signaling to αIIbβ3 and aggregation.

The integrin αIIbβ3 plays an important role in hemostasis mediating platelet adhesion, aggregation and bidirectional signaling.[235,236] Little is known about the molecular mechanisms underlying the regulation of αIIb-mediated outside-in signaling. Recently, it has been shown that this signaling is enhanced in platelets of a patient lacking the terminal 39 residues of the β3 CYTO domain, as detected by thromboxane production and granule secretion and requires ligand cross-linking of αIIbβ3 and platelet aggregation.[227] A synthetic CYTO-targeted agent, the cell-permeable palmitylated β3 peptide corresponding to the CYTO sequence R724-R734, has been demonstrated to effectively and specifically inhibit this outside-in signaling,[227] thus supporting basic principles and feasibility of the SCHOOL model-suggested CYTO-targeted strategy.

All integrin α subunits are known to contain a highly conserved KXGFFKR motif in their CYTO domains that plays a crucial role in the regulation of integrin affinity for their ligands.[226,237-239] A synthetic CYTO-targeted agent, the palmitylated peptide corresponding to the K989-R995 sequence of the CYTO domain of the platelet integrin GPIIb (αIIb) subunit has been shown to specifically induce platelet activation and aggregation equivalent to that of strong agonists such as thrombin.[226] The authors conclude that this lipid-modified peptide imitates the CYTO domain of GPIIb and, in a highly specific and effective manner, initiates parallel but independent signaling

pathways, one leading to ligand binding and platelet aggregation and the other to intracellular signaling events such as TXA2 synthesis and secretion.[226]

An example of using a synthetic peptide to inhibit protein-protein homointeractions in the intracellular milieu has been recently reported in studies of Ebola virus (EBOV), a filovirus that causes sporadic outbreaks of a fatal hemorrhagic fever in Africa.[230,240] Viral protein 30 (VP30), one of seven structural proteins of this enveloped virus,[240] is the constituent of the nucleocapsid and represents an EBOV-specific transcription activation factor.[241] The essential role of homooligomerization for the function of VP30 and the significance of the self-assembly of VP30 for viral transcription and propagation have been recently reported.[230] Interestingly, it has been also shown that the homooligomerization of VP30 can be dose dependently inhibited by a 25-mer peptide derived from the presumed oligomerization interface region.[230] Importantly, when this peptide is transfected into EBOV-infected cells, the peptide inhibits viral replication, suggesting that inhibition of VP30 oligomerization represents a target for EBOV antiviral drugs.[230] This confirms that, as proposed by the SCHOOL model for MIRR-mediated TM signaling and cell activation,[27-29] protein-protein homodimerization/homooligomerization interface(s) can represent an important point of intervention in the CYTO milieu and be targeted by synthetic peptides, their derivatives and peptidomimetics.

Another potential application of the CYTO-targeted strategy involves the use of CYTO-targeted agents to modulate TLR4 signaling. This receptor is activated by monophosphoryl lipid A, derived from the active moiety (lipid A) of bacterial endotoxin (lipopolysaccharide, LPS). As recently demonstrated,[242] LPS binds to a secreted glycoprotein MD-2, which in turn binds to TLR4 and induces aggregation and signal transduction. It has been also shown that TLR4 can form homodimers.[243] Despite both TLR4 monomers and dimers are able to activate NF-κB, this activation is significantly enhanced upon homodimerization.[243] However, NF-κB activation by TLR4 monomer, but not homodimer, is completely inhibited by dominant negative MyD88, suggesting that TLR4 homodimers and monomers can activate NF-κB through different mechanisms.[243] Using the protein complementation assay, a novel method to detect protein-protein interactions in vivo,[244] the TLR4 homodimerization has been shown to be mediated by the TLR4 CYTO domain.[245] I suggest that, similar to other applications mentioned above, CYTO-targeted agents can be used to modulate TLR4-mediated signaling and cell activation, thus modulating the host immune response to LPS.

Conclusions

Despite numerous models of MIRR signaling suggested for particular MIRRs and a growing interest in targeting MIRR signaling as a potential treatment strategy for many immune disorders, the molecular mechanisms that underlie MIRR triggering and subsequent TM signal transduction were unknown for a long time, preventing our improved understanding of these fundamentally important processes and therefore the development of novel pharmacological approaches.

Discovery of an unusual and unique biophysical phenomenon, the existence of specific homointeractions between the intrinsically disordered CYTO domains of MIRR signaling subunits,[31,33] defined the last piece in the puzzle of MIRR triggering and TM signaling and led me to the development of a general model of MIRR-mediated immune cell activation, the SCHOOL model.[27-30,57,58] Suggesting MIRR triggering as an outcome of ligand-induced interplay between three major driving forces represented by well-defined protein-protein interactions that strikingly fall within the similar micromolar affinity range and are characterized by relatively rapid kinetics, the model finally unravels a long-standing mystery of MIRR-mediated TM signal transduction. Importantly, assuming that the molecular mechanisms underlying TM signaling and cell activation mediated by all receptors that belong to the MIRR family are similar, the SCHOOL model can be readily applied to any particular member of this receptor family. In doing so, the model suggests molecular mechanisms for the vast majority of unexplained immunological observations accumulated to date (see also Chapter 12) and reveals novel universal therapeutic targets for a diverse variety of disorders mediated by immune cells, thus opening new horizons in both funda-

mental and clinical research in different fields such as immunology, structural biology, virology, hematology and others.

My central hypothesis is that the similar structural architecture of the MIRRs dictates similar mechanisms of MIRR triggering and subsequent signaling and cell activation and therefore suggests the existence of similar therapeutic targets in seemingly unrelated diseases. This makes possible the development of novel pharmacological approaches as well as the transfer of clinical knowledge, experience and therapeutic strategies between various immune disorders. In addition, this hypothesis significantly improves our understanding of the immunomodulatory activity of many human viruses. Thus, the lessons learned from the SCHOOL model and viral pathogenesis indicate that a general drug design approach may be used to treat a variety of different and seemingly unrelated immune diseases. The model unraveled the striking similarity of the molecular mechanisms underlying immunomodulatory activities of the TCR TM peptides first introduced by Manolios et al in 1997[78] and viral fusion peptides that appeared to be used by different viruses not only to entry target cells but also to modulate and escape the host immune response. This suggests the possibility to design, synthesize and apply highly specific and effective therapeutic agents and strongly supports the feasibility, utility and both fundamental and clinical importance of the TM-targeted strategy suggested by the SCHOOL model.

Application of this model to the platelet collagen receptor GPVI has already resulted in the development of a novel concept of platelet inhibition and the invention of novel platelet inhibitors. Importantly, the similar basic principles based on our current knowledge of the structural assembly of MIRRs and the molecular mechanisms of MIRR signaling suggested by the SCHOOL model were used to explain immunomodulatory activity of TCR TM peptides and to design, synthesize and apply new GPVI-targeted platelet inhibitors. Again, within the model, a similar approach can be applied to any particular receptor of the MIRR family and therefore to any disease or medical conditions mediated by this receptor. This is not only a comprehensive example of the usability and predictive power of the SCHOOL model but also supports my central hypothesis in the context of our ability to develop general pharmacological approaches and transfer clinical knowledge, experience and therapeutic strategies between seemingly disparate immune-mediated diseases.

In summary, I would like to highlight that the SCHOOL model (described in Chapter 12 in more detail) provides a set of basic principles underlying MIRR-mediated signaling and indicates that a general drug design approach could be used to treat many different, seemingly unrelated, immune diseases. Considering the multiplicity and diversity of the MIRRs involved in the pathogenesis of numerous human diseases, the proposed model can contribute significantly to the improvement of existing therapies and the design of new therapeutic strategies for malignancies, diverse immune system disorders, including those with infections caused by various viruses and other MIRR-mediated medical conditions.

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