CHAPTER 12

Signaling Chain Homooligomerization (SCHOOL) Model

Alexander B. Sigalov*

Abstract

Multichain immune recognition receptors (MIRRs) represent a family of surface receptors expressed on different cells of the hematopoietic system and function to transduce signals leading to a variety of biologic responses. The most intriguing and distinct structural feature of MIRR family members is that extracellular recognition domains and intracellular signaling domains are located on separate subunits. The biochemical cascades triggered by MIRRs are understood in significant detail, however, the mechanism by which extracellular ligand binding initiates intracellular signal transduction processes is not clear and no model fully explains how MIRR signaling commences.

In this Chapter, I describe a novel mechanistic model of MIRR-mediated signal transduction, the signaling chain homooligomerization (SCHOOL) model. The basic concept of this model assumes that the structural similarity of the MIRRs provides the basis for the similarity in the mechanisms of MIRR-mediated transmembrane signaling. Within the SCHOOL model, MIRR triggering is considered to be a result of the ligand-induced interplay between (1) intrareceptor transmembrane interactions between MIRR recognition and signaling subunits that stabilize and maintain receptor integrity and (2) interreceptor homointeractions between MIRR signaling subunits that lead to the formation of oligomeric signaling structures, thus triggering the receptors and initiating the signaling cascade. Thus, the SCHOOL model is based on specific protein-protein interactions—biochemical processes that can be influenced and controlled. In this context, this plausible and easily testable model is fundamentally different from those previously suggested for particular MIRRs and has several important advantages. The basic principles of transmembrane signaling learned from the SCHOOL model may be used in different fields of immunology and cell biology to describe, explain and predict immunological phenomena and processes mediated by structurally related but functionally different membrane receptors. Important applications of the SCHOOL model in clinical immunology, molecular pharmacology and virology are described in the Chapters 20 and 22 of this book.

Introduction

Immune cells respond to the presence of foreign antigens with a wide range of responses, including the secretion of preformed and newly formed mediators, phagocytosis of particles, endocytosis, cytotoxicity against target cells, as well as cell proliferation and/or differentiation. Antigen recognition by immune cells is mediated by the interaction of soluble, particulate and cellular antigens with an array of membrane-bound signaling receptors. Key among these receptors is the family of

*Alexander B. Sigalov—Department of Pathology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester 01655, MA, USA. Email: alexander.sigalov@umassmed.edu

Multichain Immune Recognition Receptor Signaling: From Spatiotemporal Organization to Human Disease, edited by Alexander B. Sigalov.

structurally related but functionally different multichain immune recognition receptors (MIRRs) that are expressed on many different immune cells, including T-and B-cells, natural killer (NK) cells, mast cells, macrophages, basophils, neutrophils, eosinophils, dendritic cells and platelets.[1,2] Figure 1 shows typical examples of MIRRs including 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). For more information on the structure and function of these and other MIRRs, I refer the reader to Chapters 1-5 of this book and recent reviews.[3-22]

A distinct but common structural characteristic of MIRRs is that the extracellular recognition (or ligand-binding) domains and the intracellular signaling domains of these multisubunit complexes are intriguingly located on separate subunits (Figs. 1 and 2). The MIRR ligand-binding subunits are integral membrane proteins with small intracellular domains that are themselves inert with regard to signaling. Signaling is achieved through the association of the ligand-binding chains with signal-transducing subunits that contain in their cytoplasmic domains one or more copies of the immunoreceptor tyrosine-based activation motifs (ITAMs) with two appropriately spaced tyrosines ($YxxL/Ix_{6-8}YxxL/I$; where x denotes nonconserved residues)[23] or the YxxM motif,[24,25] found in the DAP10 cytoplasmic domain[25] (Fig. 1). The association of the MIRR subunits in resting cells is driven mostly by the noncovalent transmembrane (TM) interactions between recognition and signaling components (Fig. 2) and plays a key role in receptor assembly, integrity and surface expression.[2,9-11,13,18,21,26-37]

The MIRR-mediated activation signal can be divided into four parts: (1) the extracellular recognition of a multivalent antigen resulting in the aggregation, or clustering, of the MIRRs, (2) MIRR triggering and TM signal transduction, (3) phosphorylation of the ITAM or YxxM tyrosine residues by protein tyrosine kinases (PTKs) and activation of specific intracellular pathways and (4) the activation of genes in the nucleus. The extracellular recognition of an antigen, the MIRR-triggered biochemical cascades and the mechanisms of gene activation are understood in significant detail. However, the mechanism by which the MIRR transduces ordered information such as antigen recognition from outside the cell via receptor TM and juxtamembrane (JM) regions into intracellular biochemical events (part 2) is not well defined. In other words, the key question remains unanswered: what is the molecular mechanism by which clustering of the extracellular recognition domains of MIRRs leads to receptor triggering and tyrosine phosphorylation of the intracellular ITAMs or YxxMs, thus initiating specific pathways and resulting in immune cell functional outcomes? It is also not known how this putative mechanism can explain the intriguing ability of immune cells to discern and differentially respond to slightly different ligands.

MIRR-mediated signal transduction plays an important role in health and disease making these receptors attractive targets for rational intervention in a variety of immune disorders.[7,10,13,38-42] Thus, future therapeutic strategies depend on our detailed understanding of the molecular mechanisms underlying MIRR triggering and subsequent TM signal transduction. In addition, knowing these mechanisms would give us a new handle in dissecting the basic structural and functional aspects of the immune response.

Despite numerous models of MIRR-mediated TM signal transduction suggested for particular MIRRs (e.g., TCR, BCR, Fc receptors, NK receptors, etc.), no current model fully explains at the molecular level how ligand-induced TM signal transduction commences. As a consequence, these models are mostly descriptive, do not explain mechanistically a vast majority of the specific processes behind "outside-in" MIRR signaling and do not reveal clinically important points of therapeutic intervention. In addition, since the term "MIRR" was first introduced in 1992[1] and MIRR-triggered signaling pathways were hypothesized to be similar,[1,43-46] no general mechanistic model of MIRR-mediated immune cell activation has been suggested up to date. This impedes our

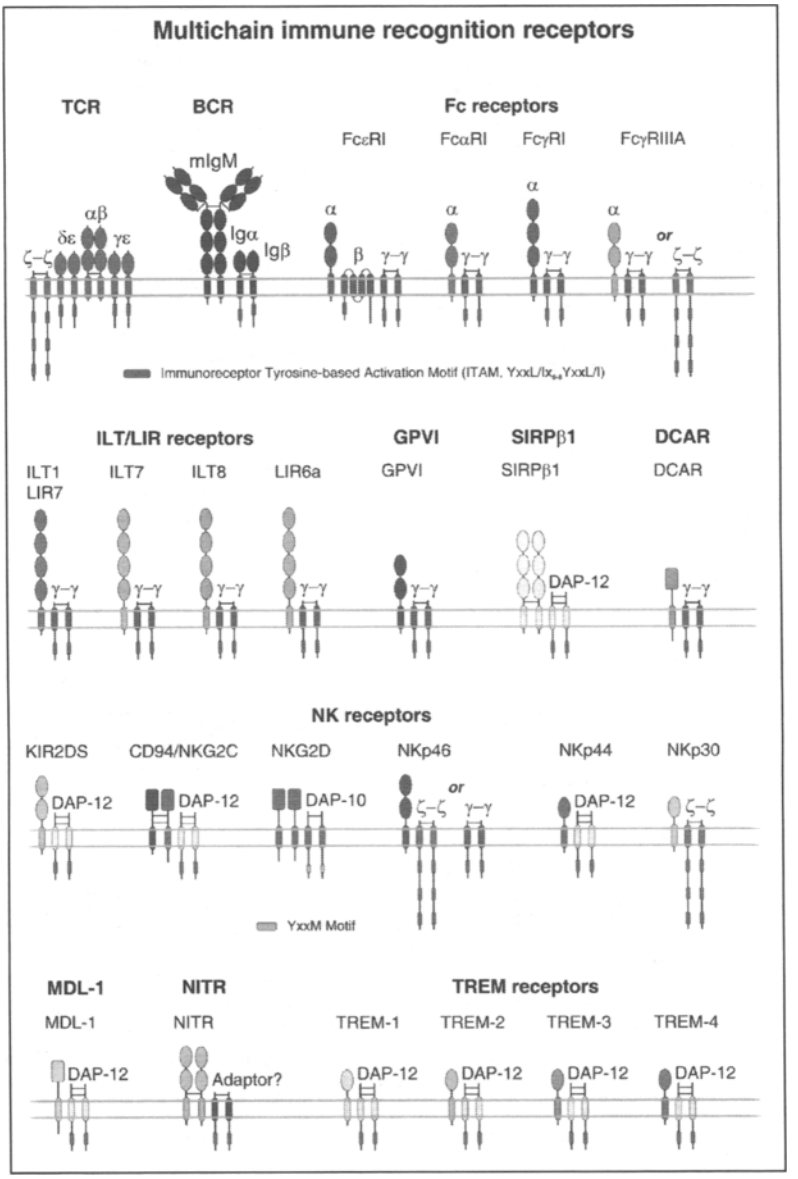

Figure 1. Multichain immune recognition receptors (MIRRs). Schematic presentation of the MIRRs expressed on many different immune cells including T- and B-cells, natural killer cells, mast cells, macrophages, basophils, neutrophils, eosinophils, dendritic cells and platelets. Abbreviations: TCR, T-cell receptor; BCR, B-cell receptor; ILT, Ig-like transcript; LIR, leukocyte Ig-like receptor; GPVI, glycoprotein VI; DNAX adapter proteins of 10 and 12 kD, DAP-10 and DAP-12, respectively; signal regulatory protein, SIRP; dendritic cell immunoactivating receptor, DCAR; NK, natural killer cells; KIR, killer cell Ig-like receptor; myeloid DAP12-associating lectin 1, MDL-1; novel immune-type receptor, NITR; TREM receptors, triggering receptors expressed on myeloid cells. Reprinted 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.

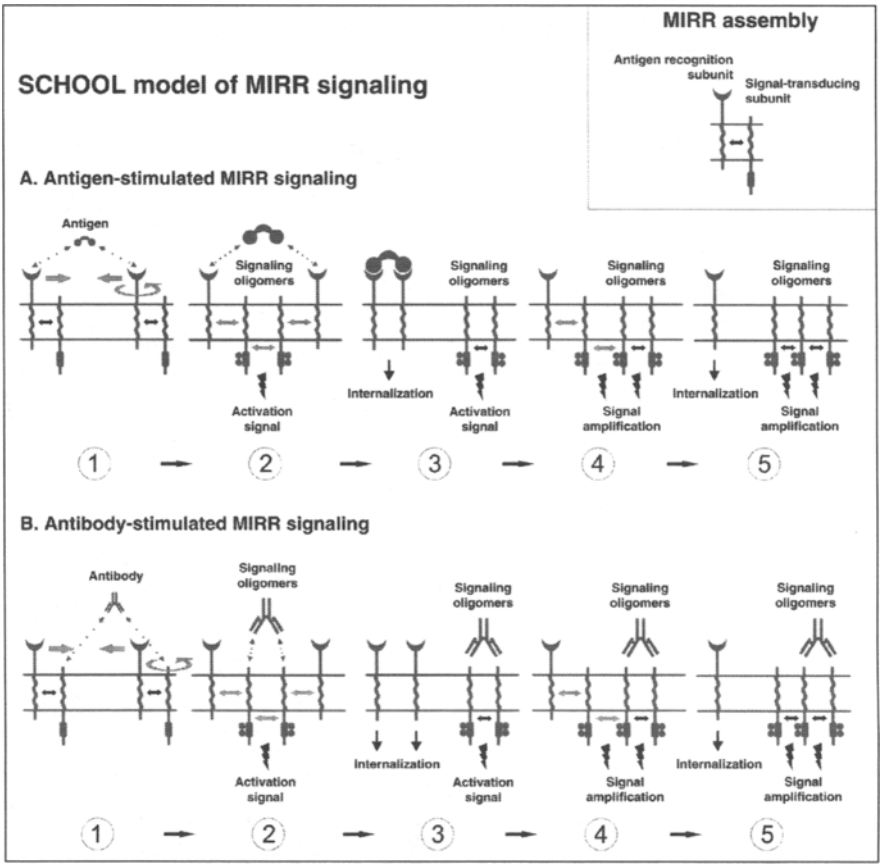

Figure 2. Structural assembly of MIRRs (the inset) and the signaling chain homooligomerization (SCHOOL) model of MIRR signaling. The model proposes that formation of competent MIRR signaling subunit oligomers driven by the homooligomerization of signaling subunits is necessary and sufficient to trigger the receptors and induce transmembrane (TM) signal transduction and downstream sequence. MIRR clustering and receptor reorientation (stage 1) induced by ligand (A) or antibodies to MIRR recognition (not shown) or signaling (B) subunits (e.g., anti-TCRα, anti-TCRβ, anti-CD3ε, anti-Igβ, etc.) lead to formation of a dimeric/oligomeric intermediate in which signaling chains from different receptor units start to trans-homointeract and form signaling oligomers (stage 2). All interchain interactions in this intermediate are shown by light gray arrows reflecting their transition state. Upon formation of signaling oligomers, protein tyrosine kinases phosphorylate the tyrosine residues in the cytoplasmic signaling motifs, the immunoreceptor tyrosine-based activation motifs (ITAMs, shown as gray rectangles), that leads to generation of the activation signal, dissociation of signaling oligomers and internalization of the engaged MIRR binding domains (stages 2 and 3). Next, the signaling oligomers sequentially homointeract with the relevant signaling subunits of nonengaged receptors resulting in formation of higher-order signaling oligomers, thus propagating and amplifying the signals (stages 4 and 5). This also leads to the release and subsequent internalization of the nonengaged ligand-binding MIRR subunits. Small solid black arrows indicate specific intersubunit hetero- and homointeractions between TM and cytoplasmic domains, respectively. The TM interactions between MIRR antigen recognition and signal-transducing subunits have a key role in receptor assembly and integrity on resting cells while cytoplasmic homointeractions represent a main driving force of MIRR triggering. Circular arrows indicate ligand-induced receptor reorientation. Phosphate groups are shown as gray circles.

advanced understanding of the immune response and even more important, prevents the potential transfer of therapeutic strategies between seemingly disparate immune disorders.

Central Hypothesis

My central hypothesis is that the similar structural architecture of the MIRRs dictates similar mechanisms of MIRR triggering and subsequent TM signal transduction 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 immune disorders. In addition, this hypothesis significantly improves our understanding of the immune modulatory activity of human viruses such as human immunodeficiency virus (HIV) and human cytomegalovirus (CMV) and assumes that the lessons learned from viral pathogenesis can be used for the development of new therapeutic approaches.

In this chapter, I describe a novel mechanistic model of MIRR triggering and subsequent TM signal transduction, the signaling chain homooligomerization (SCHOOL) model.[2,47-49] The model suggests similar mechanisms for all known MIRRs (Fig. 2) and reveals new therapeutic targets in MIRR triggering pathways[2,49] that are described in Chapter 20. Important applications of this model in basic and clinical virology are considered in Chapter 22.

SCHOOL Model of MIRR Signaling

Ligand-induced dimerization/oligomerization of cell surface receptors is frequently employed in signal transduction,[50,51] with dimerization of receptors being the most frequent. For MIRRs, binding of multivalent but not monovalent ligand and subsequent receptor clustering/oligomerization are also thought to be required for induction of the signaling cascade, with MIRR dimerization constituting a necessary and sufficient step for triggering cell activation.[43,50,52-76] Thus, the receptor dimer can be considered as an "elementary stimulatory unit" leading to an immune response. Intracellularly, the need for MIRR dimerization is consistent with the suggested structural hypothesis of cross-phosphorylation[43,77] that assumes that the kinase(s) responsible for catalyzing ITAM Tyr residue phosphorylations exist associated with the receptors, however, for steric reasons they cannot phosphorylate tyrosine residues on chains of the same receptor complex. Upon dimerization/oligomerization, these kinases phosphorylate the tyrosines of a distinct receptor complex (cross-phosphorylation, or transphosphorylation), thus triggering the receptor.[43]

Basic Concepts and Principles

The SCHOOL model suggests that formation of competent MIRR signaling subunit oligomers, rather than MIRR oligomers per se, is necessary and sufficient to trigger the receptors and induce TM signal transduction and the downstream signaling sequence.[2,47,48] Within the model, this oligomerization is driven by the specific homotypic interactions I discovered in 2001 and first reported in 2004,[78] of intrinsically disordered cytoplasmic domains of MIRR signaling subunits. Later, the natural propensity of the TCR ζ cytoplasmic domain to homodimerize has been confirmed by other investigators.[79] Surprisingly, in contrast to other unfolded proteins,[80] the homodimerization/oligomerization of the unstructured protein molecules studied is not accompanied by a structural transition to a folded form,[78,81,82] thus opposing the generally accepted view on the behavior of intrinsically disordered proteins and representing a very unique and unusual biophysical phenomenon itself. Hypothesizing a crucial physiological role of these homointeractions in MIRR triggering and cell activation, the SCHOOL model[2,47,48] indicates that MIRR engagement by multivalent antigen or anti-MIRR-signaling subunit antibodies (e.g., anti-CD3ε or anti-Igβ antibodies for TCRs and BCRs, respectively) leads to receptor clustering coupled with a multi-step structural reorganization driven by the homooligomerization of MIRR signaling subunits (Fig. 2). The model also assumes that the diversity of the immune cell response is partly provided by the combinatorial nature of MIRR-mediated signaling. Signal diversification may be achieved through different patterns of MIRR signaling subunit oligomerization[2,47,48] in

combination with distinct activation signals provided by different MIRR signaling modules[83-94] and/or different ITAMs located on the same signaling module (e.g., TCR ζ chain).[95] Thus, according to the model, the more signaling subunits that are in the MIRR complex, the higher is the diversity of immune cell functional outcomes in response to different ligands.

Within the proposed model, MIRR triggering is considered to be the result of the ligand-induced interplay between (1) intrareceptor TM interactions that stabilize and maintain receptor integrity,[2,9-11,13,18,19,21,26-37,96-100] and (2) interreceptor homointeractions between the cytoplasmic domains of MIRR signaling subunits[78,81] that lead to the formation of oligomeric signaling structures and dissociation of the signaling subunits from their respective recognition subunits. Formation of these signaling oligomers triggers phosphorylation of ITAMs, thus initiating the signaling cascade.

Main Stages of MIRR Triggering/Signaling

According to the SCHOOL model, MIRR triggering and TM signaling induced by binding to multivalent antigen or anti-MIRR antibodies can be divided into five main stages (Fig. 2):

1. *Dynamic lateral clustering and rotation.* Antigen/antibody brings two or more MIRRs together in sufficient proximity and correct relative orientation toward each other to promote the interreceptor homointeractions between signaling subunits. Once initiated, these homointeractions weaken the intrareceptor TM interactions between recognition and signaling subunits.
2. *Intermediate complex formation.* A signaling-competent oligomeric intermediate complex is formed, bringing together the cytoplasmic domains of the signaling subunits, protein kinases and various adaptor/effector proteins, to create a competent, activated receptor complex. In the signaling subunit oligomers formed, the ITAM Tyr residues become phosphorylated, thus starting the signaling cascade.
3. *Dissociation and internalization.* Signaling oligomers dissociate from the engaged ligand-recognition subunits, which are internalized.
4. *Interactions with nonengaged receptors, lateral signal propagation and amplification.* 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.
5. *Dissociation and internalization.* Signaling oligomers dissociate from the nonengaged ligand-recognition subunits, which later are internalized.

This plausible and easy testable model is fundamentally different from those previously suggested for particular MIRRs (TCR, BCR, FcεRI, GPVI, etc.) and has several important advantages.

First, this model is based on specific protein-protein interactions—biochemical processes that constitute the foundation for the majority of cell recognition and signal transduction processes in health and disease. Protein-protein interactions can be influenced and controlled[101] and specific inhibition and/or modulation of these interactions provides a promising novel approach for rational drug design, as revealed by the recent progress in the design of inhibitory antibodies, peptides and small molecules.[102,103] Second, assuming that the general principles underlying MIRR-mediated TM signaling mechanisms are similar, the SCHOOL model can be applied to any particular receptor of the MIRR family, including but not limiting to those shown in (Fig. 1). Third, based on specific protein-protein interactions, the model reveals new therapeutic targets for the treatment of a variety of disorders mediated by immune cells (see Chapter 20). Fourth, this model represents a powerful tool to dissect molecular mechanisms of MIRR-mediated signaling and related cell functional outcomes in response to antigen. Finally, an important application of the 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. These and other clinically important features of the SCHOOL model will be discussed in more detail in Chapters 20 and 22.

Main Restraints of MIRR Triggering/Signaling Imposed by the SCHOOL Model

Interactions between TM helixes of recognition and signaling MIRR subunits maintain receptor integrity in unstimulated cells and determine the relative positions of these subunits in the receptor complex (angles, distances, etc.), thus dictating the overall geometry and topology of MIRRs.[2,9-11,13,18,21,26-37,99,100] Within the SCHOOL model, the overall structural architecture of MIRRs, in combination with the requirement to initiate interreceptor homointeractions between MIRR signaling subunits (Fig. 2), impose several restraints for MIRR triggering:

- 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 and
- sufficient lifetime of an individual receptor in MIRR dimers/oligomers.

The importance of these factors for productive MIRR triggering is strongly supported by a growing body of evidence.[4,32,54,56,62,67,68,73,90,104-134] Interestingly, relative receptor orientation also has been shown to be critically important for the activation of other dimeric/oligomeric TM receptors.[135-139]

Therefore, the restraints imposed by the model play an especially important role during the first stage of MIRR triggering (Fig. 2), at which 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 might explain the ability of MIRRs to differentially activate a variety of signaling pathways depending on the nature of the stimulus.

Within the proposed model, the signaling oligomers formed dissociate from ligand-binding chains, which later are internalized (Fig. 2, stage 3). This mechanism provides a structural and mechanistic basis for our improved understanding of many immunological phenomena, such as adaptive T-cell tolerance or anergy,[140-143] differential biological role of CD3 chains,[144] ligand- or antibody-induced exposure of a cryptic polyproline sequence in the cytoplasmic domain of CD3ε,[113,145-147] BCR desensitization,[148-151] cytomegaloviral (CMV) escape from NK attack[152] and others. The dissociation mechanism allows the initially formed signaling oligomers to sequentially homointeract with the signaling subunits of nonengaged receptors (Fig. 2, stages 4 and 5) resulting in formation of higher-order signaling oligomers, thus propagating and amplifying the signal. Also, this leads to dissociation and subsequent internalization of the nonengaged ligand-binding subunits. Thus, as with bacterial chemoreceptors,[153-155] the SCHOOL model-based mechanism of MIRR-mediated cell activation suggests spreading (propagation) activation signal from engaged to nonengaged receptors within receptor clusters.

Finally, it should be noted that similar spatial, structural and temporal restraints are imposed within the proposed model for MIRR triggering by not only antigen (Fig. 2A) but also the anti-MIRR (Fig. 2B) antibodies such as anti-TCRα, anti-TCRβ, anti-CD3ε, anti-Igβ and others. This may explain differential immune cell functional outcomes mediated by MIRRs depending on the specificity of the antibodies.[107,108,111-113,156-160]

Supportive Evidence

I developed the SCHOOL model as a general model for the structurally related MIRR family members, namely, for all receptors that have extracellular recognition and intracellular signaling

modules located on separate receptor subunits. For this reason, in order to support the main concept and assumptions of the model, I use a rapidly growing body of evidence coming from studies of various MIRRs.

Clustering and Proximity

In order to trigger the MIRR, within the SCHOOL model, two or more receptors should be clustered/oligomerized in sufficient proximity to each other to initiate homointeractions between signaling subunits with subsequent formation of competent signaling subunit oligomers (Fig. 2).[2,47,48] To date, these spatial restraints imposed by the model on MIRR triggering and initiation of the signaling cascade are consistent with the experimental data observed.

T-Cell Receptor

There is a growing line of structural, biophysical and cellular evidence suggesting that ligand-specific TCR oligomerization is critical to generate a functional signal and that TCR dimerization constitutes a necessary and sufficient step for triggering T-cell activation (see also Chapters 6 and 11).[52,57-60,65-68,72,74,105,161-167] These findings clearly demonstrate that dimeric/oligomeric antigens are able to stimulate T-cells, whereas monomeric fail to do so. Interestingly, a correlation between antigenicity and repetitiveness of major histocompatibility complex (MHC)-bound peptides (pMHCs) has been also shown.[105] For dimeric pMHC class I and II complexes, the ability to trigger T-cells has been reported to decrease with increasing length of the connecting spacer.[168,169] Recently, by testing well-defined dimeric, tetrameric and octameric pMHC complexes containing rigid polyproline spacers of different lengths, it has been also shown that their ability to activate cytotoxic T-lymphocytes decreases as the distance between their subunit MHC complexes increases.[104] Intriguingly, the preTCR complex has been shown to form oligomers spontaneously, in a ligand-independent manner.[170,171] This oligomerization is mediated by specific charged residues in the extracellular domain of the preTCRα chain and is necessary and sufficient to induce autonomous signaling and stimulate preTCR function.[170,171] Recently, TCR-coreceptor complexes from naïve or activated CD4+ or CD8+ T-cells have been found to exist as either dimers or tetramers, whereas no monomers or multimers were detected.[167]

B-Cell Receptor

Similar to the TCR-induced signaling, the BCR activation signal is shown to be triggered by cross-linking of receptors through multivalent antigen,[4,54,56,114-116,172] thus confirming the necessity of BCR clustering for competent signaling and cell activation (see also Chapter 6). Interestingly, as it has been shown in 2007 for the preBCR, the ability of the purified recombinant receptor to dimerize indicates that accessory protein(s) are not required for dimerization and by extension, preBCR signaling through multimerization can occur in a ligand-independent fashion.[55] Showing strong similarities to the observations reported for the preTCR-mediated signaling,[170,171] these findings are well consistent with the molecular mechanisms proposed by the SCHOOL model.

Fc Receptors

Multichain Fc receptors, such as FcεRI, FcαRI, FcγRI and FcγRIII, have been known to initiate cell signaling following interactions with multivalent ligands that induce their clustering (see also Chapter 3).[29,62,63,77,88,111,117-121,173-175] FcεRI aggregates as small as dimers have been reported to be capable of providing an effective activation signal to cause mediator secretion.[111] Using a set of chemically well defined ligands of valences 1-3, the magnitude of the cellular response has been demonstrated to dramatically increase as the valency of a ligand raises from two to three.[62] Trivalent ligands with rigid double-stranded DNA spacers have been shown to effectively stimulate FcεRI-mediated degranulation responses in a length-dependent manner, providing direct evidence for receptor transphosphorylation as a key step in the mechanism of signaling by this receptor, whereas long bivalent ligands with flexible spacers has been demonstrated to be very potent inhibitors of mast cell degranulation stimulated by multivalent antigen.[122] In other studies, the spacing of receptors in ligand-specific FcεRI aggregates has been also shown to be important for generating the activation signal.[174]

NK Receptors

Multivalent ligand-induced receptor oligomerization is presumed to be a common mechanism for initiating NK receptor-mediated signaling.[123-125] Also, structural and biochemical studies of NKG2D receptor[69,176,177] have demonstrated that the receptor exists as a dimer not only in the crystal but also at the surface of unstimulated NK cells. However, in contrast to preBCR and preTCR, this ligand-independent dimerization does not trigger the receptor and initiate downstream signaling, suggesting that dimerization is necessary but not sufficient to trigger the receptor.

Glycoprotein VI

Collagen, a natural ligand of GPVI, contains the GPVI-binding GPO (glycine-proline-hydroxyproline) motifs that form about 10% of the fibrillar collagen sequence and thus represent multiple GPVI-binding sites.[178] Using a series of collagen-like model peptides containing GPO motifs of increasing length within $(GPP)_n$ sequences, Smethurst et al[179] have demonstrated that platelet aggregation and protein tyrosine phosphorylation can be induced only by cross-linked peptides that contain two or more GPO triplets. Multimeric snake venom proteins such as convulxin also strongly activate GPVI in a multimer size-dependent manner,[180,181] suggesting that clustering of GPVI receptors through multiple binding events leads to activation. Structural studies have revealed a dimeric state of GPVI and 2 parallel grooves on the GPVI dimer surface as collagen-binding sites with an orientation and spacing of these grooves precisely matching the dimensions of an intact collagen fiber.[64] These findings provide a structural basis for GPVI signaling mechanisms in which collagen-induced GPVI clustering triggers a signaling cascade via the FcRγ-chain. In 2007, GPVI–FcRγ-chain oligomerization on the surface of unstimulated platelets has been directly demonstrated,[71] suggesting that, like dimerization of NKG2D, oligomerization of GPVI is necessary but not sufficient to trigger the receptor.

Other MIRRs

Human TREM-1 receptor has been shown to exist as a "head-to-tail" dimer in crystal, suggesting that the dimeric TREM-1 most likely contains two distinct ligand-binding sites.[70] High-avidity ligands are thought to trigger TREM-1 and TREM-2, suggesting that formation of multivalent ligand-receptor complexes is a necessary step in TREM-1-mediated cell activation.[18,125] Murine paired immunoglobulin-like receptor (PIR)-A and human leucocyte immunoglobulin-like receptor (LILR)-A2 (ILT/LIR7) complexed with the FcRγ signaling chain through their transmembrane domains are also required to be clustered by a multivalent ligand in order to initiate TM signaling.[19,182] Recently, it has been shown that integrin signaling in neutrophils and macrophages requires ITAM-containing adaptors, DAP-12 and FcRγ, suggesting that integrin signaling-mediated activation of cellular responses in these cells proceeds by an MIRR-like mechanism.[183] Homomeric associations involving transmembrane domains have been reported to represent a driving force for integrin activation, thus providing a structural basis for the coincidence of ligand-induced integrin clustering and activation.[184,185]

Orientation

A rapidly growing body of experimental evidence strongly supports the importance of interreceptor orientation within ligand-specific MIRR dimers/oligomers for receptor triggering and generation of an activation signal. These findings are in good agreement with the orientational restraints imposed by the SCHOOL model on the initiation of interreceptor homointeractions between signaling subunits in order to trigger MIRRs (Fig. 2).[2,47,48] Suggesting the importance of relative orientation,[2,47,48] the model explains for the first time why random encounters of MIRRs by lateral diffusion or oligomeric forms of MIRRs existing in unstimulated cells[44,69,186-189] and platelets[71] do not result in MIRR triggering and cell activation.

T-Cell Receptor

Despite direct biophysical measurements of the interreceptor relative orientation in ligand-specific TCR dimers/oligomers have not yet been performed, several lines of evidence indicate that relative orientation plays an important role in TCR-mediated cell activation. Using monoclonal

antibodies (mAbs) specific for the TCR, it has been shown that T-cell activation does not correlate with the affinity of the mAbs but rather with the recognized epitope.[160] In other studies, triggering of different epitopes of the TCR-CD3-ζ_2 receptor complex has been also reported to induce different modes of T-cell activation,[156-159] suggesting that TCR signaling is not a simple on-off switch through cross-linking/clustering. In addition, high concentrations of anti-TCR, but not anti-CD3, induce a proliferative response without antibody cross-linking.[158] Also, anti-TCR and anti-CD3 have been demonstrated to be different in their capacity to induce responsiveness to interleukin-4 (IL-4)[159] and in their requirement for costimulatory signals.[156] Yang and Parkhouse have reported that stimulation of T-cells with a panel of anti-CD3 mAb recognizing different epitopes has differential functional consequences, demonstrating for the first time that differences in activation mechanisms not only exist between TCR and CD3, but also between epitopes within CD3 and postulating that occupancy of different CD3 epitopes may result in different degrees of conformational change in the receptor complex.[107] In thymocytes, only anti-TCRβ Ab but not anti-TCRα reagents cause long-term TCR downmodulation.[108] Using three-dimensional fluorescence quantitation methods, signaling-induced reorientation of T-cell receptors that cannot be mediated by simple passive diffusion has been shown to take place during immunological synapse formation.[190] In 2007, a change in the orientation of the TCR with respect to the membrane induced by binding to pMHC has been proposed to play an important role in TCR signaling.[90] Conclusions about the importance of interreceptor orientation in the ligand-specific TCR dimers/oligomers have been also made in 2007 by Minguet et al[191] who suggested the so-called permissive geometry model of TCR signaling (see also Chapter 11). In contrast to these studies, Cochran et al[168] have reported that intermolecular orientation is not critical for triggering T-cell activation. However, to address this issue, the authors have used in their studies pMHC dimers coupled via flexible chemical cross-linkers that do not prevent rotation of pMHC molecules around their long axis. This assumption is further supported by the authors' findings that estimated distances for the used cross-linkers in fully extended conformations (50, 70 and 90 Å) did not correlate with the apparent hydrodynamic diameter values experimentally determined for the corresponding crosslinked pMHC dimers in the surprisingly narrow range of 70 to 75 Å.[168] Thus, these dimers cannot be considered as conformationally constrained suggesting a lack of control over the interreceptor orientation in these experiments.[168]

The three-dimensional structures of the three A6-TCR/peptide/HLA-A2 complexes that generate very different T-cell signals have been found to be remarkably similar to each other and to the wild-type agonist complex, suggesting that different signals are not generated by different ligand-induced conformational changes in the αβTCR.[192] This is in agreement with the SCHOOL model proposing that different signaling oligomers can be formed and therefore different T-cell signals can be generated depending on the intermolecular relative orientation in the ligand-specific TCR dimers/oligomers rather than ligand-induced extracellular conformational changes.[2,47,48]

In summary, a vast majority of the experimental findings reported so far strongly support an importance of interreceptor relative orientation in ligand-specific TCR clusters for TCR triggering and cell activation.

B-Cell Receptor

BCRs have been proposed and confirmed to organize into oligomeric clusters on the B-cell surface.[44,187-189] The observed basal BCR clustering does not result in receptor triggering and subsequent cell activation suggesting that, like with TCR and EpoR, a member of cytokine receptor superfamily,[135] the oligomerization of the BCR is necessary but not sufficient for receptor activation[189] and that interreceptor relative orientation in the BCR dimers/oligomers plays an important role in receptor triggering. The differential effects of the point mutations in various parts of the TM sequence of BCR membrane Ig (mIg) have been reported to differentially affect B-cell activation induced by mono- or polyvalent anti-mIg antibodies, thus providing more evidence for importance of correct intermolecular orientation in BCR signaling.[35]

Fc Receptors

As shown for FcεRI, it is not only the number of crosslinked FcεRIs that determines the magnitude of mediator secretion-causing signal induced by different mAbs, but also the relative orientation of receptors within the produced dimers, thus suggesting the importance of the orientational restraint in ligand-specific FceRI dimers/oligomers for generating competent activation signal.[111,112,119,126,127,193] Further, in the IgA receptor, FcαRI, a positively charged arginine residue within the TM domain of ligand recognition α chain promotes association with the signaling FcRγ chain.[99] Studies on signaling through mutants of the FcαRI have shown that a vertical relocation of this TM positive charge does not have any significant effect on proximal and distal receptor functions, whereas a lateral transfer of the positive charge completely abrogates these functions.[32] A possible explanation for these findings is that a vertical relocation of the noncovalent electrostatic bond does not change interreceptor relative orientation within the receptor dimers/oligomers formed upon multivalent ligand stimulation while lateral transfer does.

NK Receptors

Existence of dimeric NKG2D receptor complexes in both NKG2D crystals and at the surface of unstimulated NK cells[69,176,177] suggests that not only dimerization but also relative orientation of receptors within ligand-specific NKG2D dimers/oligomers plays an important role in receptor triggering.

Glycoprotein VI

Similar to NKG2D receptor complexes, GPVI has been found to form a back-to-back dimer in the GPVI crystal[64] and to exist in an oligomeric state on the surface of unstimulated platelets,[71] suggesting an important role of interreceptor relative orientation within these oligomers in GPVI signaling.

Other Receptors

The type I TM glycoprotein gp130 is the commonly used signaling receptor chain of all IL-6-type cytokines (i.e., IL-6).[194] Intriguingly, signal transduction via IL-6 requires not only gp130 homodimerization but also the correct relative orientation of the gp130 cytoplasmic regions in ligand-specific receptor dimer, suggesting that subtle changes in the orientation of the receptor chains relative toward each other might result in very different responses.[138] Enforcement of gp130 dimerization is not sufficient for receptor activation but additional conformational requirements have to be fulfilled.[195] Thus, like dimerization of the MIRRs, dimerization of the cytokine receptors by monoclonal antibodies is in most cases not enough to induce signal transduction.[196]

Interestingly, many members of the tumor necrosis factor receptor superfamily were once thought to signal through ligand-induced receptor trimerization. However, recently, these receptors have been shown to exist as pre-assembled oligomers on the cell surface.[197,198] This suggests that, upon the binding of the trimeric ligand, not only oligomerization (trimerization) of these single-chain receptors but also the correct intermolecular relative orientation within trimers plays a crucial role in signaling.

Oligomerization of Signaling Subunits

According to the SCHOOL model, homooligomerization of the cytoplasmic domains of MIRR signaling subunits drives formation of competent signaling oligomers, thus leading to triggering of the receptor and initiation of the signaling cascade (Fig. 2).[2,47,48] Importantly, this homooligomerization also plays a crucial role in amplification and lateral propagation of the activation signal(s) (Fig. 2). The model also suggests that depending on the nature of stimuli, different signaling subunits can be oligomerized and become phosphorylated, thus triggering distinct signaling pathways and resulting in different functional outcomes.[2,47-49] The experimental data obtained to date for different MIRRs strongly support the main concept of the SCHOOL model.

The ability of TCR ζ cytoplasmic domain to oligomerize was first reported in 2004[78] and later confirmed in cell studies on the activity of membrane-anchored chimeric β_2m/peptide molecules fused with the cytoplasmic domain of ζ chain.[79] Similarly, the propensity of the BCR Igα and Igβ

signaling subunits to oligomerize[78] has been recently confirmed and demonstrated to result in the ability of the BCR Igα/Igβ heterodimer to assemble into oligomers.[199]

Both in vitro and in vivo studies have shown that dimerization of CD3ε is critical and sufficient to substitute for a preTCR signal and drive double-positive transition, suggesting that the property of the preTCR responsible for β-selection is the autonomous formation of oligomers, which brings CD3 signaling subunits in close proximity to each other.[170,171] These findings confirm the ability of CD3ε to dimerize, first reported in 2004 for the CD3ε cytoplasmic domain[78] and proves the physiological importance of this dimerization suggested by the SCHOOL model.[2,47-49]

As reported,[200] FcεRI signaling β and γ subunits independently dissociate from a ligand-binding α chain immediately after crosslinking with multivalent ligand. Moreover, these signaling subunits dissociate in the oligomerized form. Interestingly, only γ chains are oligomerized on surfaces of cells stimulated with a suboptimal concentration of antigen, while β chains remain dispersed.[200] In contrast, stimulation of cells with an optimal concentration of antigen results in the distinct oligomerization of both signaling subunits.[200]

In cytokine receptor signaling, dimerization of not just extracellular but rather cytoplasmic domains of the gp130 signaling subunit is critically required to trigger the receptor and initiate the signaling cascade.[138,195,196] Recently, ligand-induced formation of surface receptor oligomers has been reported for the Fas receptor.[201] This single-chain receptor has a cytoplasmic death domain (DD) that, upon receptor stimulation with a trivalent ligand, binds to the homologous DD of the adaptor protein Fas-associated death domain protein (FADD) and homotrimerizes, thus initiating the caspase signaling cascade. Interestingly, a mutation in Fas cytoplasmic domain (T225K) linked to autoimmune lymphoproliferative syndrome impairs receptor oligomerization and inhibits Fas-mediated signaling but retains the ability to interact with FADD.[201] This indicates that homointeractions between Fas cytoplasmic tails have an important role in the receptor triggering. Similarly, cytoplasmic domain-mediated dimerization of toll-like receptor 4 (TLR4) has been recently reported to play an important role in the TLR4 triggering and signal transduction.[202,203]

Dissociation

Within the SCHOOL model, dissociation of competent signaling oligomers from both engaged and nonengaged ligand-recognition subunits upon multivalent ligand stimulation, plays an important role in MIRR triggering, signal amplification and propagation and initiation of the signaling cascade (Fig. 2).[2,47,48] Experimental data accumulated to date strongly support this suggestion.

In activated T-cells, the CD3 and ζ signaling chains has been shown to independently dissociate from the remaining receptor subunits.[204-207] Further, TCRs lacking ζ are endocytosed more rapidly than completely assembled receptors,[208] in line with the SCHOOL model. For BCR, it has been reported that, upon binding of moderate- to low-affinity antigen, the Igα/Igβ subunits physically dissociate from mIg resulting in BCR desensitization.[148] Interestingly, although desensitized cells fail to respond to receptor ligation by a high dose of antigen or by anti-Igλ antibodies, the dissociated Igα/Igβ signaling complex retains signaling function if aggregated by anti-Igβ antibodies.[148] In this context, similar mechanisms are proposed by the SCHOOL model to be involved in the BCR desensitization,[148,149,151] T-cell clonal anergy[141,209,210] and in the inhibition of T-cell activation by the so-called TCR core peptide (CP).[211] The ligand-mediated physical dissociation of the activated BCR complex has been later confirmed in other studies.[212] In 2005,[213] using primary murine B-cells, it has been found that while >95% of the mIg is internalized following anti-Ig-induced aggregation, 20-30% of Igβ remains on the surface, suggesting that mIg and Igβ may function independently following the initial stages of signal transduction. As mentioned, upon crosslinking of the FcεRI with multivalent ligand, oligomerized signaling β and γ chains immediately dissociate from a ligand-recognition α chain.[200]

Duration of the Ligand-Receptor Contact

The SCHOOL model suggests that the multivalent 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 homointeractions

between signaling subunits, thus initiating the downstream signaling cascade (Fig. 2).[2,47,48] It should be noted that duration of the MIRR-ligand interaction generally correlates with the strength (affinity/avidity) of the ligand. Clearly, the strength of the ligand determines not only duration of the ligand-MIRR contact but also lifetime of an individual receptor in the engaged MIRR dimer/oligomer. These important aspects of the model are also consistent with the experimental data accumulated so far.

In T-cells, the results of multiple reports show a broad correlation between the duration of TCR-ligand interaction and ligand potency.[214-216] A similar interpretation is possible for the data on a revised model of kinetic proofreading in which the duration of TCR engagement regulates the efficiency with which signals trickle through the rapidly reversible early activation pathways to induce later responses[217] (see also Chapter 8). It is also known that the off-rate of ligand binding plays a role in determining the specificity of the TCR-generated signal in a population of T-cells that can discriminate between self and nonself in the thymus.[218] Also, the number of TCR ITAMs required for efficient positive or negative selection has been reported to vary depending upon the affinity of the TCR/ligand interaction.[219] In studies on T-cell activation by bacterial superantigens, a simple relationship between the affinity of the *Staphylococcus enterotoxin* C3 (SEC3)-TCR interaction and the functional responses has been proposed; stronger binding results in stronger T-cell responses.[220] As recently shown, short-lived pMHC ligands induce anergy in T-cell clones in vitro and specific memory T-cells in vivo.[221] Total signal strength has been demonstrated to determine the capacity of primed T-cells to respond to homeostatic cytokines, to survive cytokine withdrawal and to accumulate in vivo.[222] The strength of antigen stimulation is also known to regulate T-cell progression through thresholds of proliferation, differentiation and death.[223]

Similar to T-cells, the B-cell response to antigen varies as a function of antigen/BCR interaction affinity.[224] As demonstrated, above the threshold, concentration of antigen required to trigger a response decreases as the affinity increases.[224] BCR signal strength has been shown to determine B-cell fate.[225] Importantly, continuous receptor signaling of a defined amplitude appears to be critical for development and survival of mature B-cells.[226] It is also known that, upon binding of moderate- to low- but not high-affinity antigen, the Igα/Igβ subunits physically dissociate from mIg resulting in BCR desensitization.[148] A critical role of receptor affinity in antigen-driven selection of B-cell clones in vivo has been also suggested based on studies of stable B-cell transfectants.[227] Recently, the strength of the initial BCR-triggered activation signal has been proposed to finally determine the eventual duration of BCR signaling and the rate of its transmission through downstream pathways.[228]

A great body of evidence shows that the capacity of downstream signaling by an individual FcεRI depends on its capacity to remain in a cluster and is therefore influenced by the ligand affinity/avidity.[62,121,128-131,229] The ability of a similar signaling mechanism to trigger distinct FcεRI-mediated mast cell responses like mediator release and survival has been reported to be determined by the FcRγ signal strength or duration.[129,230] Interestingly, recent findings redefine FcαRI as a bifunctional inhibitory/activating receptor of the immune system that mediates both anti- and proinflammatory functions of IgA, depending on ligand multimericity and duration of multivalent ligand-induced receptor signaling.[132] In platelets, affinity/avidity of interaction of GPVI with collagen or convulxin has been suggested to play an important role in receptor signaling and GPVI-mediated platelet activation.[133,181]

For more information on the important role of the ligand-MIRR complex lifetime in MIRR triggering I refer the reader to Chapter 8 of this book and recent reviews.[216,229,231-233]

SCHOOL Model: Trinity of Description, Explanation and Prediction

Based on well-defined biochemical processes such as specific protein-protein interactions, the SCHOOL model represents the first general mechanistic model of MIRR signaling and can be also defined as a dynamic, continuous, spatially homogeneous, descriptive and explanatory model.[234] This model describes and explains molecular mechanisms and the main driving forces of TM signal transduction for functionally unrelated receptors that share a common organizing

principle—extracellular recognition module(s) and intracellular signaling module(s) are found on separate subunits and are noncovalently associated through their TM domains. Thus, the basic principles of TM signaling learned from the model can be used in different fields of immunology and cell biology to describe processes that are mediated by structurally related but functionally different membrane receptors.[2,47-49] Besides the ability to describe general principles of MIRR-mediated signal transduction, the SCHOOL model provides a mechanistic explanation for specific processes behind "outside-in" MIRR signaling that remain unclear. Since it was first published in 2004,[48] the model has also predicted several experimental observations that have been later reported for different immune cells.

By definition, the utility of scientific models is evaluated on their abilities to explain past observations, predict future observations and control events as well as on their simplicity, or even aesthetic appeal. The distinct features of the SCHOOL model demonstrating its utility are described in detail below for specific MIRRs (see also Chapters 20 and 22).

SCHOOL Model of TCR Signaling

Description

The TCR is a multisubunit complex composed of the ligand-binding clonotypic αβ heterodimer, as well as the heterodimeric CD3δε and CD3γε signaling components and the disulfide-linked ζ homodimer that contain one (ε, γ and δ) or three (ζ) ITAMs, respectively (Figs. 1 and 3; Chapter 1). This receptor complex provides an intriguing ability of T-cells to discern and differentially respond to MHC-bound peptides that can differ by only a single amino acid. The mechanism by which the precise ligand-binding specificities of the TCR are converted into the distinct intracellular signaling processes and diverse functional outcomes has been one of the most controversial topics in T-cell immunology. The SCHOOL model suggests not only the mechanism of TCR triggering and cell activation that can explain the majority of immunological phenomena observed experimentally but also proposes distinct ways to control and modulate the T-cell-mediated immune response.

The overall rigid geometry and topology of the TCR is defined by electrostatic interactions between TCRαβ TM domains and TM domains of different signaling dimers: CD3γε, CD3δε and ζ_2.[26,27,35] Interestingly, the TCR ζ subunit seems to have a unique and dynamic relationship with the TCR-CD3 complex since only this signaling homodimer appears to turn over independently from the rest of the TCR complex on the cell surface.[235] Assuming that different TCR signaling modules provide distinct signaling and T-cell functional outcomes,[47,48,83] the SCHOOL model of T-cell activation suggests[47,48] that depending on the nature of activating stimuli, two or more TCRs can be clustered to dimer/oligomer in different relative orientations that promote homointeractions between different signaling subunits. This results in formation of distinct CD3 and/or ζ signaling oligomers and their activation through the phosphorylation of the corresponding ITAM tyrosines (Fig. 3), thus initiating distinct signaling cascades and leading to distinct functional outcomes.

Within the model (Fig. 3), two or more TCRs are clustered to dimer/oligomer with sufficient interreceptor proximity upon binding with multivalent ligand and simultaneously rotate around the receptor axis perpendicular to the membrane to adopt a correct relative orientation toward each other, permissive of initiating the trans-homointeractions between ζ molecules. Until the ζ ITAM tyrosines are phosphorylated by protein tyrosine kinase (PTK), this process is reversible and its reversibility can depend on duration of the TCR-ligand contact that generally correlates with the strength (affinity/avidity) of the ligand and sufficient lifetime of a receptor in TCR dimers/oligomers. At this point of bifurcation, two alternative pathways (Fig. 3, stages IV and III) leading to partial or full T-cell activation, respectively, can take place depending on the nature of activating stimuli. As a result, either ζ or both ζ and CD3 signaling oligomers are formed with subsequent phosphorylation of ITAM tyrosines by PTKs and dissociation from remaining TCR-CD3 complexes or TCRαβ chains. At this irreversible stage, downstream signaling events are triggered. Later, the remaining TCR-CD3 complexes or TCRαβ chains are internalized. According to the proposed model, at least two different activation signals (shown in the Fig. 3

as signals A and B) can be provided from the ζ and CD3 signaling oligomers and both signals are required for full activation of T-cells. Thus, distinct signaling is achieved through ζ and CD3 signaling oligomers and/or through various combinations of signaling chains in oligomeric CD3 structures (Fig. 3). Then, the signaling oligomers formed from the initially engaged TCR dimer/oligomer can sequentially homointeract with the relevant signaling subunits of nonengaged TCRs resulting in formation of higher-order signaling oligomers with their subsequent phosphorylation and dissociation from ligand-binding subunits. This process leads to amplification and lateral propagation of the activation signal(s). Later, the remaining nonengaged TCR-CD3 complexes or TCRαβ chains are internalized.

Thus, in the context of the model, TCR clustering by the MHCs bound to agonist, partial agonist or antagonist peptides results in formation of receptor dimers/oligomers with similar intercreceptor proximity but different intermolecular orientation. This leads (or does not) to initiation of homointeractions between different signaling subunits with their subsequent oligomerization and activation, providing distinct signaling and T-cell functional outcomes. This mechanism is also proposed for T-cell activation mediated by other stimuli such as anti-TCRα, anti-TCRβ, anti-CD3ε, etc.

Comparison to Other Models

There exist numerous models of TCR triggering and their modifications, including but not limiting to a kinetic proofreading model,[217,231,233,236-240] serial triggering model,[110,241-244] serial encounter model,[245] conformational models,[44,113,145-147,191,246-253] permissive geometry model[191] and clustering[52,59,60,75,161,191] and segregation[254-256] models. Most of these models are discussed in detail in Chapters 6-11 of this book. However, despite the rapidly growing number of models and their modifications, no current model explains at the molecular level: (1) how ligand-induced TCR TM signaling commences and (2) how this process occurs differentially for altered ligands or in altered cellular contexts. Some of the models suggested so far were rejected in further studies, such as a conformational model that suggests a lipid-dependent folding transition of the TCR ζ cytoplasmic domain to be a molecular switch linking ligand-induced TCR clustering and phosphorylation of the ζ ITAM tyrosines.[249] Later studies have shown that binding of the ζ cytoplasmic domain to stable lipid bilayers is not accompanied by a structural transition to a folded form and that phosphorylated ζ is still able to bind to lipid, thus contradicting this model.[82] In addition, most of the current models have been developed by investigators to describe their own experimental data. As a consequence, these models are mostly descriptive and often fail by trying to explain most of the immunological data accumulated to date. Many of the models suggested to date simply describe a phenomenon but not the mechanisms underlying the phenomenon. Examples include clustering models[52,59,60,75,161,191] that describe a requirement for multivalent ligand to trigger TCR but do not explain the specific molecular mechanisms underlying those observations. Importantly, the lack of these mechanisms in a vast majority of the existing models does not permit to identify clinically important points of therapeutic intervention. Table 1 illustrates comparative features of the currently existing models and demonstrates how these distinctive models for the first time can be readily combined into one model, the SCHOOL model of TCR triggering and TM signaling.

Utility

The powerful ability of the SCHOOL model to describe, explain and predict TCR-related immunological phenomena, providing a mechanistic explanation at the molecular level, is illustrated in Table 2. Selected examples are also described below in more detail.

Clinically relevant TCR CP, or TCR mimic peptide, represents a synthetic peptide corresponding to the sequence of the TM region of the ligand-binding TCRα chain critical for TCR assembly and function (Chapter 16). This and similar TM peptides capable of inhibiting antigen-stimulated TCR-mediated T-cell activation were first reported in 1997.[257] Since that time, despite extensive basic and clinical studies of these peptides,[211,258-267] the molecular mechanisms of action of these clinically relevant peptides have not been elucidated until 2004 when the SCHOOL model was first introduced.[48] Within this model,[2,47-49] the TCR CP competes with the TCRα chain for

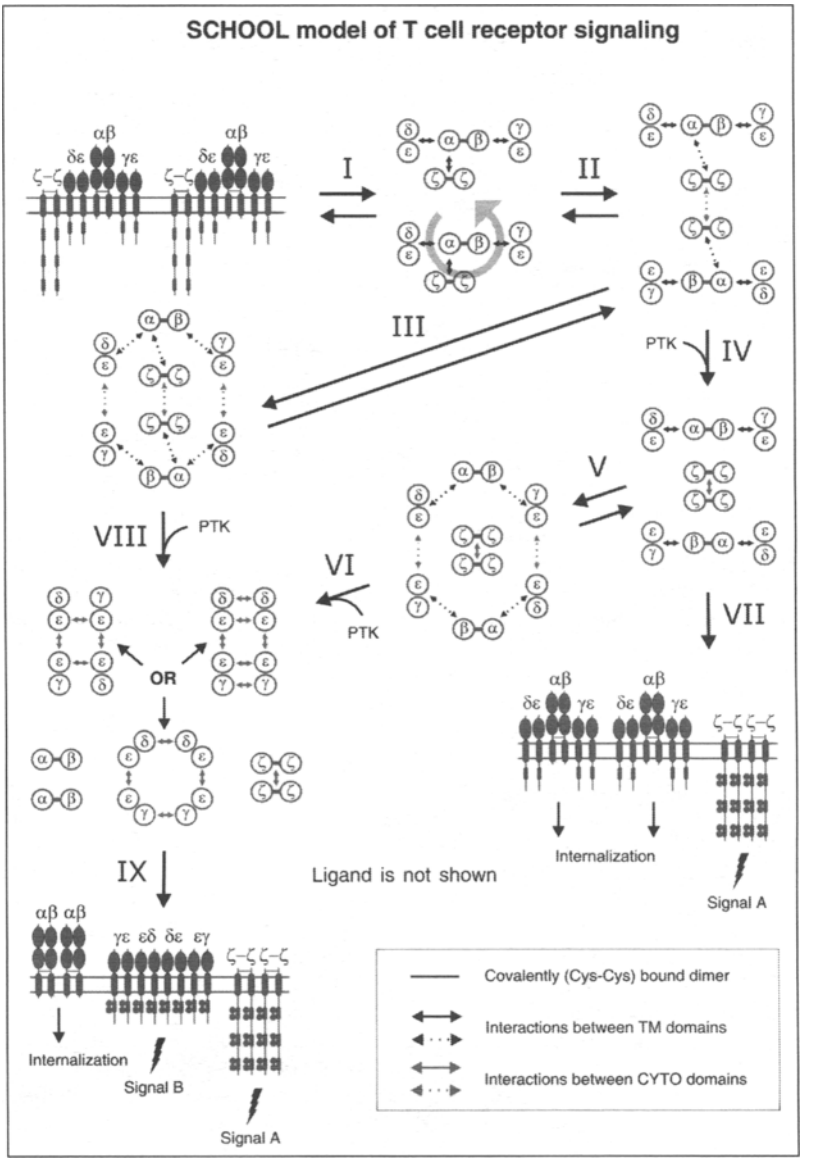

Figure 3. SCHOOL model of the T-cell receptor (TCR) activation. Immunoreceptor tyrosine-based activation motifs (ITAMs) are shown as gray rectangles. TCR-CD3-ζ components are represented as whole polypeptides and as a simplified axial view. All interchain interactions in intermediate complexes are shown by dotted arrows reflecting their transition state. Circular arrow indicates ligand-induced receptor reorientation. Interaction with multivalent ligand (not shown) clusters the receptors and pushes them to reorientate (I) and bring signaling subunits into a correct relative orientation and in sufficient proximity in the formed receptor oligomer (for illustrative purposes, receptor dimer is shown), thus starting the trans-homointeractions between ζ molecules (II). Then, two alternative pathways can take a place depending on the nature of activating stimuli. First is going through a stage IV resulting in formation of ζ_2 dimer (dimer of dimers) and phosphorylation of the ζ ITAM tyrosines, thus triggering downstream signaling events. Continued on next page.

Figure 3, continued from previous page. Then, the signaling ζ oligomers formed subsequently dissociate from the TCR-CD3 complex, resulting in internalization of the remaining engaged TCR-CD3 complexes (VII). This pathway leads to partial (or incomplete) T-cell activation. Alternatively, the intermediate complex formed at the stage II can undergo further rearrangements, starting trans-homointeractions between CD3 proteins (III) and resulting in formation of an oligomeric intermediate. Again, the stages I, II and III can be reversible or irreversible depending on interreceptor proximity and relative orientation of the receptors in TCR dimers/oligomers as well as on time duration of the TCR-ligand contact and lifetime of the receptor in TCR dimers/oligomers that generally correlate with the nature of the stimulus and its specificity and affinity/avidity. Next, in the signaling oligomers formed (III), the ITAM tyrosines undergo phosphorylation by PTKs that leads to generation of the activation signal, dissociation of signaling oligomers and internalization of the remaining engaged TCRαβ chains (VIII, XI). This pathway provides at least two different activation signals from the ζ and CD3 signaling oligomers (signals A and B), respectively and results in full T-cell activation. The distinct signaling through ζ and CD3 oligomers (or through various combinations of signaling chains in CD3 oligomeric structures) might be also responsible for distinct functions such as T-cell proliferation, effector functions, T-cell survival, pathogen clearance, TCR anergy, etc. In addition, the signaling oligomers formed can sequentially interact with the signaling subunits of nonengaged TCRs resulting in formation of higher-order signaling oligomers, thus amplifying and propagating the activation signal (not shown). Also, this leads to the release and subsequent internalization of the remaining nonengaged TCR complexes and/or TCRαβ chains (not shown). Abbreviations: PTK, protein tyrosine kinase. Phosphate groups are shown as filled gray circles. Reprinted from Trends Immunol, 25, Sigalov AB, Multichain immune recognition receptor signaling: Different players, same game? 583-589, copyright 2004 with permission from Elsevier.

binding to CD3δε and ζ hetero- and homodimers, respectively, thus resulting in disconnection and predissociation of the signaling subunits from the remaining receptor complex. The proposed mechanism is the only mechanism consistent with all experimental and clinical data reported up to date for TCR TM peptides and their lipid and/or sugar conjugates. The model also 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 the corresponding signaling subunits as well (see also Chapter 20).[2,47-49] This was recently confirmed experimentally[265,268] 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. Importantly, the SCHOOL model is the first model that not only clearly explains the molecular mechanisms of action of TCR TM peptides[2,47-49] (see also Chapter 20) but also extends 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[2,49,269] (Chapters 20 and 22).

Interestingly, the model suggests a molecular explanation for the apparent discrepancies in in vitro and in vivo activities of cell-permeable chemical inducers of dimerization.[270-272] In 1993, it has been reported that in vitro chemically induced dimerization/oligomerization of the TCR ζ cytoplasmic domain results in T-cell activation, as measured with a reporter gene assay.[270] Later, activation of a chimeric receptor, containing binding domains for chemical inducers of dimerization fused to the cytoplasmic tail of TCR ζ chain, after stimulation with chemical dimerizers in Jurkat cells has been confirmed to show tyrosine phosphorylation of the TCR ζ chain chimera, recruitment of phosphorylated Zap70 and generation of NFAT.[271] However, in vivo studies demonstrated that signaling did not lead to increased expression of activation markers, T-cell proliferation, or apoptosis.[271] The authors concluded that signaling through ζ alone is not sufficient to generate downstream events leading to full T-cell activation or thymocyte selection; instead, additional CD3 components must be required to induce a functional response in primary thymocytes and peripheral T-cells.[271] Within the model, formation of both CD3γε/δε and ζ signaling oligomers is needed to provide competent activation signal(s) resulting in full cell activation (signals A and B, Fig. 3). Formation of only ζ signaling oligomers leads to partial T-cell activation (signal A, Fig. 3).

Table 1. Comparative features of different models of TCR triggering

Model	Requirements/Restraints Imposed (+) or not (–) by a Model			
	Ligand Multivalency	Relative Interreceptor Orientation in TCR oligomers	Duration of Ligand-TCR Contact/Lifetime of TCR Oligomers	Ligand Affinity/Avidity
Kinetic proofreading	–	–	+	+
Serial triggering	–	–	+	+
Serial encounter	–	–	+	+
Conformational	–	–	+	+
Permissive geometry	+	+	–	–
Clustering	+	–	–	+
Segregation	–	–	+	+
SCHOOL	+	+	+	+

The model also explains the apparent discrepancy in CD3 TM peptide activity between in vitro and in vivo T-cell inhibition.[265] 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 (ε, δ and γ) TM peptides decrease signs of inflammation in an adjuvant-induced arthritis rat model in vivo and inhibit the immune response.[265] Within the SCHOOL model, the CD3δ and CD3γ TM peptides disconnect the corresponding signaling subunits (CD3δ and CD3γ, respectively) from the remaining receptor complex. Thus, these subunits do not participate in further processes upon antigen stimulation. On the other hand, the previously reported in vitro activation studies with T-cells lacking CD3γ and/or CD3δ cytoplasmic domains indicate that antigen-stimulated induction of cytokine secretion and T-cell proliferation are intact,[273-275] thus explaining the absence of inhibitory effect of the CD3δ and CD3γ TM peptides in the in vitro activation assays used.[265] However, in vivo deficiency either of CD3δ or CD3γ results in severe immunodeficiency disorders.[144,276-278] This could explain the inhibitory effect observed in the in vivo studies for all three CD3 TM peptides.[265] Thus, these experimental data confirm that our ability to selectively "disconnect" specific signaling subunits using the MIRR TM peptides in line with the SCHOOL model can provide a powerful tool to study MIRR functions and immune cell signaling.[2,49]

Interestingly, studies of T-lymphocytes expressing a TCR with a mutant TCR β TM domain have shown that upon antigen stimulation, these cells are similar to wild-type cells in terms of IL-2 secretion, IL-2 receptor expression and early activation and signaling events such as CD69 expression, Ca^{2+} flux and CD3ε and ζ phosphorylation, but are specifically defective in undergoing activation-induced cell death.[279] Considering that in the TCR-CD3-ζ_2 complex, the TCR β TM domain is critical for interaction with the CD3γε signaling heterodimer,[27] one can suggest the impaired association of the CD3γε with the TCR β chain in a mutant TCR. Upon antigen stimulation, this impaired (weakened) association prevents formation of CD3γε signaling oligomers and thus excludes CD3γ (but not CD3ε, because in the CD3εδ heterodimer of the TCR-CD3-ζ_2 complex, there is another CD3ε chain capable of signaling independently of CD3ε in the CD3γε) from further participation in signaling. Thus, within the model, only those signaling events that involve CD3γ (i.e., apoptotic response but not early activation and signaling events[274,275,280,281]) should be affected by a mutation of the TCR β TM domain. This is in a good agreement with the data reported.[279] Also, in this context, functional effect of this mutation should be and is very similar to the one observed by Collier et al for CD3γ TM peptide,[265] therefore providing more evidence for importance and utility of the proposed model and the MIRR TM peptides in studies on immune signaling.

The remarkable feature of the SCHOOL model is that it has a high predictive quality (Table 2 and Chapter 20) by generalizing molecular mechanisms of action and therefore potential therapeutic targets for all MIRRs.[2,47-49]

SCHOOL Model of FceRI Receptor Signaling: Description and Utility

Structurally, all Fc receptors can be divided into two major categories: single- (i.e., FcγRIIA and FcγRIIA) and multichain (i.e., FcεRI, FcαRI, FcγRI and FcγRIIIA) receptors. Multichain Fc receptors, in turn, can be divided into two subcategories: receptors that contain one (FcαRI, FcγRI, FcγRIIIA) or two (FcεRI) signaling subunits. To date, no general model has been suggested to explain at the molecular level how Fc receptor-mediated signaling commences.

As a general model of MIRR signaling, the SCHOOL model describes the molecular mechanisms underlying the receptor triggering for all multichain Fc receptors.[2,47,48] The model also suggests that the FcεRI receptor that contains two different signaling subunits, β and γ (or FcRγ), has more capabilities to induce distinct signaling pathways and, therefore, lead to different functional outcomes as compared to the Fc receptors that contain only one signaling subunit (FcRγ) (Fig. 1). Below I consider the SCHOOL model of FcεRI signaling in detail.

The FcεRI receptor consists of a ligand-binding α subunit and two kinds of signaling subunits, a β chain and disulfide-linked homodimeric γ chains (Figs. 1 and 4). It plays a pivotal role in the initiation of allergic reactions when antigen crosslinks IgE antibodies bound to FcεRI on tissue mast cells or blood basophils (Chapter 3).[40,282-284]

In resting cells, like with TCR, intrareceptor TM interactions between FcεRI α, β and γ chains define the overall rigid geometry and topology of the FcεRI.[29-31,33,37] Within the proposed model, upon stimulation with multivalent ligand, two or more FcεRIs are brought into close proximity and adopt a correct relative orientation, initiating the interreceptor trans-homointeractions between signaling subunits and weaking the intrareceptor TM interactions (stages I and II, Fig. 4). Then, depending on the duration of the FcεRI-ligand interaction (affinity/avidity of the ligand), the receptors can either go back to a resting state or forward to an active state, in which β and/or γ signaling oligomers are formed (stages III and IV, Fig. 4), thus promoting ITAM Tyr phosphorylation and generation of activation signal. Assuming that two different FcεRI signaling subunits, γ and β, provide distinct signaling,[87,88,200,285-291] the model suggests[47,48] that depending on the nature of ligand, the FcεRIs can be clustered to dimer/oligomer in different relative orientations that, in turn, promote homotypic interactions between different signaling subunits (Fig. 4). This leads to formation of distinct, γ and/or β, signaling oligomers, phosphorylation of the corresponding ITAM tyrosines and generation of different activation signals (signals A and B, Fig. 4), resulting in diverse functional outcomes. The formed β and/or γ oligomers can sequentially interact with β and/or γ subunits of nonengaged FcεRIs, thus amplifying and propagating the activation signal.

Interestingly, several mathematical models have been recently developed for the early signaling events mediated by FcεRI.[292-295] Through model simulations, it has been shown how changing the ligand concentration and consequently the concentration of receptor aggregates, can change the nature of a cellular response as well as its amplitude. These models are largely based on the recently suggested sequence of early events in FcεRI signaling.[121,296] Combining the basic organizing principles of the SCHOOL model with the existing mathematical models might significantly improve our understanding the spatiotemporal organization of FcεRI-mediated signal transduction as well as our ability to predict how this system will behave under a variety of experimental conditions.

Selected examples illustrating the ability of the SCHOOL model to provide a mechanistic explanation for FcεRI-related immunological phenomena are shown in Table 3. These and other findings mentioned above strongly support the validity and utility of the proposed activation model for the FcεRI.

SCHOOL Model of BCR Signaling: Description and Utility

The BCR is a multimeric complex composed of mIg noncovalently associated with a disulfide-linked Igα/Igβ heterodimer that is responsible for signal transduction. In the resting state, like with other MIRRs, intrareceptor TM interactions between mIg and Igα/Igβ subunits define the overall

Table 2. Molecular mechanisms suggested or predicted by the SCHOOL model to underlie selected T-cell-mediated immunological phenomena and observations. Abbreviations: Ag, antigen; CP, core peptide; FP, fusion peptide; IFN, interferon; IS, immunological synapse; HIV, human immunodeficiency virus; mAb, monoclonal antibody; pMHC, major histocompatibility complex (MHC)-bound peptide; TCR, T-cell antigen receptor; TM, transmembrane; ζ_{cyt}, TCR ζ cytoplasmic domain

Phenomenon	Observation	Mechanism
Inhibitory effect of TCR CP	TCR CP inhibits Ag-stimulated TM signal transduction and efficiently abrogates T-cell-mediated immune responses in mice and man in vitro and in vivo.[211,257,261,265,266]	TCR CP disrupts TCRα-CD3δε and TCRα-ζ TM interactions resulting in predissociation of these signaling subunits from the remaining complex and thus preventing the formation of signaling oligomers upon Ag stimulation and, consequently, inhibiting T-cell activation (Chapter 20).[2,47-49]
Diversity of TCR-mediated cell response	Precise ligand-binding specificities of the TCR are converted into diverse functional outcomes.[309-311] Different TCR signaling subunits engage partially distinct signaling pathways.[91-94] CD3 signaling subunits play differential biological role as revealed by human immunodeficiencies.[144]	Slightly different ligands bring two or more TCRs 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 and leading to diverse T-cell functional outcomes.[2,47,48] Thus, the signaling pathway and the direction of the response depends on the type of TCR signaling subunit(s) that is (are) oligomerized and ITAM-phosphorylated upon ligand stimulation.
T-cell clonal anergy	Ag-unresponsive anergic T-cells fail to produce IL-2 but produce comparable amounts of IFN-γ and proliferate to similar extents in response to immobilized anti-CD3/CD28 mAbs.[141] Ag-induced tolerance in vivo is accompanied by altered early TCR-mediated signaling events.[209] T-cell anergy is induced by activating but not by non-activating anti-CD3.[210]	Ag stimulation induces dissociation of TCR CD3 and/or ζ signaling subunits from the remaining TCRαβ subunits and/or TCRαβ-CD3 complexes, thus preventing Ag[141]- or anti-TCR[143]- but not anti-CD3 mAbs[141]-mediated formation of signaling oligomers and generation of activation signal (Fig. 2B). Depending on epitope location, anti-CD3 stimulation can induce formation of CD3 but not ζ signaling oligomers, thus leading to partial cell activation and preventing Ag-mediated T-cell response. Depending on dissociated subunit(s), TCR-mediated signaling events in anergic cells and therefore the functional outcomes can be altered differently.

Continued on next page

Table 2. Continued

Phenomenon	Observation	Mechanism
Comodulation of nonengaged TCRs	Activation of T-cells with pMHC, bacterial superantigens, or anti-Vβ antibodies downmodulates not only directly stimulated (engaged) TCR complexes but also unstimulated (nonengaged) ones.[207,241,312-315] In the IS, only a small fraction of the TCR is bound to specific pMHCs.[255]	Upon ligand stimulation, signaling oligomers dissociate from the remaining engaged TCRs that undergo internalization. Then, the dissociated signaling oligomers sequentially interact with the signaling subunits of nonengaged TCRs resulting in the release and subsequent internalization of the remaining nonengaged TCRαβ-CD3 complexes or TCRαβ chains. Internalization and intracellular fate may be different for TCR-CD3 complexes lacking ζ chain or for TCRαβ chains remaining on the cell surface after dissociation of either ζ or both ζ and CD3 signaling oligomers, respectively.[47,48]
TCR signaling initiation and following lateral signal propagation and amplification	TCR signaling is initiated and sustained in microclusters and is terminated in the TCR-rich central supramolecular activation cluster (cSMAC), a structure from which TCR are sorted for degradation.[134]	The initially formed signaling oligomers initiate TCR signaling, dissociate from the remaining engaged TCRs and interact with the signaling subunits of nonengaged TCRs, thus propagating the activation signal to nonengaged receptors and resulting in signal amplification and lateral propagation.
Exposure of the $CD3\varepsilon_{cyt}$ epitope	Ligand engagement of TCR-results in exposure of a cryptic proline-rich $CD3\varepsilon_{cyt}$ epitope that is a binding site for the adaptor protein, Nck.[113,145] The $CD3\varepsilon_{cyt}$ epitope is recognized by antibody APA1/1 and is only detected when the TCR is fully activated.[146,147] In the IS, distribution of APA1/1 epitope is more restricted than ζ, CD3ε and tyrosine-phosphorylated proteins.[146]	During full T-cell activation, dissociation of CD3εγ and/or CD3εδ signaling oligomers from TCRαβ chains (Fig. 3) induces the release/unmasking of the $CD3\varepsilon_{cyt}$ epitope. Thus, within the SCHOOL model, the ligand-induced exposure of the epitope is effect not cause of TCR triggering. During partial T-cell activation, formation of only ζ signaling oligomers and their dissociation from the remaining TCR-CD3 complexes (Fig. 3) do not release/unmask the $CD3\varepsilon_{cyt}$ epitope.

Continued on next page

Table 2. Continued

Phenomenon	Observation	Mechanism
Action of HIV-1 gp41 FP	HIV-1 FP colocalizes with CD4 and TCR molecules, coprecipitates with the TCR and inhibits Ag-specific T-cell proliferation and proinflammatory cytokine secretion in vitro.[316] The peptide blocks the TCR/CD3 TM interactions needed for antigen-triggered T-cell activation.[317]	Similarly to the TCR-CP, the HIV-1 gp41 FP disrupts TCRα-CD3δε and TCRα-ζ_2 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.[2,269] This effect is specific: anti-CD3ε antibody-stimulated T-cell activation is not affected by the peptide.
Pre-TCR signaling	Spontaneous preTCR oligomerization mediated by the preTCR α chain results in ligand-independent receptor triggering and TM signaling crucial for early T-cell development.[170,171] Forced dimerization of CD3ε is sufficient to simulate preTCR function and promote β-selection.[170]	Oligomerization of the preTCR through the preTCR α chain brings CD3 and ζ signaling subunits in close proximity and proper relative orientation, thus promoting formation of signaling oligomers and generating the activation signal. Remarkably, as predicted by the SCHOOL model, formation of CD3ε dimers/oligomers is necessary and sufficient to induce the CD3ε ITAM Tyr phosphorylation and lead to cell response.
Epitope-dependent mAb stimulation	T-cell activation induced by mAbs specific for the TCR does not correlate with the affinity of the mAbs but rather with the recognized epitope.[160] Triggering of different epitopes of the TCR-CD3-ζ_2 receptor complex depends on the mAb specificity and induces different modes of T-cell activation.[107,156-159] In thymocytes, only anti-TCRβ but not anti-TCRα Ab reagents cause long-term TCR downmodulation.[108]	Clustering/oligomerization of TCRs by different antibodies results in different intermolecular relative orientations within receptor cluster/oligomer that promote (or do not) homointeractions between different signaling subunits, leading to the formation of different CD3 and/or ζ signaling subunit oligomers and therefore to different functional outcomes. If intermolecular relative orientation in the antibody-crosslinked TCR cluster/oligomer does not promote homointeractions between CD3 and/or ζ signaling subunits, this antibody will not stimulate T-cell response.

Continued on next page

Table 2. Continued

Phenomenon	Observation	Mechanism
Coexistence of mono- and multivalent (oligomeric) TCRs in resting cells	Monovalent TCRs coexist in intact resting cells with multivalent complexes with two or more ligand-binding TCRαβ subunits,[186,318] raising a question: why does this basal TCR clustering not lead to receptor triggering whereas ligand-induced clustering does?	In resting cells, receptors within multivalent TCR complex have the relative orientation that does not promote homointeractions between CD3 and/or ζ signaling chains. Upon stimulation with multivalent ligand, these receptors adopt proper orientation relative to each other, starting homotypic interactions between signaling subunits and resulting in generation of the activation signal. A similar mechanistic explanation can also account for the existence of dimeric or tetrameric TCR-CD3-coreceptor complexes in naïve CD4+ or CD8+ T-cells.[167]

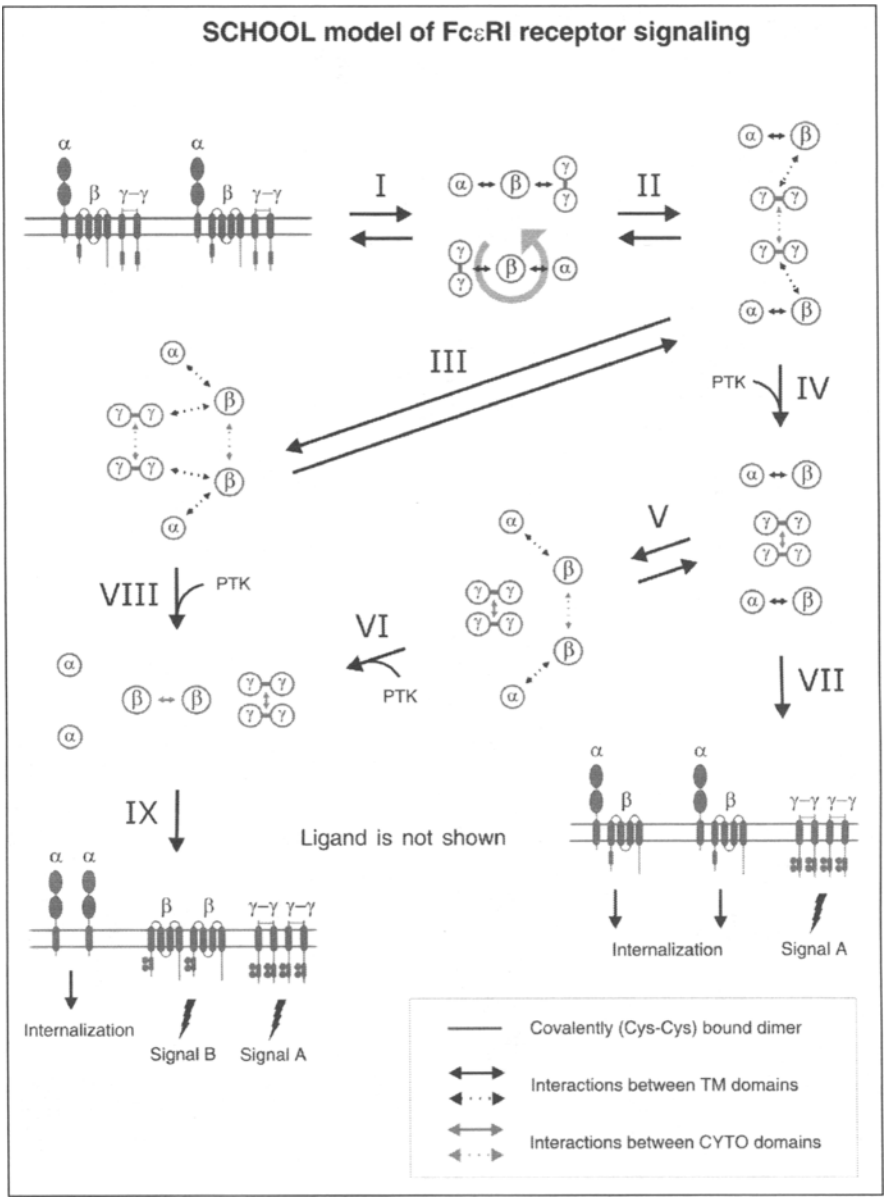

Figure 4. SCHOOL model of the high affinity IgE receptor (FcεRI) activation. Immunoreceptor tyrosine-based activation motifs (ITAMs) are shown as gray rectangles. FceRI α, β and γ components are represented as whole polypeptides and as a simplified axial view. All interchain interactions in intermediate complexes are shown by dotted arrows reflecting their transition state. Circular arrow indicates ligand-induced receptor reorientation. Interaction with multivalent ligand (not shown) clusters the receptors and pushes them to reorientate (I) and bring β and γ signaling subunits into a correct relative orientation and in sufficient proximity in the formed receptor oligomer (for illustrative purposes, receptor dimer is shown), thus starting the trans-homointeractions between γ homodimers (II). Then, two alternative pathways can take a place depending on the nature of activating stimuli. Continued on next page.

Figure 4, continued from previous page. First is going through a stage IV resulting in formation of γ_2 dimer (dimer of dimers) and phosphorylation of the γ ITAM tyrosines, thus triggering downstream signaling events. Then, the signaling γ oligomers formed subsequently dissociate from the α/β complex, resulting in internalization of the remaining engaged complexes (VII). This pathway leads to generation of the activation signal A. Alternatively, the intermediate complex formed at the stage II can undergo further rearrangements, starting trans-homointeractions between β chains (III) and resulting in formation of an oligomeric intermediate. Stages I, II and III can be reversible or irreversible depending on interreceptor proximity and relative orientation of the receptors in FcεRI dimers/oligomers as well as on time duration of the receptor-ligand contact and lifetime of the receptor in FcεRI dimers/oligomers that generally correlate with the nature of the stimulus and its specificity and affinity/avidity. Next, in the signaling oligomers formed (III), the β ITAM tyrosines undergo phosphorylation by protein tyrosine kinases (PTKs) that leads to generation of the activation signal, dissociation of signaling oligomers and internalization of the engaged α chains (VIII, XI). This pathway provides two different activation signals from the γ and β signaling oligomers (signals A and B), respectively and results in full cell activation. In addition, the signaling oligomers formed can sequentially interact with the signaling subunits of nonengaged FcεRIs resulting in formation of higher-order signaling oligomers, thus amplifying and propagating the activation signal (not shown). Also, this leads to the release and subsequent internalization of the nonengaged α and/or αβ chains (not shown). Abbreviations: PTK, protein tyrosine kinase. Phosphate groups are shown as filled gray circles.

rigid geometry and topology of the BCR.[28,34,35,297] In cells, this receptor transduces signals leading to a variety of biologic responses minimally including antigen receptor editing, apoptotic death, developmental progression, cell activation, proliferation and survival. Despite several BCR triggering and cell activation models that have been suggested,[44,116,172,298-300] no model fully explains the molecular mechanisms underlying spatiotemporal organization of BCR-triggered TM signal transduction.

Within the SCHOOL model, two or more BCRs are brought into close proximity and adopt a correct relative orientation upon receptor engagement with multivalent ligand (Fig. 5A). At this point, the trans-homointeractions between Igα and Igβ molecules are initiated, weaking the TM interactions within the BCR (Fig. 5A, stages I and II). Then, depending on the duration of ligand-BCR interaction and therefore on the affinity/avidity of ligand, the receptors can go either back to resting state or forward to active state, in which signaling Igα/Igβ oligomers are formed, thus promoting ITAM Tyr phosphorylation and generation of activation signal (Fig. 5A, stage III). Considering that Igα and Igβ chains can play different physiological roles,[84-86,301] the model suggests that depending on the nature of stimuli, different Igα/Igβ signaling oligomers can form, thus resulting in phosphorylation of Igα and/or Igβ ITAM tyrosines and induction of distinct signaling pathways. Further, once formed, these oligomers can sequentially interact with Igα/Igβ subunits of nonengaged BCRs, thus propagating and amplifying the activation signal and favoring the formation and stabilization of supramolecular complexes that can promote sustained signaling. In this context, it can also be suggested that the more BCRs are initially engaged and/or the higher is the affinity/avidity of antigen, the faster is signaling cluster formation.

In contrast to Igβ and other ITAM-containing proteins, the dynamic equilibrium between monomeric and oligomeric species of Igα is slow and this protein forms stable homooligomers (mostly, dimers and tetramers) even at very low protein concentrations.[78] Formation of the stable Igα/Igβ clusters/oligomers may be particularly important for sustained signaling during the synapse formation between B-cell and antigen-displaying target cell and subsequent antigen acquisition.[302] Also, as shown recently,[303] plasma membrane association of Igα/Igβ complexes results in generation of biologically relevant basal signaling while the ability of the BCR to interact with both conventional as well as nonconventional extracellular ligands is eliminated.

As illustrated in Table 4 by several selected examples, the proposed model is capable of providing a mechanistic explanation for BCR-related immunological phenomena. Thus, a vast majority of the experimental findings reported so far strongly support the validity and utility of this activation model for the BCR.

Table 3. Molecular mechanisms suggested or predicted by the SCHOOL model to underlie selected FcεRI-mediated immunological phenomena and observations

Phenomenon	Observation	Mechanism
FcεRI-mediated signaling and cellular responses	Signaling capacity of FcεRIs depends on the driving forces leading to their clustering and also on fine tuning provided by both lifetime (or receptor capacity to remain in a cluster that is influenced by the ligand affinity) and interreceptor relative orientation in the FcεRI dimers/oligomers.[127,229] There is no simple correlation between multivalent ligand-promoted FcεRI clustering and FcεRI-mediated cellular responses, such as cell degranulation.[126] The ratio of late to early FcεRI-stimulated events correlates with the affinity of a ligand for the receptor-bound IgE.[131] Orientational restraint in ligand-specific FceRI dimers/oligomers determines the magnitude of mediator secretion-causing signal induced by different mAbs.[111,112,119,126,127,193]	Triggering FcεRI requires close proximity of the receptors and a correct relative orientation in the FceRIs clustered (or altered in preexisting clusters) by multivalent ligand binding (Fig. 4).[2,47,48] It also requires the ligand-receptor contact to last long enough to initiate the trans-homointeractions between signaling subunits and weaken the intrareceptor TM interactions, thus resulting in formation of signaling FcεRIβ and/or FcεRIγ oligomers and generation of activation signal (Fig. 4).[2,47,48] Receptor clustering induced upon binding to multivalent ligand is necessary but not sufficient for the initiation of FcεRI signaling. To commence signal transduction, two or more clustered receptors should adopt a correct relative orientation toward each other, permissive of initiating the trans-homointeractions between β and/or γ subunits and remain in a cluster long enough to promote these interactions and therefore formation of signaling oligomers (Fig. 4).[2,47,48]
Different roles of β and γ subunits in signaling	The FcεRI β and γ subunits play different roles in signaling.[285-291] The γ chain aggregation alone can evoke cellular responses,[288,291] while the β chain acts as an amplifier for signaling.[87] Also, β chains can elicit a signal in a γ chain-independent manner.[200]	Depending on the nature of ligand (i.e., its specificity, affinity and avidity), the FcεRIs are clustered to dimer/oligomer in different relative orientations that promote homotypic interactions between different signaling subunits (Fig. 4). As a result, different, β and/or γ, signaling oligomers are formed, generating distinct activation signals and therefore distinct signaling pathways.[2,47,48]
Lateral propagation of activation signal	FcεRI-activated mast cells propagate signals from small signaling domains around dimerized/oligomerized receptors; formation of large FcεRI aggregates promotes both strong receptor triggering and rapid termination of the signaling responses.[63]	The initially formed β and/or γ signaling oligomers initiate FcεRI signaling, dissociate from the remaining engaged receptors and interact with the signaling subunits of nonengaged FcεRIs, thus propagating the activation signal to nonengaged receptors and resulting in signal amplification and lateral propagation.

Abbreviations: FcεRI, high affinity IgE receptor, mAb, monoclonal antibody; TM, transmembrane

SCHOOL Model of GPVI Signaling: Description and Utility

Studies of patients deficient in GPVI identified this platelet membrane protein as a physiological collagen receptor. This receptor is noncovalently associated with FcRγ, the ITAM-containing homodimeric signaling module. The GPVI-FcRγ receptor complex induces platelet activation when it binds to collagen or other agonists and GPVI-deficient platelets lack specifically collagen-induced aggregation and the ability to form thrombi on a collagen surface under flow conditions.[10,304] 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, future therapeutic strategies targeting platelet-mediated disease will depend on our detailed understanding of the molecular mechanisms underlying GPVI triggering and subsequent TM signal transduction. In addition, knowing these mechanisms would give us a new handle in dissecting the basic structural and functional aspects of thrombus formation.

In 2006, GPVI has been reported to form a back-to-back dimer in the GPVI crystal.[64] Based on these findings, a model for GPVI signaling has been suggested, in which GPVI clustering triggers a signaling cascade via the FcRγ chain coreceptor.[64] Despite its apparent similarity to the SCHOOL model,[48] it does not explain the existence of oligomeric GPVI-FcRγ complexes at the surface of unstimulated platelets[71] and does not suggest specific protein-protein interactions involved in the molecular mechanisms underlying the GPVI-triggered signaling. These findings[64,71] raise an important and intriguing question: why does the observed basal receptor dimerization not lead to receptor triggering and subsequent platelet activation whereas agonist-induced receptor crosslinking/clustering does?

Despite extensive studies of the GPVI-FcRγ receptor complex and its mechanism of action,[10,178,305,306] the only model that can answer this question and even more important, mechanistically explain how GPVI-mediated TM signaling begins, is the SCHOOL model.[2,47-49,307] Within this 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,[30] that maintain receptor integrity in platelets under basal conditions and homointeractions between FcRγ subunits that lead to formation of signaling oligomers and initiation of a signaling response (Fig. 5B). Binding of the multivalent ligand (collagen) to two or more GPVI-FcRγ receptor complexes pushes the receptors to cluster, rotate and adopt an appropriate orientation relative to each other (Fig. 5B, stages I and II), 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 generation of the activation signal (Fig. 5B, stage III) and subsequent dissociation of FcRγ signaling oligomers and downmodulation of the remaining engaged GPVI subunits (Fig. 5B, stage IV). Later, the dissociated oligomeric FcRγ chains can interact with FcRγ subunits of the nonengaged GPVI-FcRγ complexes, resulting in formation of higher-order signaling oligomers and their subsequent phosphorylation, thus providing lateral signal propagation and amplification (not shown).

Thus, for the preformed oligomeric receptor complexes existing in unstimulated platelets as found by Berlanga et al[71] the proposed 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 close proximity and appropriate relative orientation permissive of initiating the FcRγ homointeractions and receptor triggering.

Intriguingly, suggesting how binding to collagen triggers the GPVI-mediated signal cascade at the molecular level, the SCHOOL model reveals GPVI-FcRγ TM interactions as a novel therapeutic target for the prevention and treatment of platelet-mediated thrombotic events (Chapter 20).[2,49,307,308] Preliminary experimental results provided support for this novel concept of platelet inhibition and resulted in the development of novel class of promising platelet inhibitors.[307,308]

Thus, the experimental evidence accumulated to date on the GPVI-mediated TM signal transduction and platelet activation strongly support the validity and utility of the proposed activation model for this receptor.

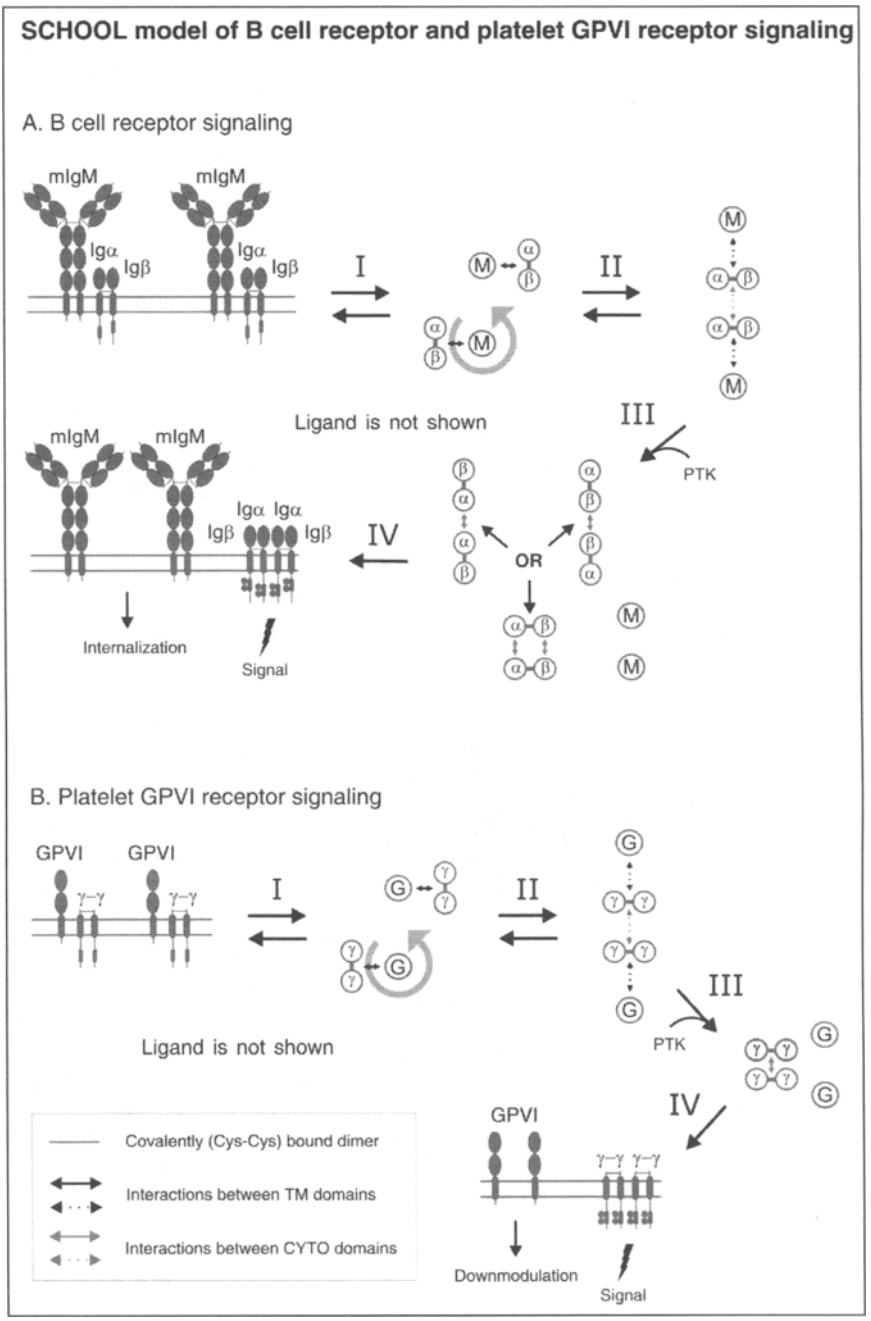

Figure 5. SCHOOL model of the B-cell receptor (BCR, panel A) and platelet collagen receptor glycoprotein VI (GPVI, panel B) activation. Immunoreceptor tyrosine-based activation motifs (ITAMs) are shown as gray rectangles. Receptor components are represented as whole polypeptides and as a simplified axial view. All interchain interactions in intermediate complexes are shown by dotted arrows reflecting their transition state. Continued on next page.

Figure 5, continued from previous page. Circular arrows indicate ligand-induced receptor reorientation. Interaction with multivalent ligand (not shown) clusters the receptors and pushes them to reorientate (I) and bring signaling subunits into a correct relative orientation and in sufficient proximity in the receptor oligomer (for illustrative purposes, receptor dimers are shown), thus starting the trans-homointeractions between Igα/Igβ heterodimers (panel A, II) or FcRγ homodimers (panel B, II). On a stage III, formation of signaling oligomers results in phosphorylation of the ITAM tyrosines, thus triggering downstream signaling events. Then, the signaling oligomers formed subsequently dissociate from the mIg or GPVI (panels A and B, respectively), resulting in generation of the activation signal and internalization of the remaining engaged receptor chains (IV). Stages I and II can be reversible or irreversible depending on interreceptor proximity and relative orientation of the receptors in ligand-specific dimers/oligomers as well as on time duration of the receptor-ligand contact and lifetime of the receptor in these dimers/oligomers that generally correlate with the nature of the stimulus and its specificity and affinity/avidity. In contrast to homodimeric FcRγ signaling subunit in GPVI-FcRγ receptor complex, the BCR signaling module contains two different signaling chains, Igα and Igβ, providing possibility of the signal and cell response diversity depending on the particular set of the Igα and/or Igβ ITAM tyrosines that become phosphorylated. Further, the signaling oligomers formed can sequentially interact with the signaling subunits of nonengaged receptors resulting in formation of higher-order signaling oligomers, thus amplifying and propagating the activation signal (not shown). Also, this leads to the release and subsequent internalization/downmodulation of the nonengaged mIg or GPVI chains (not shown). Abbreviations: PTK, protein tyrosine kinase. Phosphate groups are shown as filled gray circles.

SCHOOL Model of Other MIRR Signaling

As illustrated in Figure 1, a structural assembly of many MIRRs, such as FcαRI, FcγRI, FcγRIIIA, ILT/LIR receptors, DCAR, NK and TREM receptors, etc., is very similar to that of the GPVI receptor; all these receptors have a ligand-recognition subunit and one homodimeric signaling subunit. Thus, the basic principles of GPVI triggering and TM signaling suggested by the SCHOOL model can be easily applied to these and other, structurally related, MIRRs. Selected examples illustrating the capability of the SCHOOL model to provide a mechanistic explanation for immunological phenomena mediated by these receptors are shown in Table 5.

Conclusions

Despite growing interest in targeting MIRR signaling as a potential treatment strategy for different immune-mediated diseases (see also Chapters 20 and 22), the molecular mechanisms underlying MIRR triggering and subsequent TM signal transduction are unknown, impeding our fundamental understanding of MIRR-mediated immunological phenomena and thus preventing the development of novel pharmacological approaches.

Considering MIRR triggering as a result of ligand-induced interplay between well-defined protein-protein interactions, the proposed SCHOOL model is the first general model that provides a set of basic principles of MIRR signaling and mechanistically explains how MIRR-mediated signaling commences and what the main driving forces and restraints of MIRR triggering/signaling are. Furthermore, this model is the first model that can describe, explain and predict numerous MIRR-mediated immunological phenomena. Thus, this model represents a powerful tool that can be used in dissecting the basic structural and functional aspects of the immune response and using this knowledge in both fundamental and clinical fields. In addition, revealing the main driving forces and fundamental stages of MIRR triggering and TM signal transduction, the model identifies effective ways of modulating the immune response.

Importantly, by generalizing mechanistic features of MIRR signaling, the SCHOOL model shows how the similar structural architecture of the MIRRs dictates similar mechanisms of MIRR triggering and subsequent TM signal transduction and furthermore, reveals similar therapeutic targets in seemingly unrelated diseases (see also Chapter 20). This permits the transfer of accumulated knowledge and pharmacological approaches between seemingly disparate immune disorders and builds the molecular basis for existing and future therapeutic strategies. Impressively,

Table 4. Molecular mechanisms suggested or predicted by the SCHOOL model to underlie selected BCR-mediated immunological phenomena and observations

Phenomenon	Observation	Mechanism
BCR-mediated signaling and cellular responses	B-cell response is induced by multivalent but not monovalent ligand stimulation[56] and Ag valency influences B-cell responses by modulating the stability of BCR-signaling microdomains and BCR trafficking.[54]	Triggering BCR requires multivalent ligand-induced clustering of the BCRs in a close proximity and a correct relative orientation in the formed clusters (or reorientation of the receptors in preexisting oligomers/clusters) (Fig. 5A).[2,47,48] Also, the more BCRs are initially engaged, the faster is Igα/Igβ signaling oligomer formation and the stronger is the amplified activation signal.
Formation of Igα/Igβ oligomers	Signaling Igα/Igβ heterodimer assembles into oligomers upon ligand stimulation.[199]	Upon multivalent ligand stimulation, BCRs are clustered in close proximity and correct relative orientation, thus promoting homotypic interactions between Igα/Igβ signaling subunits. This leads to formation of signaling oligomers and phosphorylation of the ITAM tyrosines, thus initiating the signaling cascade.[2,47,48]
Comodulation of nonengaged BCRs	Unligated BCRs cluster with BCRs engaged by multivalent ligands.[56] The extent of BCR internalization is not correlated with Ag valency, suggesting that BCR signaling and internalization are distinct processes.[56] Upon anti-Ig-induced BCR clustering, >95% of the mIg is internalized, whereas 20-30% of Igβ remains on the surface.[213]	Similar to TCR (Table 2), upon multivalent ligand stimulation, signaling Igα/Igβ oligomers dissociate from the remaining engaged mIgs that undergo internalization. Then, the dissociated oligomers sequentially interact with the signaling subunits of nonengaged receptors resulting in their activation and therefore the signal amplification and propagation. This also leads to the release and subsequent internalization of the remaining nonengaged mIg chains.[47,48]
B-cell tolerance/BCR desensitization	Monomeric hen egg lysozyme (HEL) efficiently engages the specific BCR, however, presentation of HEL-derived epitopes is impaired compared to multivalent antigens.[319] Soluble monovalent antigen, administered intravenously, induces B-cell tolerance.[320,321] Upon binding of moderate- to low-affinity Ag, physical dissociation of the Igα/Igβ subunits from mIg results in BCR desensitization.[148] However, these desensitized cells can be still activated by anti-Igβ antibodies.[148]	Monovalent or moderate to low-affinity Ag stimulation induces dissociation of BCR Igα/Igβ signaling subunits from the remaining mIg, thus preventing Ag- or anti-Ig- but not anti-Igβ mAbs-mediated formation of signaling oligomers and generation of activation signal (Fig. 2B).[47,48] The remaining mIg chains are internalized. Within the SCHOOL model, the ligand-induced dissociation of signaling subunits from ligand recognition subunits is suggested to be a general molecular mechanism underlying T- and B-cell tolerance, BCR desensitization and TM peptide-modulated T and other immune cell response.[2,47,48,269,307]

Abbreviations: Ag, antigen; BCR, B-cell antigen receptor; mAb, monoclonal antibody; mIg, membrane immunoglobulin; TM, transmembrane

Table 5. Molecular mechanisms suggested or predicted by the SCHOOL model to underlie selected MIRR-mediated immunological phenomena and observations

Phenomenon	Observation	Mechanism
FcαRI-mediated signaling and cellular responses	Vertical relocation of the TM positive charge responsible for FcαRI-FcRγ association does not effect on calcium flux, MAPK phosphorylation and IL-2 release, whereas its lateral transfer completely abrogates these functions.[32]	Vertical relocation of the noncovalent electrostatic bond does not affect interreceptor relative orientation within the FcαRI dimers/oligomers formed upon multivalent ligand stimulation, whereas lateral transfer does, thus preventing formation of FcRγ signaling oligomers and initiation of signaling cascade.
NKR-mediated signaling and cellular response	Short CPs derived from the TM sequence of NKRs inhibit NK cell cytolytic activity.[268]	NKR CPs disrupt the TM interactions between NKR ligand-binding subunits and associated homodimeric signaling subunits, such as ζ–ζ, γ–γ or DAP-12 (Chapter 20).[2]
Immune escape in hCMV pathogenesis	hCMV tegument protein pp65 interacts directly with NKp30, leading to dissociation of the linked ζ signaling subunit and, consequently, to reduced killing.[152]	Binding to pp65 protein affects the NKp30-ζ$_2$ TM interactions resulting in dissociation of the ζ signaling subunit from the remaining complex and thus preventing the formation of ζ signaling oligomers upon ligand stimulation and, consequently, inhibiting NK cell cytolytic activity (Chapter 20).[2]
TREM-mediated signaling	Structurally similar receptors, TREM-1 and TREM-2 (Fig. 1) that contain the same signaling subunit, DAP-12, show activating and inhibitory functions.[18,125]	Depending on the affinity/avidity of the ligand, ligand stimulation can result in: 1) receptor clustering, formation of oligomeric signaling subunits and generation of the activation signal (TREM-1, activating function), or 2) dissociation of signaling subunit from the engaged receptor and unmasking a specific "inhibitory" epitope(s) in the cytoplasmic tail of ligand recognition subunit (TREM-2, inhibitory function).

Abbreviations: Ag, antigen; CPs, core peptides; hCMV, human cytomegalovirus; DAP-12, DNAX activation protein 12; mAb, monoclonal antibody; MAPK, mitogen-activated protein kinase; NKRs, natural killer cell receptors; TM, transmembrane; TREM, triggering receptors expressed on myeloid cells

applications of this model have already illustrated how do the similar molecular mechanisms of MIRR signaling revealed by the model work in seemingly unrelated fields, such as the treatment of T-cell-mediated skin diseases, HIV entry into target cells and the development of a novel concept of platelet inhibition (see also Chapters 20 and 22).

In conclusion, I sincerely hope that the model and issues presented in this Chapter will stimulate debate and new research to further test and apply the proposed model, thus opening new horizons in our knowledge about the immune system and generating new perspectives for the effective prevention and/or treatment of numerous immune disorders.

Acknowledgements

I would like to thank Walter M. Kim for critical reading of this manuscript.

References

1. Keegan AD, Paul WE. Multichain immune recognition receptors: Similarities in structure and signaling pathways. Immunol Today 1992; 13:63-68.
2. Sigalov AB. Immune cell signaling: A novel mechanistic model reveals new therapeutic targets. Trends Pharmacol Sci 2006; 27:518-524.
3. Krogsgaard M, Davis MM. How T-cells 'see' antigen. Nat Immunol 2005; 6:239-245.
4. DeFranco AL. B-cell activation 2000. Immunol Rev 2000; 176:50-9.
5. Dal Porto JM, Gauld SB, Merrell KT et al. B-cell antigen receptor signaling 101. Mol Immunol 2004; 41:599-613.
6. Takai T. Fc receptors and their role in immune regulation and autoimmunity. J Clin Immunol 2005; 25:1-18.
7. Takai T. Fc receptors: Their diverse functions in immunity and immune disorders. Springer Semin Immunopathol 2006; 28:303-304.
8. Colonna M, Nakajima H, Navarro F et al. A novel family of Ig-like receptors for HLA class I molecules that modulate function of lymphoid and myeloid cells. J Leukoc Biol 1999; 66:375-381.
9. Borrego F, Kabat J, Kim DK et al. Structure and function of major histocompatibility complex (MHC) class I specific receptors expressed on human natural killer (NK) cells. Mol Immunol 2002; 38:637-660.
10. Moroi M, Jung SM. Platelet glycoprotein VI: Its structure and function. Thromb Res 2004; 114:221-233.
11. Barclay AN, Brown MH. The SIRP family of receptors and immune regulation. Nat Rev Immunol 2006; 6:457-464.
12. Kanazawa N, Tashiro K, Miyachi Y. Signaling and immune regulatory role of the dendritic cell immunoreceptor (DCIR) family lectins: DCIR, DCAR, dectin-2 and BDCA-2. Immunobiology 2004; 209:179-190.
13. Biassoni R, Cantoni C, Falco M et al. Human natural killer cell activating receptors. Mol Immunol 2000; 37:1015-1024.
14. Biassoni R, Cantoni C, Marras D et al. Human natural killer cell receptors: Insights into their molecular function and structure. J Cell Mol Med 2003; 7:376-387.
15. Aoki N, Kimura S, Xing Z. Role of DAP12 in innate and adaptive immune responses. Curr Pharm Des 2003; 9:7-10.
16. Bakker AB, Baker E, Sutherland GR et al. Myeloid DAP12-associating lectin (MDL)-1 is a cell surface receptor involved in the activation of myeloid cells. Proc Natl Acad Sci USA 1999; 96:9792-9796.
17. van den Berg TK, Yoder JA, Litman GW. On the origins of adaptive immunity: Innate immune receptors join the tale. Trends Immunol 2004; 25:11-16.
18. Klesney-Tait J, Turnbull IR, Colonna M. The TREM receptor family and signal integration. Nat Immunol 2006; 7:1266-1273.
19. Takai T. Paired immunoglobulin-like receptors and their MHC class I recognition. Immunology 2005; 115:433-440.
20. Nakahashi C, Tahara-Hanaoka S, Totsuka N et al. Dual assemblies of an activating immune receptor, MAIR-II, with ITAM-bearing adapters DAP12 and FcRgamma chain on peritoneal macrophages. J Immunol 2007; 178:765-770.
21. Fujimoto M, Takatsu H, Ohno H. CMRF-35-like molecule-5 constitutes novel paired receptors, with CMRF-35-like molecule-1, to transduce activation signal upon association with FcRgamma. Int Immunol 2006; 18:1499-1508.
22. Stewart CA, Vivier E, Colonna M. Strategies of natural killer cell recognition and signaling. Curr Top Microbiol Immunol 2006; 298:1-21.
23. Reth M. Antigen receptor tail clue. Nature 1989; 338:383-384.
24. Songyang Z, Shoelson SE, Chaudhuri M et al. SH2 domains recognize specific phosphopeptide sequences. Cell 1993; 72:767-778.
25. Wu J, Cherwinski H, Spies T et al. DAP10 and DAP12 form distinct, but functionally cooperative, receptor complexes in natural killer cells. J Exp Med 2000; 192:1059-1068.
26. Manolios N, Bonifacino JS, Klausner RD. Transmembrane helical interactions and the assembly of the T-cell receptor complex. Science 1990; 249:274-277.
27. Call ME, Pyrdol J, Wiedmann M et al. The organizing principle in the formation of the T-cell receptor-CD3 complex. Cell 2002; 111:967-979.
28. Michnoff CH, Parikh VS, Lelsz DL et al. Mutations within the NH2-terminal transmembrane domain of membrane immunoglobulin (Ig) M alters Ig alpha and Ig beta association and signal transduction. J Biol Chem 1994; 269:24237-24244.
29. Daeron M. Fc receptor biology. Annu Rev Immunol 1997; 15:203-234.
30. Feng J, Garrity D, Call ME et al. Convergence on a distinctive assembly mechanism by unrelated families of activating immune receptors. Immunity 2005; 22:427-438.

31. Feng J, Call ME, Wucherpfennig KW. The assembly of diverse immune receptors is focused on a polar membrane-embedded interaction site. PLoS Biol 2006; 4:e142.
32. Bakema JE, de Haij S, den Hartog-Jager CF et al. Signaling through mutants of the IgA receptor CD89 and consequences for Fc receptor gamma-chain interaction. J Immunol 2006; 176:3603-3610.
33. Varin-Blank N, Metzger H. Surface expression of mutated subunits of the high affinity mast cell receptor for IgE. J Biol Chem 1990; 265:15685-15694.
34. Stevens TL, Blum JH, Foy SP et al. A mutation of the mu transmembrane that disrupts endoplasmic reticulum retention. Effects on association with accessory proteins and signal transduction. J Immunol 1994; 152:4397-4406.
35. Zidovetzki R, Rost B, Pecht I. Role of transmembrane domains in the functions of B- and T-cell receptors. Immunol Lett 1998; 64:97-107.
36. Blum JH, Stevens TL, DeFranco AL. Role of the mu immunoglobulin heavy chain transmembrane and cytoplasmic domains in B-cell antigen receptor expression and signal transduction. J Biol Chem 1993; 268:27236-27245.
37. Ra C, Jouvin MH, Kinet JP. Complete structure of the mouse mast cell receptor for IgE (Fc epsilon RI) and surface expression of chimeric receptors (rat-mouse-human) on transfected cells. J Biol Chem 1989; 264:15323-15327.
38. Rudd CE. Disabled receptor signaling and new primary immunodeficiency disorders. N Engl J Med 2006; 354:1874-1877.
39. Gomes MM, Herr AB. IgA and IgA-specific receptors in human disease: structural and functional insights into pathogenesis and therapeutic potential. Springer Semin Immunopathol 2006; 28:383-395.
40. Honda Z. Fcepsilon- and Fcgamma-receptor signaling in diseases. Springer Semin Immunopathol 2006; 28:365-375.
41. Moretta A, Bottino C, Vitale M et al. Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. Annu Rev Immunol 2001; 19:197-223.
42. Clemetson KJ. Platelet receptors and their role in diseases. Clin Chem Lab Med 2003; 41:253-260.
43. Ortega E. How do multichain immune recognition receptors signal? A structural hypothesis. Mol Immunol 1995; 32:941-945.
44. Reth M. Oligomeric antigen receptors: A new view on signaling for the selection of lymphocytes. Trends Immunol 2001; 22:356-360.
45. Langlet C, Bernard AM, Drevot P et al. Membrane rafts and signaling by the multichain immune recognition receptors. Curr Opin Immunol 2000; 12:250-255.
46. Dykstra M, Cherukuri A, Pierce SK. Rafts and synapses in the spatial organization of immune cell signaling receptors. J Leukoc Biol 2001; 70:699-707.
47. Sigalov A. Multi-chain immune recognition receptors: Spatial organization and signal transduction. Semin Immunol 2005; 17:51-64.
48. Sigalov AB. Multichain immune recognition receptor signaling: Different players, same game? Trends Immunol 2004; 25:583-589.
49. Sigalov AB. Transmembrane interactions as immunotherapeutic targets: Lessons from viral pathogenesis. Adv Exp Med Biol 2007; 601:335-344.
50. Heldin CH. Dimerization of cell surface receptors in signal transduction. Cell 1995; 80:213-223.
51. Metzger H. Transmembrane signaling: The joy of aggregation. J Immunol 1992; 149:1477-1487.
52. Boniface JJ, Rabinowitz JD, Wulfing C et al. Initiation of signal transduction through the T-cell receptor requires the multivalent engagement of peptide/MHC ligands [corrected]. Immunity 1998; 9:459-466.
53. Holowka D, Baird B. Antigen-mediated IGE receptor aggregation and signaling: A window on cell surface structure and dynamics. Annu Rev Biophys Biomol Struct 1996; 25:79-112.
54. Thyagarajan R, Arunkumar N, Song W. Polyvalent antigens stabilize B-cell antigen receptor surface signaling microdomains. J Immunol 2003; 170:6099-6106.
55. Bankovich AJ, Raunser S, Juo ZS et al. Structural insight into preB cell receptor function. Science 2007; 316:291-294.
56. Puffer EB, Pontrello JK, Hollenbeck JJ et al. Activating B-cell signaling with defined multivalent ligands. ACS Chem Biol 2007; 2:252-262.
57. Deng L, Langley RJ, Brown PH et al. Structural basis for the recognition of mutant self by a tumor-specific, MHC class II-restricted T-cell receptor. Nat Immunol 2007; 8:398-408.
58. Adams EJ, Chien YH, Garcia KC. Structure of a gammadelta T-cell receptor in complex with the nonclassical MHC T22. Science 2005; 308:227-231.
59. Bachmann MF, Ohashi PS. The role of T-cell receptor dimerization in T-cell activation. Immunol Today 1999; 20:568-576.
60. Bachmann MF, Salzmann M, Oxenius A et al. Formation of TCR dimers/trimers as a crucial step for T-cell activation. Eur J Immunol 1998; 28:2571-2579.

61. DeFranco AL, Gold MR, Jakway JP. B-lymphocyte signal transduction in response to anti-immunoglobulin and bacterial lipopolysaccharide. Immunol Rev 1987; 95:161-176.
62. Posner RG, Savage PB, Peters AS et al. A quantitative approach for studying IgE-FcepsilonRI aggregation. Mol Immunol 2002; 38:1221-1228.
63. Draberova L, Lebduska P, Halova I et al. Signaling assemblies formed in mast cells activated via Fcepsilon receptor I dimers. Eur J Immunol 2004; 34:2209-2219.
64. Horii K, Kahn ML, Herr AB. Structural basis for platelet collagen responses by the immune-type receptor glycoprotein VI. Blood 2006; 108:936-942.
65. Cochran JR, Cameron TO, Stern LJ. The relationship of MHC-peptide binding and T-cell activation probed using chemically defined MHC class II oligomers. Immunity 2000; 12:241-250.
66. Fahmy TM, Bieler JG, Schneck JP. Probing T-cell membrane organization using dimeric MHC-Ig complexes. J Immunol Methods 2002; 268:93-106.
67. Kiessling LL, Gestwicki JE, Strong LE. Synthetic multivalent ligands as probes of signal transduction. Angew Chem Int Ed Engl 2006; 45:2348-2368.
68. Klemm JD, Schreiber SL, Crabtree GR. Dimerization as a regulatory mechanism in signal transduction. Annu Rev Immunol 1998; 16:569-592.
69. Garrity D, Call ME, Feng J et al. The activating NKG2D receptor assembles in the membrane with two signaling dimers into a hexameric structure. Proc Natl Acad Sci USA 2005; 102:7641-7646.
70. Radaev S, Kattah M, Rostro B et al. Crystal structure of the human myeloid cell activating receptor TREM-1. Structure 2003; 11:1527-1535.
71. Berlanga O, Bori-Sanz T, James JR et al. Glycoprotein VI oligomerization in cell lines and platelets. J Thromb Haemost 2007; 5:1026-1033.
72. Symer DE, Dintzis RZ, Diamond DJ et al. Inhibition or activation of human T-cell receptor transfectants is controlled by defined, soluble antigen arrays. J Exp Med 1992; 176:1421-1430.
73. Schweitzer-Stenner R, Tamir I, Pecht I. Analysis of Fc(epsilon)RI-mediated mast cell stimulation by surface-carried antigens. Biophys J 1997; 72:2470-2478.
74. Patrick SM, Kim S, Braunstein NS et al. Dependence of T-cell activation on area of contact and density of a ligand-coated surface. J Immunol Methods 2000; 241:97-108.
75. Germain RN. T-cell signaling: the importance of receptor clustering. Curr Biol 1997; 7:R640-644.
76. Alam SM, Davies GM, Lin CM et al. Qualitative and quantitative differences in T-cell receptor binding of agonist and antagonist ligands. Immunity 1999; 10:227-237.
77. Pribluda VS, Pribluda C, Metzger H. Transphosphorylation as the mechanism by which the high-affinity receptor for IgE is phosphorylated upon aggregation. Proc Natl Acad Sci USA 1994; 91:11246-11250.
78. Sigalov A, Aivazian D, Stern L. Homooligomerization of the cytoplasmic domain of the T-cell receptor zeta chain and of other proteins containing the immunoreceptor tyrosine-based activation motif. Biochemistry 2004; 43:2049-2061.
79. Berko D, Carmi Y, Cafri G et al. Membrane-anchored beta 2-microglobulin stabilizes a highly receptive state of MHC class I molecules. J Immunol 2005; 174:2116-2123.
80. Talbott M, Hare M, Nyarko A et al. Folding is coupled to dimerization of Tctex-1 dynein light chain. Biochemistry 2006; 45:6793-6800.
81. Sigalov AB, Zhuravleva AV, Orekhov VY. Binding of intrinsically disordered proteins is not necessarily accompanied by a structural transition to a folded form. Biochimie 2007; 89:419-421.
82. Sigalov AB, Aivazian DA, Uversky VN et al. Lipid-binding activity of intrinsically unstructured cytoplasmic domains of multichain immune recognition receptor signaling subunits. Biochemistry 2006; 45:15731-15739.
83. Pitcher LA, van Oers NS. T-cell receptor signal transmission: Who gives an ITAM? Trends Immunol 2003; 24:554-560.
84. Pike KA, Baig E, Ratcliffe MJ. The avian B-cell receptor complex: Distinct roles of Igalpha and Igbeta in B-cell development. Immunol Rev 2004; 197:10-25.
85. Storch B, Meixlsperger S, Jumaa H. The Ig-alpha ITAM is required for efficient differentiation but not proliferation of preB cells. Eur J Immunol 2007; 37:252-260.
86. Gazumyan A, Reichlin A, Nussenzweig MC. Ig beta tyrosine residues contribute to the control of B-cell receptor signaling by regulating receptor internalization. J Exp Med 2006; 203:1785-1794.
87. Lin S, Cicala C, Scharenberg AM et al. The Fc(epsilon)RIbeta subunit functions as an amplifier of Fc(epsilon)RIgamma-mediated cell activation signals. Cell 1996; 85:985-995.
88. Sanchez-Mejorada G, Rosales C. Signal transduction by immunoglobulin Fc receptors. J Leukoc Biol 1998; 63:521-533.
89. Lysechko TL, Ostergaard HL. Differential Src family kinase activity requirements for CD3 zeta phosphorylation/ZAP70 recruitment and CD3 epsilon phosphorylation. J Immunol 2005; 174:7807-7814.

90. Kuhns MS, Davis MM. Disruption of extracellular interactions impairs T-cell receptor-CD3 complex stability and signaling. Immunity 2007; 26:357-369.
91. Chau LA, Bluestone JA, Madrenas J. Dissociation of intracellular signaling pathways in response to partial agonist ligands of the T-cell receptor. J Exp Med 1998; 187:1699-1709.
92. Jensen WA, Pleiman CM, Beaufils P et al. Qualitatively distinct signaling through T-cell antigen receptor subunits. Eur J Immunol 1997; 27:707-716.
93. Pitcher LA, Mathis MA, Young JA et al. The CD3 gammaepsilon/deltaepsilon signaling module provides normal T-cell functions in the absence of the TCR zeta immunoreceptor tyrosine-based activation motifs. Eur J Immunol 2005; 35:3643-3654.
94. Kesti T, Ruppelt A, Wang JH et al. Reciprocal Regulation of SH3 and SH2 Domain Binding via Tyrosine Phosphorylation of a Common Site in CD3{epsilon}. J Immunol 2007; 179:878-885.
95. Chae WJ, Lee HK, Han JH et al. Qualitatively differential regulation of T-cell activation and apoptosis by T-cell receptor zeta chain ITAMs and their tyrosine residues. Int Immunol 2004; 16:1225-1236.
96. Wines BD, Trist HM, Monteiro RC et al. Fc receptor gamma chain residues at the interface of the cytoplasmic and transmembrane domains affect association with FcalphaRI, surface expression and function. J Biol Chem 2004; 279:26339-26345.
97. Kikuchi-Maki A, Catina TL, Campbell KS. Cutting edge: KIR2DL4 transduces signals into human NK cells through association with the Fc receptor gamma protein. J Immunol 2005; 174:3859-3863.
98. DeFranco AL, Mittelstadt PR, Blum JH et al. Mechanism of B-cell antigen receptor function: transmembrane signaling and triggering of apoptosis. Adv Exp Med Biol 1994; 365:9-22.
99. Morton HC, van den Herik-Oudijk IE, Vossebeld P et al. Functional association between the human myeloid immunoglobulin A Fc receptor (CD89) and FcR gamma chain. Molecular basis for CD89/FcR gamma chain association. J Biol Chem 1995; 270:29781-29787.
100. Kim MK, Huang ZY, Hwang PH et al. Fcgamma receptor transmembrane domains: role in cell surface expression, gamma chain interaction and phagocytosis. Blood 2003; 101:4479-4484.
101. Arkin M. Protein-protein interactions and cancer: Small molecules going in for the kill. Curr Opin Chem Biol 2005; 9:317-324.
102. Fry DC. Protein-protein interactions as targets for small molecule drug discovery. Biopolymers 2006; 84:535-552.
103. Loregian A, Palu G. Disruption of protein-protein interactions: Towards new targets for chemotherapy. J Cell Physiol 2005; 204:750-762.
104. Angelov GS, Guillaume P, Cebecauer M et al. Soluble MHC-peptide complexes containing long rigid linkers abolish CTL-mediated cytotoxicity. J Immunol 2006; 176:3356-3365.
105. Rotzschke O, Falk K, Strominger JL. Superactivation of an immune response triggered by oligomerized T-cell epitopes. Proc Natl Acad Sci USA 1997; 94:14642-14647.
106. Tamir I, Schweitzer-Stenner R, Pecht I. Immobilization of the type I receptor for IgE initiates signal transduction in mast cells. Biochemistry 1996; 35:6872-6883.
107. Yang H, Parkhouse RM. Differential activation requirements associated with stimulation of T-cells via different epitopes of CD3. Immunology 1998; 93:26-32.
108. Niederberger N, Buehler LK, Ampudia J et al. Thymocyte stimulation by anti-TCR-beta, but not by anti-TCR-alpha, leads to induction of developmental transcription program. J Leukoc Biol 2005; 77:830-841.
109. Carreno LJ, Gonzalez PA, Kalergis AM. Modulation of T-cell function by TCR/pMHC binding kinetics. Immunobiology 2006; 211:47-64.
110. Holler PD, Lim AR, Cho BK et al. CD8(-) T-cell transfectants that express a high affinity T-cell receptor exhibit enhanced peptide-dependent activation. J Exp Med 2001; 194:1043-1052.
111. Ortega E, Schweitzer-Stenner R, Pecht I. Possible orientational constraints determine secretory signals induced by aggregation of IgE receptors on mast cells. EMBO J 1988; 7:4101-4109.
112. Pecht I, Ortega E, Jovin TM. Rotational dynamics of the Fc epsilon receptor on mast cells monitored by specific monoclonal antibodies and IgE. Biochemistry 1991; 30:3450-3458.
113. Gil D, Schamel WW, Montoya M et al. Recruitment of Nck by CD3 epsilon reveals a ligand-induced conformational change essential for T-cell receptor signaling and synapse formation. Cell 2002; 109:901-912.
114. Tamir I, Cambier JC. Antigen receptor signaling: Integration of protein tyrosine kinase functions. Oncogene 1998; 17:1353-1364.
115. Sulzer B, Perelson AS. Immunons revisited: Binding of multivalent antigens to B-cells. Mol Immunol 1997; 34:63-74.
116. Reth M, Wienands J. Initiation and processing of signals from the B-cell antigen receptor. Annu Rev Immunol 1997; 15:453-479.
117. Nimmerjahn F. Activating and inhibitory FcgammaRs in autoimmune disorders. Springer Semin Immunopathol 2006; 28:305-319.

118. Maurer D, Fiebiger E, Reininger B et al. Fc epsilon receptor I on dendritic cells delivers IgE-bound multivalent antigens into a cathepsin S-dependent pathway of MHC class II presentation. J Immunol 1998; 161:2731-2739.
119. Lara M, Ortega E, Pecht I et al. Overcoming the signaling defect of Lyn-sequestering, signal-curtailing FcepsilonRI dimers: Aggregated dimers can dissociate from Lyn and form signaling complexes with Syk. J Immunol 2001; 167:4329-4337.
120. Metzger H. The high affinity receptor for IgE, FcepsilonRI. Novartis Found Symp 2004; 257:51-59; discussion 59-64, 98-100, 276-185.
121. Metzger H, Eglite S, Haleem-Smith H et al. Quantitative aspects of signal transduction by the receptor with high affinity for IgE. Mol Immunol 2002; 38:1207-1211.
122. Holowka D, Sil D, Torigoe C et al. Insights into immunoglobulin E receptor signaling from structurally defined ligands. Immunol Rev 2007; 217:269-279.
123. Radaev S, Sun PD. Structure and function of natural killer cell surface receptors. Annu Rev Biophys Biomol Struct 2003; 32:93-114.
124. Natarajan K, Dimasi N, Wang J et al. Structure and function of natural killer cell receptors: multiple molecular solutions to self, nonself discrimination. Annu Rev Immunol 2002; 20:853-885.
125. Turnbull IR, Colonna M. Activating and inhibitory functions of DAP12. Nat Rev Immunol 2007; 7:155-161.
126. Posner RG, Paar JM, Licht A et al. Interaction of a monoclonal IgE-specific antibody with cell-surface IgE-Fc epsilon RI: characterization of equilibrium binding and secretory response. Biochemistry 2004; 43:11352-11360.
127. Schweitzer-Stenner R, Ortega E, Pecht I. Kinetics of Fc epsilon RI dimer formation by specific monoclonal antibodies on mast cells. Biochemistry 1994; 33:8813-8825.
128. Kent UM, Mao SY, Wofsy C et al. Dynamics of signal transduction after aggregation of cell-surface receptors: studies on the type I receptor for IgE. Proc Natl Acad Sci USA 1994; 91:3087-3091.
129. Yamasaki S, Ishikawa E, Kohno M et al. The quantity and duration of FcRgamma signals determine mast cell degranulation and survival. Blood 2004; 103:3093-3101.
130. Gibbs BF, Rathling A, Zillikens D et al. Initial Fc epsilon RI-mediated signal strength plays a key role in regulating basophil signaling and deactivation. J Allergy Clin Immunol 2006; 118:1060-1067.
131. Torigoe C, Inman JK, Metzger H. An unusual mechanism for ligand antagonism. Science 1998; 281:568-572.
132. Pasquier B, Launay P, Kanamaru Y et al. Identification of F calphaRI as an inhibitory receptor that controls inflammation: dual role of FcRgamma ITAM. Immunity 2005; 22:31-42.
133. Miura Y, Takahashi T, Jung SM et al. Analysis of the interaction of platelet collagen receptor glycoprotein VI (GPVI) with collagen. A dimeric form of GPVI, but not the monomeric form, shows affinity to fibrous collagen. J Biol Chem 2002; 277:46197-46204.
134. Varma R, Campi G, Yokosuka T et al. T-cell receptor-proximal signals are sustained in peripheral microclusters and terminated in the central supramolecular activation cluster. Immunity 2006; 25:117-127.
135. Jiang G, Hunter T. Receptor signaling: when dimerization is not enough. Curr Biol 1999; 9:R568-571.
136. Livnah O, Johnson DL, Stura EA et al. An antagonist peptide-EPO receptor complex suggests that receptor dimerization is not sufficient for activation. Nat Struct Biol 1998; 5:993-1004.
137. Syed RS, Reid SW, Li C et al. Efficiency of signalling through cytokine receptors depends critically on receptor orientation. Nature 1998; 395:511-516.
138. Greiser JS, Stross C, Heinrich PC et al. Orientational constraints of the gp130 intracellular juxtamembrane domain for signaling. J Biol Chem 2002; 277:26959-26965.
139. Gay NJ, Gangloff M, Weber AN. Toll-like receptors as molecular switches. Nat Rev Immunol 2006; 6:693-698.
140. Mescher MF, Agarwal P, Casey KA et al. Molecular basis for checkpoints in the CD8 T-cell response: Tolerance versus activation. Semin Immunol 2007; 19:153-161.
141. Colombetti S, Benigni F, Basso V et al. Clonal anergy is maintained independently of T-cell proliferation. J Immunol 2002; 169:6178-6186.
142. Choi S, Schwartz RH. Molecular mechanisms for adaptive tolerance and other T-cell anergy models. Semin Immunol 2007; 19:140-152.
143. Chiodetti L, Choi S, Barber DL et al. Adaptive tolerance and clonal anergy are distinct biochemical states. J Immunol 2006; 176:2279-2291.
144. Recio MJ, Moreno-Pelayo MA, Kilic SS et al. Differential biological role of CD3 chains revealed by human immunodeficiencies. J Immunol 2007; 178:2556-2564.
145. Gil D, Schrum AG, Alarcon B et al. T-cell receptor engagement by peptide-MHC ligands induces a conformational change in the CD3 complex of thymocytes. J Exp Med 2005; 201:517-522.

146. Risueno RM, Gil D, Fernandez E et al. Ligand-induced conformational change in the T-cell receptor associated with productive immune synapses. Blood 2005; 106:601-608.
147. Risueno RM, van Santen HM, Alarcon B. A conformational change senses the strength of T-cell receptor-ligand interaction during thymic selection. Proc Natl Acad Sci USA 2006; 103:9625-9630.
148. Vilen BJ, Nakamura T, Cambier JC. Antigen-stimulated dissociation of BCR mIg from Ig-alpha/Ig-beta: implications for receptor desensitization. Immunity 1999; 10:239-248.
149. Vilen BJ, Famiglietti SJ, Carbone AM et al. B-cell antigen receptor desensitization: Disruption of receptor coupling to tyrosine kinase activation. J Immunol 1997; 159:231-243.
150. Cambier JC, Fisher CL, Pickles H et al. Dual molecular mechanisms mediate ligand-induced membrane Ig desensitization. J Immunol 1990; 145:13-19.
151. Vilen BJ, Burke KM, Sleater M et al. Transmodulation of BCR signaling by transduction-incompetent antigen receptors: Implications for impaired signaling in anergic B-cells. J Immunol 2002; 168:4344-4351.
152. Arnon TI, Achdout H, Levi O et al. Inhibition of the NKp30 activating receptor by pp65 of human cytomegalovirus. Nat Immunol 2005; 6:515-523.
153. Levit MN, Liu Y, Stock JB. Stimulus response coupling in bacterial chemotaxis: Receptor dimers in signalling arrays. Mol Microbiol 1998; 30:459-466.
154. Sourjik V, Berg HC. Functional interactions between receptors in bacterial chemotaxis. Nature 2004; 428:437-441.
155. Sourjik V. Receptor clustering and signal processing in E. coli chemotaxis. Trends Microbiol 2004; 12:569-576.
156. Kawaguchi M, Eckels DD. Differential activation through the TCR-CD3 complex affects the requirement for costimulation of human T-cells. Hum Immunol 1995; 43:136-148.
157. Lanier LL, Ruitenberg JJ, Allison JP et al. Distinct epitopes on the T-cell antigen receptor of HPB-ALL tumor cells identified by monoclonal antibodies. J Immunol 1986; 137:2286-2292.
158. Schlitt HJ, Kurrle R, Wonigeit K. T-cell activation by monoclonal antibodies directed to different epitopes on the human T-cell receptor/CD3 complex: Evidence for two different modes of activation. Eur J Immunol 1989; 19:1649-1655.
159. Schwinzer R, Franklin RA, Domenico J et al. Monoclonal antibodies directed to different epitopes in the CD3-TCR complex induce different states of competence in resting human T-cells. J Immunol 1992; 148:1322-1328.
160. Yoon ST, Dianzani U, Bottomly K et al. Both high and low avidity antibodies to the T-cell receptor can have agonist or antagonist activity. Immunity 1994; 1:563-569.
161. Reich Z, Boniface JJ, Lyons DS et al. Ligand-specific oligomerization of T-cell receptor molecules. Nature 1997; 387:617-620.
162. Garboczi DN, Ghosh P, Utz U et al. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. Nature 1996; 384:134-141.
163. Abastado JP, Lone YC, Casrouge A et al. Dimerization of soluble major histocompatibility complex-peptide complexes is sufficient for activation of T-cell hybridoma and induction of unresponsiveness. J Exp Med 1995; 182:439-447.
164. Lindstedt R, Monk N, Lombardi G et al. Amino acid substitutions in the putative MHC class II "dimer of dimers" interface inhibit CD4+ T-cell activation. J Immunol 2001; 166:800-808.
165. Hayball JD, Lake RA. The immune function of MHC class II molecules mutated in the putative superdimer interface. Mol Cell Biochem 2005; 273:1-9.
166. Watts TH. T-cell activation by preformed, long-lived Ia-peptide complexes. Quantitative aspects. J Immunol 1988; 141:3708-3714.
167. Rubin B, Knibiehler M, Gairin JE. Allosteric Changes in the TCR/CD3 Structure Upon Interaction With Extra- or Intra-cellular Ligands. Scand J Immunol 2007; 66:228-237.
168. Cochran JR, Cameron TO, Stone JD et al. Receptor proximity, not intermolecular orientation, is critical for triggering T-cell activation. J Biol Chem 2001; 276:28068-28074.
169. Cebecauer M, Guillaume P, Hozak P et al. Soluble MHC-peptide complexes induce rapid death of CD8+ CTL. J Immunol 2005; 174:6809-6819.
170. Yamasaki S, Ishikawa E, Sakuma M et al. Mechanistic basis of pre-T-cell receptor-mediated autonomous signaling critical for thymocyte development. Nat Immunol 2006; 7:67-75.
171. Yamasaki S, Saito T. Molecular basis for preTCR-mediated autonomous signaling. Trends Immunol 2007; 28:39-43.
172. Geisberger R, Crameri R, Achatz G. Models of signal transduction through the B-cell antigen receptor. Immunology 2003; 110:401-410.
173. Yamashita T, Mao SY, Metzger H. Aggregation of the high-affinity IgE receptor and enhanced activity of p53/56lyn protein-tyrosine kinase. Proc Natl Acad Sci USA 1994; 91:11251-11255.

174. Kane P, Erickson J, Fewtrell C et al. Cross-linking of IgE-receptor complexes at the cell surface: Synthesis and characterization of a long bivalent hapten that is capable of triggering mast cells and rat basophilic leukemia cells. Mol Immunol 1986; 23:783-790.
175. Kanamaru Y, Blank U, Monteiro RC. IgA Fc receptor I is a molecular switch that determines IgA activating or inhibitory functions. Contrib Nephrol 2007; 157:148-152.
176. Li P, Morris DL, Willcox BE et al. Complex structure of the activating immunoreceptor NKG2D and its MHC class I-like ligand MICA. Nat Immunol 2001; 2:443-451.
177. Strong RK. Asymmetric ligand recognition by the activating natural killer cell receptor NKG2D, a symmetric homodimer. Mol Immunol 2002; 38:1029-1037.
178. Farndale RW. Collagen-induced platelet activation. Blood Cells Mol Dis 2006; 36:162-165.
179. Smethurst PA, Onley DJ, Jarvis GE et al. Structural basis for the platelet-collagen interaction: The smallest motif within collagen that recognizes and activates platelet Glycoprotein VI contains two glycine-proline-hydroxyproline triplets. J Biol Chem 2007; 282:1296-1304.
180. Lu Q, Navdaev A, Clemetson JM et al. Snake venom C-type lectins interacting with platelet receptors. Structure-function relationships and effects on haemostasis. Toxicon 2005; 45:1089-1098.
181. Kato K, Furihata K, Cheli Y et al. Effect of multimer size and a natural dimorphism on the binding of convulxin to platelet glycoprotein (GP)VI. J Thromb Haemost 2006; 4:1107-1113.
182. Singer KL, Mostov KE. Dimerization of the polymeric immunoglobulin receptor controls its transcytotic trafficking. Mol Biol Cell 1998; 9:901-915.
183. Mocsai A, Abram CL, Jakus Z et al. Integrin signaling in neutrophils and macrophages uses adaptors containing immunoreceptor tyrosine-based activation motifs. Nat Immunol 2006; 7:1326-1333.
184. Li R, Mitra N, Gratkowski H et al. Activation of integrin alphaIIbbeta3 by modulation of transmembrane helix associations. Science 2003; 300:795-798.
185. Hantgan RR, Lyles DS, Mallett TC et al. Ligand binding promotes the entropy-driven oligomerization of integrin alpha IIb beta 3. J Biol Chem 2003; 278:3417-3426.
186. Schamel WW, Arechaga I, Risueno RM et al. Coexistence of multivalent and monovalent TCRs explains high sensitivity and wide range of response. J Exp Med 2005; 202:493-503.
187. Iber D, Gruhn T. Organisation of B-cell receptors on the cell membrane. Syst Biol (Stevenage) 2006; 153:401-404.
188. Matsuuchi L, Gold MR. New views of BCR structure and organization. Curr Opin Immunol 2001; 13:270-277.
189. Schamel WW, Reth M. Monomeric and oligomeric complexes of the B-cell antigen receptor. Immunity 2000; 13:5-14.
190. Moss WC, Irvine DJ, Davis MM et al. Quantifying signaling-induced reorientation of T-cell receptors during immunological synapse formation. Proc Natl Acad Sci USA 2002; 99:15024-15029.
191. Minguet S, Swamy M, Alarcon B et al. Full activation of the T-cell receptor requires both clustering and conformational changes at CD3. Immunity 2007; 26:43-54.
192. Ding YH, Baker BM, Garboczi DN et al. Four A6-TCR/peptide/HLA-A2 structures that generate very different T-cell signals are nearly identical. Immunity 1999; 11:45-56.
193. Ortega E, Lara M, Lee I et al. Lyn dissociation from phosphorylated Fc epsilon RI subunits: A new regulatory step in the Fc epsilon RI signaling cascade revealed by studies of Fc epsilon RI dimer signaling activity. J Immunol 1999; 162:176-185.
194. Simpson RJ, Hammacher A, Smith DK et al. Interleukin-6: Structure-function relationships. Protein Sci 1997; 6:929-955.
195. Muller-Newen G, Kuster A, Wijdenes J et al. Studies on the interleukin-6-type cytokine signal transducer gp130 reveal a novel mechanism of receptor activation by monoclonal antibodies. J Biol Chem 2000; 275:4579-4586.
196. Autissier P, De Vos J, Liautard J et al. Dimerization and activation of the common transducing chain (gp130) of the cytokines of the IL-6 family by mAb. Int Immunol 1998; 10:1881-1889.
197. Chan KF, Siegel MR, Lenardo JM. Signaling by the TNF receptor superfamily and T-cell homeostasis. Immunity 2000; 13:419-422.
198. Chan FK. Three is better than one: Preligand receptor assembly in the regulation of TNF receptor signaling. Cytokine 2007; 37:101-107.
199. Siegers GM, Yang J, Duerr CU et al. Identification of disulfide bonds in the Ig-alpha/Ig-beta component of the B-cell antigen receptor using the Drosophila S2 cell reconstitution system. Int Immunol 2006; 18:1385-1396.
200. Asai K, Fujimoto K, Harazaki M et al. Distinct aggregation of beta- and gamma-chains of the high-affinity IgE receptor on cross-linking. J Histochem Cytochem 2000; 48:1705-1716.
201. 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.

202. Lee HK, Dunzendorfer S, Tobias PS. Cytoplasmic domain-mediated dimerizations of toll-like receptor 4 observed by beta-lactamase enzyme fragment complementation. J Biol Chem 2004; 279:10564-10574.
203. Weber AN, Moncrieffe MC, Gangloff M et al. Ligand-receptor and receptor-receptor interactions act in concert to activate signaling in the Drosophila toll pathway. J Biol Chem 2005; 280:22793-22799.
204. Kishimoto H, Kubo RT, Yorifuji H et al. Physical dissociation of the TCR-CD3 complex accompanies receptor ligation. J Exp Med 1995; 182:1997-2006.
205. Kosugi A, Saitoh S, Noda S et al. Translocation of tyrosine-phosphorylated TCRzeta chain to glycolipid-enriched membrane domains upon T-cell activation. Int Immunol 1999; 11:1395-1401.
206. La Gruta NL, Liu H, Dilioglou S et al. Architectural changes in the TCR: CD3 complex induced by MHC: Peptide ligation. J Immunol 2004; 172:3662-3669.
207. Liu H, Rhodes M, Wiest DL et al. On the dynamics of TCR: CD3 complex cell surface expression and downmodulation. Immunity 2000; 13:665-675.
208. D'Oro U, Munitic I, Chacko G et al. Regulation of constitutive TCR internalization by the zeta-chain. J Immunol 2002; 169:6269-6278.
209. McKay DB, Irie HY, Hollander G et al. Antigen-induced unresponsiveness results in altered T-cell signaling. J Immunol 1999; 163:6455-6461.
210. Willems F, Andris F, Xu D et al. The induction of human T-cell unresponsiveness by soluble anti-CD3 mAb requires T-cell activation. Int Immunol 1995; 7:1593-1598.
211. Wang XM, Djordjevic JT, Kurosaka N et al. T-cell antigen receptor peptides inhibit signal transduction within the membrane bilayer. Clin Immunol 2002; 105:199-207.
212. Kim JH, Cramer L, Mueller H et al. Independent trafficking of Ig-alpha/Ig-beta and mu-heavy chain is facilitated by dissociation of the B-cell antigen receptor complex. J Immunol 2005; 175:147-154.
213. Kremyanskaya M, Monroe JG. Ig-independent Ig beta expression on the surface of B lymphocytes after B-cell receptor aggregation. J Immunol 2005; 174:1501-1506.
214. Davis MM, Boniface JJ, Reich Z et al. Ligand recognition by alpha beta T-cell receptors. Annu Rev Immunol 1998; 16:523-544.
215. Gascoigne NR, Zal T, Alam SM. T-cell receptor binding kinetics in T-cell development and activation. Expert Rev Mol Med 2001; 2001:1-17.
216. Germain RN, Stefanova I. The dynamics of T-cell receptor signaling: Complex orchestration and the key roles of tempo and cooperation. Annu Rev Immunol 1999; 17:467-522.
217. Rosette C, Werlen G, Daniels MA et al. The impact of duration versus extent of TCR occupancy on T-cell activation: a revision of the kinetic proofreading model. Immunity 2001; 15:59-70.
218. Werlen G, Hausmann B, Naeher D et al. Signaling life and death in the thymus: Timing is everything. Science 2003; 299:1859-1863.
219. Love PE, Lee J, Shores EW. Critical relationship between TCR signaling potential and TCR affinity during thymocyte selection. J Immunol 2000; 165:3080-3087.
220. Andersen PS, Geisler C, Buus S et al. Role of the T-cell receptor ligand affinity in T-cell activation by bacterial superantigens. J Biol Chem 2001; 276:33452-33457.
221. Mirshahidi S, Ferris LC, Sadegh-Nasseri S. The magnitude of TCR engagement is a critical predictor of T-cell anergy or activation. J Immunol 2004; 172:5346-5355.
222. Gett AV, Sallusto F, Lanzavecchia A et al. T-cell fitness determined by signal strength. Nat Immunol 2003; 4:355-360.
223. Langenkamp A, Casorati G, Garavaglia C et al. T-cell priming by dendritic cells: Thresholds for proliferation, differentiation and death and intraclonal functional diversification. Eur J Immunol 2002; 32:2046-2054.
224. Batista FD, Neuberger MS. Affinity dependence of the B-cell response to antigen: A threshold, a ceiling and the importance of off-rate. Immunity 1998; 8:751-759.
225. Casola S, Otipoby KL, Alimzhanov M et al. B-cell receptor signal strength determines B-cell fate. Nat Immunol 2004; 5:317-327.
226. Benschop RJ, Cambier JC. B-cell development: signal transduction by antigen receptors and their surrogates. Curr Opin Immunol 1999; 11:143-151.
227. George J, Penner SJ, Weber J et al. Influence of membrane Ig receptor density and affinity on B-cell signaling by antigen. Implications for affinity maturation. J Immunol 1993; 151:5955-5965.
228. Singh DK, Kumar D, Siddiqui Z et al. The strength of receptor signaling is centrally controlled through a cooperative loop between Ca2+ and an oxidant signal. Cell 2005; 121:281-293.
229. Kinet JP. The high-affinity IgE receptor (Fc epsilon RI): From physiology to pathology. Annu Rev Immunol 1999; 17:931-972.
230. Yamasaki S, Saito T. Regulation of mast cell activation through FcepsilonRI. Chem Immunol Allergy 2005; 87:22-31.

231. Hlavacek WS, Redondo A, Wofsy C et al. Kinetic proofreading in receptor-mediated transduction of cellular signals: Receptor aggregation, partially activated receptors and cytosolic messengers. Bull Math Biol 2002; 64:887-911.
232. Iwashima M. Kinetic perspectives of T-cell antigen receptor signaling. A two-tier model for T-cell full activation. Immunol Rev 2003; 191:196-210.
233. Hlavacek WS, Redondo A, Metzger H et al. Kinetic proofreading models for cell signaling predict ways to escape kinetic proofreading. Proc Natl Acad Sci USA 2001; 98:7295-7300.
234. Haefner J. Modeling Biological Systems: Principles and Applications, 2nd ed. New York: Springer; 2005:480.
235. Ono S, Ohno H, Saito T. Rapid turnover of the CD3 zeta chain independent of the TCR-CD3 complex in normal T-cells. Immunity 1995; 2:639-644.
236. McKeithan TW. Kinetic proofreading in T-cell receptor signal transduction. Proc Natl Acad Sci USA 1995; 92:5042-5046.
237. Rabinowitz JD, Beeson C, Lyons DS et al. Kinetic discrimination in T-cell activation. Proc Natl Acad Sci USA 1996; 93:1401-1405.
238. Rabinowitz JD, Beeson C, Wulfing C et al. Altered T-cell receptor ligands trigger a subset of early T-cell signals. Immunity 1996; 5:125-135.
239. Kersh GJ, Kersh EN, Fremont DH et al. High- and low-potency ligands with similar affinities for the TCR: the importance of kinetics in TCR signaling. Immunity 1998; 9:817-826.
240. Schamel WW, Risueno RM, Minguet S et al. A conformation- and avidity-based proofreading mechanism for the TCR-CD3 complex. Trends Immunol 2006; 27:176-182.
241. Valitutti S, Muller S, Cella M et al. Serial triggering of many T-cell receptors by a few peptide-MHC complexes. Nature 1995; 375:148-151.
242. Itoh Y, Hemmer B, Martin R et al. Serial TCR engagement and down-modulation by peptide:MHC molecule ligands: relationship to the quality of individual TCR signaling events. J Immunol 1999; 162:2073-2080.
243. Borovsky Z, Mishan-Eisenberg G, Yaniv E et al. Serial triggering of T-cell receptors results in incremental accumulation of signaling intermediates. J Biol Chem 2002; 277:21529-21536.
244. Sousa J, Carneiro J. A mathematical analysis of TCR serial triggering and down-regulation. Eur J Immunol 2000; 30:3219-3227.
245. Friedl P, Gunzer M. Interaction of T-cells with APCs: The serial encounter model. Trends Immunol 2001; 22:187-191.
246. Davis MM. A new trigger for T-cells. Cell 2002; 110:285-287.
247. Alarcon B, Gil D, Delgado P et al. Initiation of TCR signaling: Regulation within CD3 dimers. Immunol Rev 2003; 191:38-46.
248. Reth M, Wienands J, Schamel WW. An unsolved problem of the clonal selection theory and the model of an oligomeric B-cell antigen receptor. Immunol Rev 2000; 176:10-18.
249. Aivazian D, Stern LJ. Phosphorylation of T-cell receptor zeta is regulated by a lipid dependent folding transition. Nat Struct Biol 2000; 7:1023-1026.
250. Rojo JM, Janeway CA Jr. The biologic activity of anti-T-cell receptor V region monoclonal antibodies is determined by the epitope recognized. J Immunol 1988; 140:1081-1088.
251. Krogsgaard M, Prado N, Adams EJ et al. Evidence that structural rearrangements and/or flexibility during TCR binding can contribute to T-cell activation. Mol Cell 2003; 12:1367-1378.
252. Kjer-Nielsen L, Dunstone MA, Kostenko L et al. Crystal structure of the human T-cell receptor CD3 epsilon gamma heterodimer complexed to the therapeutic mAb OKT3. Proc Natl Acad Sci USA 2004; 101:7675-7680.
253. Levin SE, Weiss A. Twisting tails exposed: The evidence for TCR conformational change. J Exp Med 2005; 201:489-492.
254. Anton van der Merwe P, Davis SJ, Shaw AS et al. Cytoskeletal polarization and redistribution of cell-surface molecules during T-cell antigen recognition. Semin Immunol 2000; 12:5-21.
255. Monks CR, Freiberg BA, Kupfer H et al. Three-dimensional segregation of supramolecular activation clusters in T-cells. Nature 1998; 395:82-86.
256. Davis SJ, van der Merwe PA. The kinetic-segregation model: TCR triggering and beyond. Nat Immunol 2006; 7:803-809.
257. Manolios N, Collier S, Taylor J et al. T-cell antigen receptor transmembrane peptides modulate T-cell function and T-cell-mediated disease. Nat Med 1997; 3:84-88.
258. Ali M, De Planque MRR, Huynh NT et al. Biophysical studies of a transmembrane peptide derived from the T-cell antigen receptor. Lett Pept Sci 2002; 8:227-233.
259. Bender V, Ali M, Amon M et al. T-cell antigen receptor peptide-lipid membrane interactions using surface plasmon resonance. J Biol Chem 2004; 279:54002-54007.

260. Gerber D, Quintana FJ, Bloch I et al. D-enantiomer peptide of the TCRalpha transmembrane domain inhibits T-cell activation in vitro and in vivo. FASEB J 2005; 19:1190-1192.
261. Enk AH, Knop J. T-cell receptor mimic peptides and their potential application in T-cell-mediated disease. Int Arch Allergy Immunol 2000; 123:275-281.
262. 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.
263. Manolios N, Huynh NT, Collier S. Peptides in the treatment of inflammatory skin disease. Australas J Dermatol 2002; 43:226-227.
264. Kurosaka N, Bolte A, Ali M et al. T-cell antigen receptor assembly and cell surface expression is not affected by treatment with T-cell antigen receptor-alpha chain transmembrane peptide. Protein Pept Lett 2007; 14:299-303.
265. Collier S, Bolte A, Manolios N. Discrepancy in CD3-transmembrane peptide activity between in vitro and in vivo T-cell inhibition. Scand J Immunol 2006; 64:388-391.
266. Amon MA, Ali M, Bender V et al. Lipidation and glycosylation of a T-cell antigen receptor (TCR) transmembrane hydrophobic peptide dramatically enhances in vitro and in vivo function. Biochim Biophys Acta 2006.
267. Ali M, Salam NK, Amon M et al. T-cell antigen receptor-alpha chain transmembrane peptides: Correlation between structure and function. Int J Pept Res Ther 2006; 12:261-267.
268. Vandebona H, Ali M, Amon M et al. Immunoreceptor transmembrane peptides and their effect on natural killer (NK) cell cytotoxicity. Protein Pept Lett 2006; 13:1017-1024.
269. Sigalov AB. Interaction between HIV gp41 fusion peptide and T-cell receptor: Putting the puzzle pieces back together. FASEB J 2007; 21:1633-1634; author reply 1635.
270. Spencer DM, Wandless TJ, Schreiber SL et al. Controlling signal transduction with synthetic ligands. Science 1993; 262:1019-1024.
271. Soldevila G, Castellanos C, Malissen M et al. Analysis of the individual role of the TCRzeta chain in transgenic mice after conditional activation with chemical inducers of dimerization. Cell Immunol 2001; 214:123-138.
272. Crabtree GR, Schreiber SL. Three-part inventions: Intracellular signaling and induced proximity. Trends Biochem Sci 1996; 21:418-422.
273. Buferne M, Luton F, Letourneur F et al. Role of CD3 delta in surface expression of the TCR/CD3 complex and in activation for killing analyzed with a CD3 delta-negative cytotoxic T-lymphocyte variant. J Immunol 1992; 148:657-664.
274. Luton F, Buferne M, Legendre V et al. Role of CD3gamma and CD3delta cytoplasmic domains in cytolytic T-lymphocyte functions and TCR/CD3 down-modulation. J Immunol 1997; 158:4162-4170.
275. Haks MC, Cordaro TA, van den Brakel JH et al. A redundant role of the CD3 gamma-immunoreceptor tyrosine-based activation motif in mature T-cell function. J Immunol 2001; 166:2576-2588.
276. Haks MC, Pepin E, van den Brakel JH et al. Contributions of the T-cell receptor-associated CD3gamma-ITAM to thymocyte selection. J Exp Med 2002; 196:1-13.
277. de Saint Basile G, Geissmann F, Flori E et al. Severe combined immunodeficiency caused by deficiency in either the delta or the epsilon subunit of CD3. J Clin Invest 2004; 114:1512-1517.
278. Roifman CM. CD3 delta immunodeficiency. Curr Opin Allergy Clin Immunol 2004; 4:479-484.
279. Teixeiro E, Daniels MA, Hausmann B et al. T-cell division and death are segregated by mutation of TCRbeta chain constant domains. Immunity 2004; 21:515-526.
280. Torres PS, Zapata DA, PachecoCastro A et al. Contribution of CD3 gamma to TCR regulation and signaling in human mature T-lymphocytes. Int Immunol 2002; 14:1357-1367.
281. Rodriguez-Tarduchy G, Sahuquillo AG, Alarcon B et al. Apoptosis but not other activation events is inhibited by a mutation in the transmembrane domain of T-cell receptor beta that impairs CD3zeta association. J Biol Chem 1996; 271:30417-30425.
282. Sutton BJ, Gould HJ. The human IgE network. Nature 1993; 366:421-428.
283. Gould HJ, Sutton BJ, Beavil AJ et al. The biology of IGE and the basis of allergic disease. Annu Rev Immunol 2003; 21:579-628.
284. Kraft S, Novak N. Fc receptors as determinants of allergic reactions. Trends Immunol 2006; 27:88-95.
285. Adamczewski M, Paolini R, Kinet JP. Evidence for two distinct phosphorylation pathways activated by high affinity immunoglobulin E receptors. J Biol Chem 1992; 267:18126-18132.
286. Dombrowicz D, Lin S, Flamand V et al. Allergy-associated FcRbeta is a molecular amplifier of IgE- and IgG-mediated in vivo responses. Immunity 1998; 8:517-529.
287. Eiseman E, Bolen JB. Signal transduction by the cytoplasmic domains of Fc epsilon RI-gamma and TCR-zeta in rat basophilic leukemia cells. J Biol Chem 1992; 267:21027-21032.

288. Jouvin MH, Adamczewski M, Numerof R et al. Differential control of the tyrosine kinases Lyn and Syk by the two signaling chains of the high affinity immunoglobulin E receptor. J Biol Chem 1994; 269:5918-5925.
289. Scharenberg AM, Lin S, Cuenod B et al. Reconstitution of interactions between tyrosine kinases and the high affinity IgE receptor which are controlled by receptor clustering. EMBO J 1995; 14:3385-3394.
290. Shiue L, Green J, Green OM et al. Interaction of p72syk with the gamma and beta subunits of the high-affinity receptor for immunoglobulin E, Fc epsilon RI. Mol Cell Biol 1995; 15:272-281.
291. Wilson BS, Kapp N, Lee RJ et al. Distinct functions of the Fc epsilon R1 gamma and beta subunits in the control of Fc epsilon R1-mediated tyrosine kinase activation and signaling responses in RBL-2H3 mast cells. J Biol Chem 1995; 270:4013-4022.
292. Goldstein B, Faeder JR, Hlavacek WS. Mathematical and computational models of immune-receptor signalling. Nat Rev Immunol 2004; 4:445-456.
293. Goldstein B, Faeder JR, Hlavacek WS et al. Modeling the early signaling events mediated by FcepsilonRI. Mol Immunol 2002; 38:1213-1219.
294. Faeder JR, Hlavacek WS, Reischl I et al. Investigation of early events in Fc epsilon RI-mediated signaling using a detailed mathematical model. J Immunol 2003; 170:3769-3781.
295. Hlavacek WS, Faeder JR, Blinov ML et al. The complexity of complexes in signal transduction. Biotechnol Bioeng 2003; 84:783-794.
296. Nadler MJ, Matthews SA, Turner H et al. Signal transduction by the high-affinity immunoglobulin E receptor Fc epsilon RI: coupling form to function. Adv Immunol 2000; 76:325-355.
297. Liu KJ, Parikh VS, Tucker PW et al. Role of the B-cell antigen receptor in antigen processing and presentation. Involvement of the transmembrane region in intracellular trafficking of receptor/ligand complexes. J Immunol 1993; 151:6143-6154.
298. Pleiman CM, D'Ambrosio D, Cambier JC. The B-cell antigen receptor complex: Structure and signal transduction. Immunol Today 1994; 15:393-399.
299. Cambier JC. Signal transduction by T- and B-cell antigen receptors: Converging: Structures and concepts. Curr Opin Immunol 1992; 4:257-264.
300. Cambier JC, Pleiman CM, Clark MR. Signal transduction by the B-cell antigen receptor and its coreceptors. Annu Rev Immunol 1994; 12:457-486.
301. Kraus M, Pao LI, Reichlin A et al. Interference with immunoglobulin (Ig)alpha immunoreceptor tyrosine-based activation motif (ITAM) phosphorylation modulates or blocks B-cell development, depending on the availability of an Igbeta cytoplasmic tail. J Exp Med 2001; 194:455-469.
302. Batista FD, Iber D, Neuberger MS. B-cells acquire antigen from target cells after synapse formation. Nature 2001; 411:489-494.
303. Bannish G, Fuentes-Panana EM, Cambier JC et al. Ligand-independent signaling functions for the B- lymphocyte antigen receptor and their role in positive selection during B-lymphopoiesis. J Exp Med 2001; 194:1583-1596.
304. Kato K, Kanaji T, Russell S et al. The contribution of glycoprotein VI to stable platelet adhesion and thrombus formation illustrated by targeted gene deletion. Blood 2003; 102:1701-1707.
305. Gawaz M. Role of platelets in coronary thrombosis and reperfusion of ischemic myocardium. Cardiovasc Res 2004; 61:498-511.
306. Gibbins JM, Okuma M, Farndale R et al. Glycoprotein VI is the collagen receptor in platelets which underlies tyrosine phosphorylation of the Fc receptor gamma-chain. FEBS Lett 1997; 413:255-259.
307. Sigalov AB. More on: glycoprotein VI oligomerization: A novel concept of platelet inhibition. J Thromb Haemost 2007; 5:2310-2312.
308. Sigalov AB. Inhibiting Collagen-induced Platelet Aggregation and Activation with Peptide Variants. US 12/001,258 and PCT PCT/US2007/025389 patent applications were filed on 12/11/2007 and 12/12/2007, respectively, claiming a priority to US provisional patent application 60/874,694 filed on 12/13/2006.
309. Leitenberg D, Balamuth F, Bottomly K. Changes in the T-cell receptor macromolecular signaling complex and membrane microdomains during T-cell development and activation. Semin Immunol 2001; 13:129-138.
310. Janeway CA Jr, Bottomly K. Responses of T-cells to ligands for the T-cell receptor. Semin Immunol 1996; 8:108-115.
311. Janeway CA Jr, Bottomly K. Signals and signs for lymphocyte responses. Cell 1994; 76:275-285.
312. Niedergang F, Dautry-Varsat A, Alcover A. Peptide antigen or superantigen-induced down-regulation of TCRs involves both stimulated and unstimulated receptors. J Immunol 1997; 159:1703-1710.
313. Bonefeld CM, Rasmussen AB, Lauritsen JP et al. TCR comodulation of nonengaged TCR takes place by a protein kinase C and CD3 gamma di-leucine-based motif-dependent mechanism. J Immunol 2003; 171:3003-3009.

314. San Jose E, Borroto A, Niedergang F et al. Triggering the TCR complex causes the downregulation of nonengaged receptors by a signal transduction-dependent mechanism. Immunity 2000; 12:161-170.
315. von Essen M, Nielsen MW, Bonefeld CM et al. Protein kinase C (PKC) alpha and PKC theta are the major PKC isotypes involved in TCR down-regulation. J Immunol 2006; 176:7502-7510.
316. Quintana FJ, Gerber D, Kent SC et al. HIV-1 fusion peptide targets the TCR and inhibits antigen-specific T-cell activation. J Clin Invest 2005; 115:2149-2158.
317. Bloch I, Quintana FJ, Gerber D et al. T-Cell inactivation and immunosuppressive activity induced by HIV gp41 via novel interacting motif. FASEB J 2007; 21:393-401.
318. Alarcon B, Swamy M, van Santen HM et al. T-cell antigen-receptor stoichiometry: Preclustering for sensitivity. EMBO Rep 2006; 7:490-495.
319. Kim YM, Pan JY, Korbel GA et al. Monovalent ligation of the B-cell receptor induces receptor activation but fails to promote antigen presentation. Proc Natl Acad Sci USA 2006; 103:3327-3332.
320. Eynon EE, Parker DC. Small B-cells as antigen-presenting cells in the induction of tolerance to soluble protein antigens. J Exp Med 1992; 175:131-138.
321. Gahring LC, Weigle WO. The induction of peripheral T-cell unresponsiveness in adult mice by monomeric human gamma-globulin. J Immunol 1989; 143:2094-2100.