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Review Article

Physical-Chemical Regulation of Membrane Receptors Dynamics in Viral Invasion and Immune Defense

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Abstract

Mechanical cues dynamically regulate membrane receptors functions to trigger various physiological and pathological processes from viral invasion to immune defense. These cues mainly include various types of dynamic mechanical forces and the spatial confinement of plasma membrane. However, the molecular mechanisms of how they couple with biochemical cues in regulating membrane receptors functions still remain mysterious. Here, we review recent advances in methodologies of single-molecule biomechanical techniques and in novel biomechanical regulatory mechanisms of critical ligand recognition of viral and immune receptors including SARS-CoV-2 spike protein, T cell receptor (TCR) and other co-stimulatory immune receptors. Furthermore, we provide our perspectives of the general principle of how force-dependent kinetics determine the dynamic functions of membrane receptors and of biomechanical-mechanism-driven SARS-CoV-2 neutralizing antibody design and TCR engineering for T-cell-based therapies.

Introduction

Ligand recognition of membrane receptors triggers various crucial physiological and pathological processes from viral invasion to immunological defense.[1] For example, beta-corona viruses (e.g. severe acute respiratory syndrome coronavirus 2, SARS-CoV-2) invasion of host cells for activating viral infections is initiated by their spike protein recognition of membrane receptors on host cells (e.g. angiotensin-converting enzyme 2, ACE2, ligand for both SARS-CoV and SARS-CoV-2).[2] T cell receptors (TCR) expressed on T lymphocytes (e.g. cytotoxic $CD8^+$ T cells or CTL) specifically recognize viral- or tumor-mutation-associated antigenic peptides presented by major histocompatibility complex class I (MHC class I) molecules, triggering a series of antigen-specific adaptive immune defense to eliminate viral infected or transformed target cells.[3–7] NKG2D (Natural Killer Group 2, member D) receptors, as co-stimulatory receptors

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commonly expressed on the surface of natural killer (NK) or T cells, recognizes autologous ligands from the MIC (MHC class I chain-associated, MICA and MICB) and ULBP (UL16 binding protein, ULBP1-6) families expressed on stressed, transformed, or infected cells, to mediate the killing process of virus-infected or tumor cells.[8–10] During these processes, mechanical cues, as essential factors, dynamically regulate receptor-ligand binding kinetics to delicately tune membrane receptor's functions and precisely activate viral invasion or immune defenses against foreign attacks.[11–14]

With the development of single-molecule force spectroscopy techniques (SMFS) and molecular dynamics simulations, membrane receptor-ligand interactions have been resolved with high-sensitivity, high-specificity, and high spatial and temporal resolutions, revealing unprecedented dynamical biophysical regulatory mechanisms that are unable to be disclosed by qualitative biochemical analysis[14–20]. Mechanical cues exerted on receptor-ligand binding complex mainly include different dynamic mechanical forces and the spatial confinement of cellular plasma membrane.[18,21,22] Mechanical tensile or traction force generated by cytoskeletal actomyosin contraction, cell membrane bending and cell migration or shear force provided by blood flow could deform or change receptors and/or ligands' conformations, differentially modulating the dissociation pathway of receptor-ligand interactions.[13,23,24] The plasma membrane of cells provides a unique microenvironment that biomechanically restricts the orientation of the ectodomain of membrane receptor and the spatial diffusion or movement only within the two-dimensional (2D) membrane, thus inevitably impacting the association and dissociation processes of receptor-ligand interactions and their binding kinetics[14,20,25–28]. Furthermore, mechanical regulation of receptor-ligand interactions executes crucial effects on transmembrane signaling of membrane receptors.[29–31] The underlying mechanisms have been dissected by biophysical methodologies that simultaneously record binding kinetics and binding-triggered cell signaling, such as fBFP (fluorescent biomembrane force probe),[18,32] BATTLES (Biomechanically-Assisted T-cell Triggering for Large-scale Exogenous-pMHC Screening),[18,33,34] and theoretical models.[35–40]

Here, we summarize recent advances in the development of single-molecule force spectroscopy techniques and crucial functional mechanisms underlying receptor-ligand interactions for triggering viral infection and immune defense. We propose that understanding the biomechanical characteristics of how mechanical and chemical cues couple in regulation of membrane receptor-ligand interactions may help optimize the designs of neutralizing antibodies against SARS-CoVs infection and of TCR-T or neoantigen vaccines for T-cell-based immunotherapies.

Single-molecule force spectroscopy and molecular dynamics simulations

Single-molecule force spectroscopy (SMFS) techniques, mainly containing atomic force microscopy (AFM), optical tweezers (OT), magnetic tweezers (MT), and biomembrane force probe (BFP) have revolutionized receptor-ligand binding kinetics measurements.[16,41] These SMFS techniques work like soft and mechanically sensitive springs, transducing piconewton forces to the displacements of a bead or a cantilever that can be quantitatively monitored and precisely manipulated in high spatial–temporal resolutions (for details see published reviews.[16,41] Benefited from precise force manipulations on single-molecule bonds, SMFS techniques have been applied to characterize the force-dependent receptor-ligand binding kinetics under different types of dynamic piconewton forces, and to characterize force-induced conformational changes, revealing crucial functional mechanisms of membrane receptors, such as viral (e.g. SARS-CoV-2 spike),[2,42–44] immune receptors (e.g. TCR[15,45] and NKG2D[8]) and adhesion receptors (e.g. Integrin[23], PSGL-1[46], LFA-1.[47]).

Along with the development of SMFS techniques, research on membrane receptor's functions keep carrying forward.[48] Taking BFP as an example, binding kinetics of receptor-ligand interactions can be derived from different methods, mainly containing dynamic force spectroscopy for measuring force-free dissociation rates,[49] force-clamp assay for characterizing force-dependent dissociation rates,[2,9,18,50] adhesion frequency assay for binding affinities and force-free association and dissociation rates,[51] and thermal-fluctuation assay for association rates and force-free dissociation rates.[26] With the more physiological-relevant biophysical condition, TCR-pMHC binding kinetics measurements are more matched with their ligand potencies in comparison to those by SPR measurements.[51] Later in 2014, the integration of fluorescent spectroscopy into the BFP system enabled the recording of intracellular Ca^{2+} signaling and measuring TCR-pMHC binding kinetics simultaneously, digitalizing TCR triggering mechanisms more directly and revolutionizing canonical methodologies for studying the mechanisms of membrane receptors mechanosensing and triggering.[18] Our group further improved the clamping force stability and accuracy of BFP in 2020, enabling the lifetime measurements of single-molecule bonds on live cells with ultra-slow dissociation kinetics, such as the interaction between anti-PD-1 mAb and PD-1 on live T cells.[17] Thus, the rapidly developed SMFS techniques have become more efficient in

dissecting the dynamic biophysical mechanisms of membrane receptors functions.

In addition to the aforementioned SMFS techniques, molecular dynamics (MD) simulation is an efficient computational method for dissecting the conformational changes and the dissociation pathways of receptor-ligand interactions with atomic resolution.[52,53] In this system, mechanical forces can drive receptor-ligand dynamic contacts and gradually exhibit distinct dissociation pathways. Analyzing the detailed trajectories, MD can provide putative force-inducing residues at the binding interface, including the formation of new hydrophobic interactions, hydrogen bonds, salt bridges, or electrostatic interactions that cannot be observed in static structures,[2,9,50] providing testable hypothesis for low throughput SMFS experiments and functional assays to characterize mutation effects with less efforts. The force-induced allosteric alterations could provide new biophysical regulatory mechanisms of interactions between membrane receptors and ligands.

Mechanical force

Mechanical force has been shown to be a biophysical determinant of membrane receptor-ligand interactions during viral invasion and immune defense. Mechanical force induced by membrane bending has been reported to be involved in cell–cell contact as well as in viral endocytosis.[54–57] When virions attach to the epithelial layer of the lung airways, the bent cell membrane exerts tensile forces (e.g. 0 –30 pN) on the viral spike-ACE2 complex, regulating viral spike/ACE2 binding kinetics and accordingly mediating viral-host recognition, attachment, and invasion.[2] A growing number of studies suggest that T cells enforce piconewton forces (e.g. 12 –19 pN for naïve $CD8^+$ T cell) to TCR-pMHC bonds and dynamically modulate their binding kinetics, as well as their conformations to accordingly transduce signals across cell membranes.[58–62] During the aforementioned processes, mechanical force regulates the dissociation rates of receptor-ligand interactions, exhibiting catch-, slip-or ideal bonds.[2,9,15,18,50,61,63–67] Catch bonds slow down the dissociation of receptor-ligand interactions and prolong the bond lifetimes in a specialized force range due to newly formed interactions between residues on receptor and ligand during force-regulated dissociation pathway.[46,68] In contrast, slip bonds, as Bell predicted, accelerate bond dissociation as mechanical force increases.[69] Ideal bonds exhibit no effect on dissociation, which is independent of changes in mechanical forces.[14,16] However, the detailed molecular mechanisms remain largely unknown.

Mechanical force in virus invasion

COVID-19 pandemic has caused immeasurable damage worldwide, and new variants are constantly emerging, such as beta, gamma, delta, omicron, and omicron variants (https://www.gisaid.org).[70] The initial step of viral invasion is that the spike protein (including S1 and S2 subunit) of SARS-CoV-2 (SARS2-S) recognizes host cell receptors (targeting mainly ACE2 and also tyrosine-protein kinase receptor UFO (AXL), and immune system receptors, such as, toll-like receptors (TLR), C-lectin type receptors (CLR), neuropilin-1 (NRP1), and DPP4/CD26).[71–79] Receptor-binding domain (RBD) in S1 subunit is the major domain for binding host receptor ACE2, while the S2 subunit forms fusion machinery to target host-cell plasma membrane after S1/S2 detachment.[80] Both processes are potentially regulated by mechanical forces, and revealing the underlying mechanisms from the biomechanical angle would possibly provide novel strategies for preventing novel mutated SARS-CoV-2 strains that are able to evade therapeutic antibodies or vaccines protection.

Studies have shown that mechanical forces generated by cell membrane bending boost ACE2-dependent SARS-CoV-2 invasion.[2,80] Firstly, in the viral recognition stage, mechanical force exerted on a single spike/ACE2 bond is approximately between 0 and 30 pN according to theoretical calculation.[2] Compared with SARS-S-RBD (~6% amino acid sequence difference compared to SARS2-S-RBD,[81] the interactions of SARS2-S-RBD binding to ACE2 have stronger mechanical stability to resist stretching force through inducing a more pronounced catch-slip bond behavior with much longer force-dependent bond lifetimes (Figure 1(A)), which is positively correlated with their infectivity. Mechanistically, SARS2-S-RBD is more prone to adopt an open conformation under mechanical force loading, thereby promoting the formation of more H-bonds and hydrophobic interactions on the SARS2-S-RBD/ACE2 binding surface, compared to that in SARS-S-RBD/ACE2 binding (Figure 1(C)). Secondly in the fusion stage, mechanical force accelerates S1/S2 detachment to promote S2 structural rearrangement and fusion machinery formation. In this regard, our group for the first time revealed that mechanical force dramatically speeds up SARS2-S S1/S2 detachment by up to $\sim 10^3$ times faster than that in the force-free condition (Figure 1(B)).[2]

These mechanical regulations during viral invasion were further validated by the more infectious D614G mutation of SARS2-S. D614G mutation, located outside of the RBD of the S1 subunit, causes more fatality and is inherited in Alpha, Beta, Delta and recent Omincron SARS-CoV-2 virus.[82–88] A recent study showed that D614G converts the S1 protein conformation

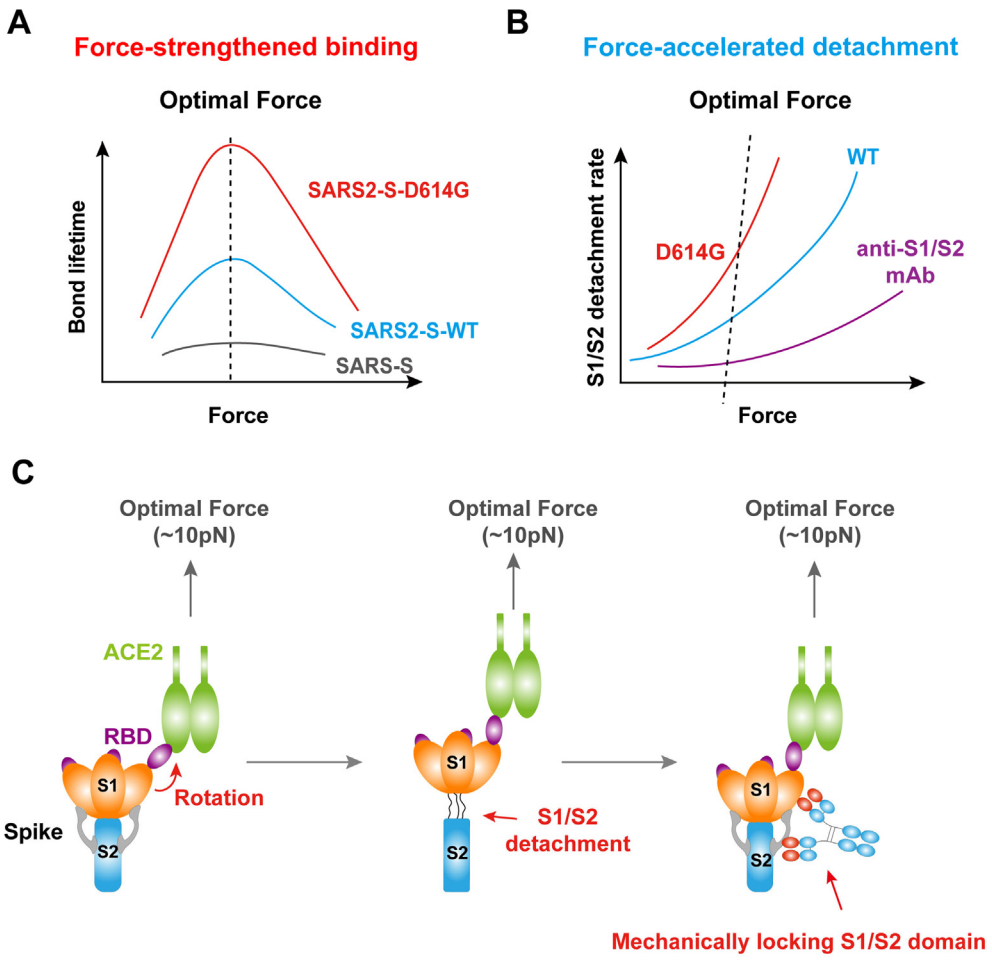

Figure 1. Mechanical forces strengthen the spike/ACE2 binding and accelerate S1/S2 detachment, providing novel intervention strategy. (A) Schematics of force-dependent bond lifetimes of the interactions of host ACE2 receptors interacting with SARS2-S-D614G (red), SARS2-S-WT (blue) and SARS-S (gray), respectively. (B) S1/S2 detachment rate versus force curves of SARS2-S-D614G (red), SARS2-S-WT (blue) and SARS2-S-WT in the presence of neutralizing antibody targeting S1/S2 (purple), respectively. (C) The dynamic structural model of force-regulated SARS2-S/ACE2 binding and conformational change of SARS2-S, force-induced S1/S2 detachment, and S1/S2-locking antibodies to impede SARS2-S/ACE2 binding.

(RBD and N-terminal domains of S1 subunit) to an ACE2-binding fusion-competent state and thus may increase viral infectivity.[89] Our group further found that mechanical force extent almost four-time longer lifetimes of SARS2-S-D614G binding with ACE2 receptors and 35 times faster force-induced S1/S2 detachment than those of wild-type SARS2-S (Figure 1(A, B)).[90] Thus, the more plausible molecular mechanism by which SARS2-S D614G mutation causes higher infection rate from biophysical standpoint is that D614G mutation fosters stronger mechanical stability of SARS2-S/ACE2 complex and better couples with mechanical force to induce much faster S1/S2 detachment. Currently, new sub-variants (e.g., omicron BA.1, BA.2, BA.2.12.1) are constantly emerging, we speculate that mechanical force are also essential for entry of other virus variants and the biophysical mechanism will be a common feature of viral invasion.

The widely adopted treatment strategy to prevent viral invasion is to block the engagement of the spike-RBD and ACE2 interactions with neutralizing antibodies.[91–94] However, due to the high mutagenicity of SARS2-S1 (especially RBD),[95] it is very likely that SARS2 evades neutralizing antibodies blockade through abolishing these mAb bindings with RBD[96–101] or that SARS2 may adopt other entry pathways by binding with other host membrane proteins (e.g. AXL) to maintain viral infectivity, which are the intrinsic limitations for these RBD-blockade designs of neutralizing antibodies.[86,102] We speculate that the aforementioned mechanical regulatory mechanisms could provide another novel intervention strategy to prevent virus infection, that is, mechanically locking the S1

subunit to S2 subunit to impede their detachment (Figure 1(C)).[2,103–106] In this way, revealing the underlying biophysical mechanisms of viral invasion, especially biomechanical activation of viral spikes, would potentially optimize the design of therapeutic antibodies.

Mechanical regulation in immune defense

Force-regulated conformational changes of immune receptors (e.g. TCR) or their ligands (e.g. pMHC, MICA) have been reported to uncover the detailed molecular mechanisms of receptor-ligand interactions during immune defense in recent studies.[9,15,50,107,108] Single-molecule force spectroscopy revealed that mechanical force strengthens agonistic pMHC-TCR (canonical αβTCR, not reverse docking αβTCR and γδTCR) interactions through catch bonds (Figure 2(A)).[50,108–111] In contrast, antagonistic pMHC-TCR interactions only exhibit slip bonds under mechanical force (Figure 2(B)).[18,112] To allosterically activate TCR-pMHC catch bonds, mechanical force induces conformational changes in pMHC to promote new contacts (hydrogen bonds) at the peptide-TCR or MHC-TCR binding interface (Figure 2(C)).[50] Mechanical stretching of single pMHC molecule results in an about 13 nm extension, and locking such extension attenuates TCR-pMHC catch bonds, demonstrating that force-induced conformational changes in pMHC contribute to the catch bonds formation.[50] Mechanical force mainly drives three sequential steps of conformational changes in an agonistic pMHC/TCR complex to activate catch bonds. First, TCR exploits its CDRβ (complementarity determining region) to establish a physical contact with the functional hotspot (e.g. the fourth or sixth residue for most mouse and human TCR-pMHC-I systems, such as peptides presented by H-2K^b, H-2K^d or HLA-A2, etc.[15,50,113–115] of the agonistic peptide. Second, force induces the MHC α1 and α2 domains to rotate towards TCR CDR loops, allosterically promoting new interactions at the TCR-MHC binding interface. Finally, force boosts the separation β2m from MHC α chain, resulting in greater rotation and extension of pMHC (Figure 2(C)). In addition to the tensile force-induced conformational changes in pMHC, the different direction of mechanical force can also result in rearrangement of the TCR-pMHC binding interface that explains the catch-bond formation.[50,116] Studies have found that shear force allosterically drives the conformational change of the FG loop in the constant region of the TCR β-subunit, controlling the catch bond formation and modulating bond lifetimes of TCR-pMHC interactions.[117] However, at the molecular or atomic level, protein molecules on the cell membrane are very flexible and mechanical force can induce membrane receptor ligand-binding domain to rotate around its anchoring point towards their ligands, which is usually not parallel to cell membrane. In other words, shear force may be eventually converted into a normal or tensile force to regulate receptor-ligand interactions. But, the detailed mechanism of how shear force is transformed into shear force remains unclear. Collectively, the force-dependent conformational changes in pMHC and TCR allosterically regulate TCR-pMHC interactions to determine TCR antigen discriminatory power.

Catch-bond engineering can serve as a promising biophysical strategy targeting cancer immunotherapy, e.g. TCR engineering in T cell-based adoptive cell therapy (ACT). A recent study exploits the biophysical parameters of TCR-pMHC interactions to develop an optimization strategy based on the force-induced conformational changes in TCR/pMHC complex and screen high-potency TCR variants through modifying low-affinity and non-reactive or affinity-matured and off-target TCRs.[34,50] Zhao et al specifically mutated polar or charged amino acids in TCR CDR region (defined as “hotspot” on the TCR) that would potentially form/impede new H-bonds and/or salt bridge interactions at the TCR-pMHC binding surface under force, instead of mutating the residues that directly contact with pMHC, to ensure their affinities with catch bond enhancement/inhibition, potentially boosting T-cell responses/avoiding excessive T-cell cross-reactivity, respectively (Figure 2(E)).[34,118,119] As a validation of the newly developed strategy, they selected a tumor-associated MAGE-A3-specific TCR (A3A-TCR) that was previously used in clinical trials with high binding affinities to its antigens and with severe off-target toxicity due to high cross-reactivity.[120] With the catch bonds engineering strategy, engineered analogs of A3A-TCR maintain binding affinity in the natural physiological range and high-efficiency activation and reduce TCR’s cross-reactivity.[34] It is the milestone that catch bond is utilized to optimize TCR for immunotherapy, providing a new biophysical angle to revolutionize T cell-mediated adoptive cell therapy. On the other hand, cancer-associated somatic mutations in pHLA-A2 were found to limit conformational extension in agonistic pHLA and impede TCR-pMHC catch bonds (Figure 2(D)),[50] which is also a promising target for the catch bond engineering strategy.

The biophysical regulatory mechanisms described above also prompt researches of other immune receptors in the innate immune system (e.g. NKG2D on lymphocyte cells). NK cells are another type of cytotoxic cells in the immune defense, which can rapidly respond to kill virus-infected or tumor cells.[121] NK cells exert tensile forces through NKG2D receptors, which interact dynamically with their ligands (e.g., MICA, MICB, ULBP1, and ULBP3), converting mechanical stimuli into biochemical signals.[122–124] MICA is the most pronounced ligand to exploit mechanical force and

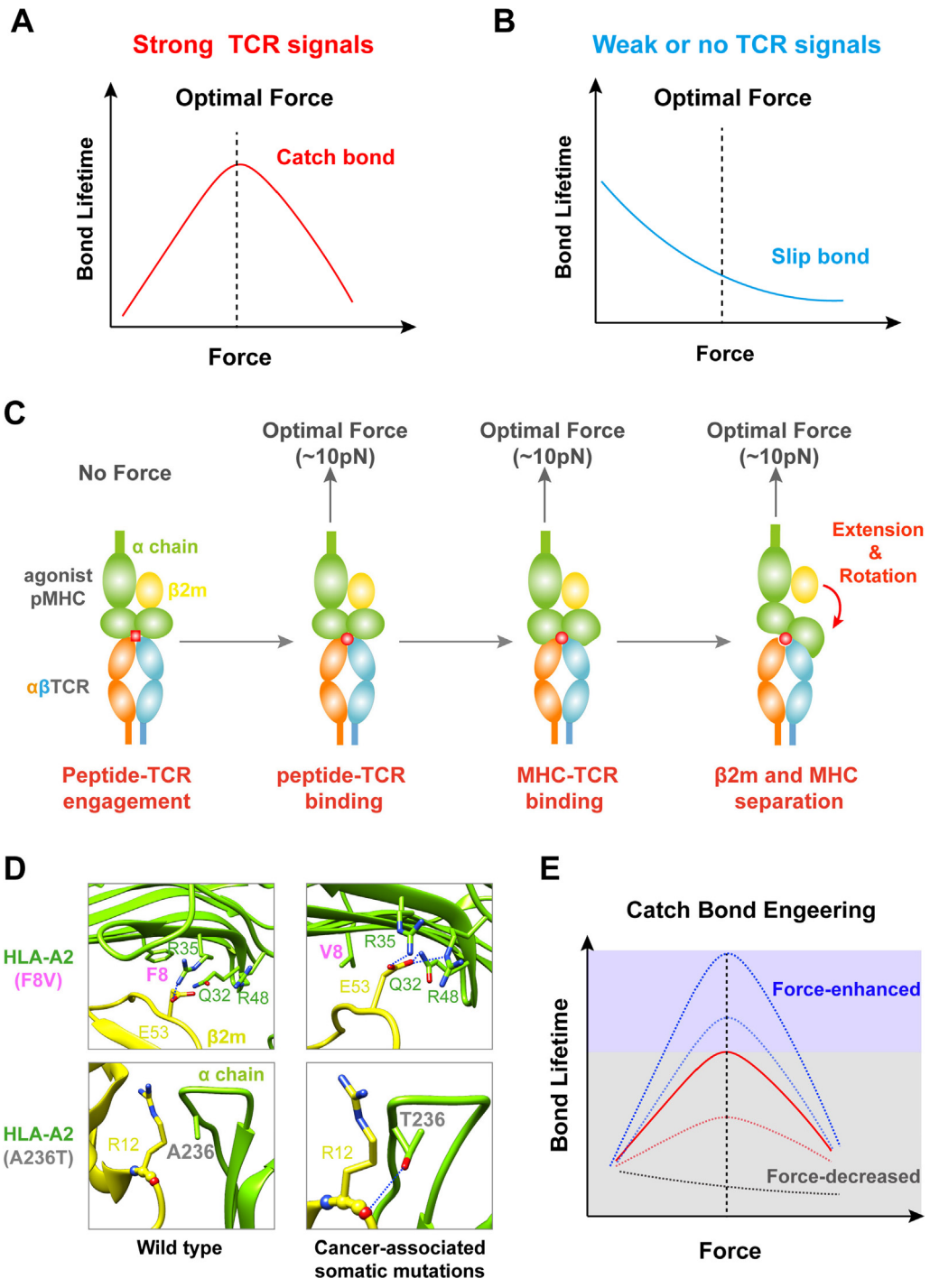

Figure 2. Force-induced conformational changes regulate catch bond formation between TCRs and pMHCs. (A, B) Bond lifetime versus force curves showing that TCR forms catch-slip bonds with agonists pMHC (red) (A) but slip bonds with antagonists pMHC (blue) (B). (C) Dynamic sequential steps in the mechanical regulation of pMHC conformation between TCR and agonistic pMHC. (D) Cancer-associated somatic mutations (A236T and F8V in HLA-A2) induce new H-bonds between α and β2 m subunits. H-bonds are indicated as blue dashed lines. (E) Mechanical forces modulate enhancement or reduction of the catch bond between TCRs and pMHCs.

has the longest bond lifetimes with NKG2D at the optimal force of ~10 pN compared with MICB, ULBP1, and ULBP3 ligands. At a low-force regime (<10 pN), mechanical force induces the catch bonds formation, while increasing mechanical force beyond 10 pN form the slip bonds to shorten the bond lifetimes for NKG2D-MICA interactions. Other ligands bound to NKG2D exhibit only slip bonds under full force spectrum.[9] Thus, mechanical force aids NKG2D the power to discriminate different

ligands, similar to but less potent than TCRs, through differential force-induced ligand conformational change of NKG2D/ligand complex.

Collectively, we believe that revealing mechanical regulations on other immune receptors, as well as the popular immune checkpoint receptors (e.g. PD-1 and CTLA4), would also promote the optimization of immunotherapies strategy from a biomechanical angle to treat not only infectious diseases, tumors but also autoimmune diseases.

Spatial confinement of cell plasma membranes

In addition to the effects of mechanical force, the spatial confinement by plasma membrane could also enforce significant impacts on the association and dissociation processes, especially their kinetic rates of receptor-ligand interactions and accordingly regulate membrane receptors functions.[107] The association rates (k_{on}) characterize how fast the receptor-ligand bond forms, while the dissociation rates characterize how long the bond lasts (k_{off}), and binding affinity (K_a) is defined by the ratio of k_{on}/k_{off}, reflecting receptor-ligand binding strength at equilibrium states.[16] In this section, we summarize recent advances about how spatial confinement of cell plasma membranes modulates the binding kinetics of receptor-ligand interactions and how SNPs (Single Nucleotide Polymorphisms) in transmembrane (TM) regions of membrane receptors exert allosteric regulatory effects on their binding kinetics under membrane confinement.

The measurement of receptor-ligand interactions in a two-dimensional (2D) manner (e.g., micropipette assay), where the binding kinetics are detected based on two apposing cell membranes that reconstitute the physiological conditions of membrane proteins, is significantly different from those in three-dimensional (3D) manners (e.g. surface plasmon resonance, SPR) using purified ligands in soluble states.[11,69] The existing regulatory mechanisms of how spatial confinement of plasma membrane regulate receptor-ligand interactions can be roughly divided into two categories. Firstly, 2D cell plasma membrane physically restricts the orientation of membrane receptors and/or ligands, affecting 2D binding affinities through changing association rates, such as interactions between human Fcγ receptor III (CD16, stimulatory receptor expressed mainly on NK cells as well as neutrophils, monocytes, macrophages, and T cells[125] or FcγRIIB (inhibitory receptor expressed on B lymphocytes[126] and their IgG ligands.[27,127] The molecule orientation changes arise primarily from differences in length or inclination of the TM regions of membrane receptors inside the membrane, altering their membrane-confined lateral mobility.[27,127,128] Secondly, the cell plasma membrane influences the spatial diffusion or movement of surface molecules with long ectodomains (e.g. CD45), regulating the binding kinetics of receptor-ligand interactions at cell–cell interfaces.[129–131] When the receptor diffuses on the plasma membrane, both the distance and the local concentration of membrane receptors and/or ligands would change in the contact zone. The diffusion or movement within 2D cell plasma membrane can be driven by many possible mechanisms according to kinetic segregation model, such as: molecule length changes, spatial reorganization, or functional clustering.[38] However, the mechanism by which molecular diffusion facilitates or prevents receptor-ligand interactions is still undefined.[132,133] Collectively, the physical regulation of the spatial confinement of cell plasma membranes reveals a novel mechanism for the regulation of physical–chemical coupling on receptor-ligand interactions.

Single nucleotide polymorphisms (SNPs) in transmembrane regions of membrane receptors can allosterically regulate receptor-ligand interactions through coupling the TM-lipid bilayer biochemical interaction with the spatial confinement of cell plasma membrane. An SNP variant of FcγRIIB, I232T, in systemic lupus erythematosus (SLE), impedes its two-dimensional binding affinities through association rates with its ligands (IgG1, IgG2, and IgG3) and suppresses immune cells activation.[127] Considering that the I232T variant is located in the transmembrane regions of the FcγRIIB receptor, and that the tilted transmembrane regions lead to a bent ectodomain conformation that impedes ligands association, SNP potentially couples with membrane confinement to affect the receptor-ligand recognition and signaling functions.[127] SNP mutations have also been identified in immune checkpoint molecules, such as LAG-3 (Lymphocyte-activation gene 3,[134] we speculate that this novel SNP-membrane confinement coupling mechanism would potentially inspirate new strategies for the treatment of immune checkpoint blockade (ICB) therapy.

Biomechanical regulation of membrane receptors transmembrane signaling

The triggering of transmembrane signaling of membrane receptors also depends on the effects of mechanical cues. How cells convert biomechanical stimuli into intracellular biochemical signals (mechanotransduction) is a crucial yet unresolved question in mechanobiology. Here, we take TCR as an example to illustrate recent advances.[18,135] At least three models have been proposed to reveal the mechanotransduction mechanisms of T cells (Figure 3). First, force-induced conformational changes in the extracellular domains of membrane receptors (e.g. TCR) sequentially propagates across the cell membrane,

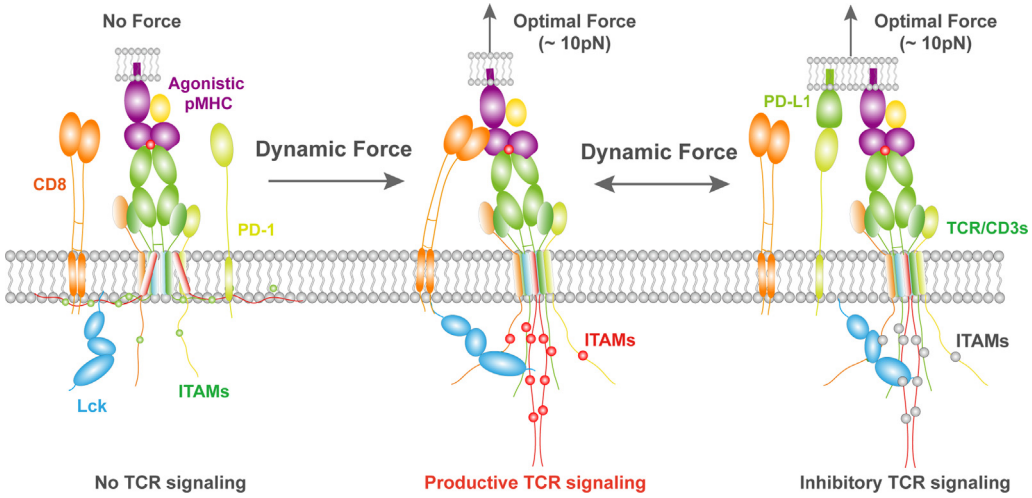

Figure 3. A dynamic model of mechano-chemical coupling for receptor-ligand interactions and transmembrane signaling. When TCR recognizes agonistic pMHC, force induces conformational changes in pMHC and the extracellular domains of TCR, which sequentially propagates across the cell membrane to induce intracellular kinase activation (Lck) and structural changes in the cytoplasmic domains (CD3). Meanwhile, CD8-associated Lck binds to phosphorylated CD3 ITAMs inside the cell membrane and to TCR-CD3 complex outside the cell membrane, forming a positive feedback mechanical loop that amplifies productive TCR signaling. However, the TCR-CD8 cooperative binding of pMHC is disrupted in the presence of PD-1, manifesting a negative or inhibitory cooperativity.

inducing structural changes in the cytoplasmic domains and downstream signaling (Figure 3).[117] In details, when a TCR recognizes an agonistic pMHC, TCR's extracellular domains (e.g. FG loop) probably transmit forces to the TCR transmembrane (TM) regions that form a compact and precisely organized structure with the CD3 subunits ($\varepsilon\delta$, $\varepsilon\gamma$, $\zeta\zeta$) within the membrane.[64,116,117] The juxtamembrane region of the CD3$\zeta\zeta$ signaling module likely acts as a mechanical pivot connecting TCRα chain, thereby transmitting biochemical and biomechanical information across the cell membrane.[6] Second, accumulation of dynamic catch bonds between TCR and pMHC triggers T cell signaling.[18] By simultaneously measuring the TCR-pMHC force-dependent binding kinetics and binding-triggered intracellular signaling, Zhu and his colleagues demonstrated that the accumulative bond lifetimes within the initial 60 seconds between TCR and agonist pMHC are the most relevant to intracellular signaling (e.g. Ca^{2+} flux), rather than the other parameters (e.g. binding affinities, peak bond lifetimes, average lifetimes, or longest lifetimes).[18] On the contrary, for antagonistic pMHC ligands and TCR interactions, the accumulative bond lifetimes are very short, and the Ca^{2+} signals are not induced within the specific time window.[18] This indicates that the synchronization between extracellular receptor-ligand interactions and intracellular kinase activation is critical for triggering T cells. Third, mechanosensing through membrane receptors induces a mechanical feedback loop that affects T cell activation. Mechanical-induced "dynamic catch" in trimolecular or multi-molecular interactions (e.g. TCR-pMHC-CD8[61] or TCR-pMHC-CD8-PD-1[136]), regulate the linkage and cis-interactions of intracellular signaling molecules (e.g. linking CD8 and TCR-CD3 via lymphocyte-specific protein tyrosine kinase, Lck) to form a mechanotransduction loop, also known as inside-out signaling, further amplifying the ligand discriminative ability of membrane receptors.[67] In addition, PD-1 specifically blocks the mechanotransduction loop during T-cell antigen recognition.[136] However, the detailed mechanisms about how mechanical forces coordinate the mechanical signaling from these co-receptor, co-stimulatory, and co-inhibitory molecules to delicately tune the intracellular signaling cascades remain unclear.

Collectively, biomechanical parameters are key determinants of membrane receptors transmembrane signaling, providing novel physical transmission mechanisms for receptor's ligand recognition and signal triggering.

Perspectives

Force fluctuations

Force fluctuations are functionally significant in numerous biological contexts, such as embryonic lineage sorting,[137] cell migration,[138] and signal transduction.[3] During embryonic development, primitive endoderm (PrE, founder of the yolk sac) and ectoderm (EPI, founder of the fetus) need to be physically separated. Dynamic cell surface fluctuations, rather than static cell surface parameters, robustly ensure physical lineage sorting.[137] During cell adhesion, the integrin-based focal adhesions (FAs) exhibit dynamic fluctuating traction through

extracellular matrix cytoskeleton motion.[138] Force exerted on receptor-ligand bonds *in vivo* potentially act in a cyclically fluctuating manner and often repeat with multiple cycles, depending on cytoskeletal actin velocity at the immune synapse or membrane fluctuations.[139–141] It is known that active actin cytoskeleton modulates the binding kinetics of receptor-ligand bonds *in situ*, but the cyclically fluctuating regulatory mechanisms to activate membrane receptors remain unclear. Membrane fluctuations are also influenced by the movement of the actin cytoskeleton and other factors, such as membrane microvilli structure, that affect receptor-ligand binding strength.[133,141–144] Besides, the frequency and amplitude of force fluctuations play critical roles in determining receptor-ligand binding and membrane receptors functions.[145] Different frequencies, amplitudes, and directions of dynamic force control different signal patterns and determine cell fates.[141] Therefore, gaining insights into how dynamic force of different frequencies, amplitudes and directions are functionally crucial to membrane receptors functions, worth for deeper exploring.

Membrane receptors dynamics buffer force fluctuations

The biophysical parameters of receptor-ligand interactions play critical effects on buffering force fluctuations. When the frequency of oscillatory force is sufficiently rapid, the signals exerted on receptor-ligand bonds may be unstable and easily disturbed.[146] Despite this interference, organisms could provide specific mechanisms and exhibit extraordinary robustness to reduce noise.[147] Thus, membrane receptors are trying to exploit the oscillatory forces to optimize their functions (e.g. Integrin or TCR).[19,21] Studies have shown that cyclical force could reinforce cell adhesion by prolonging much longer-lived bond lifetimes of integrin and its ligand interactions.[19] TCR exploits the cyclical forces exerted by actin movement significantly increase T cell signaling strength (Ca^{2+} flux), providing a novel cyclic mechanical reinforcement possibility of T cell triggering.[21,139] When the cells are treated with Latrunculin A (LatA), which inhibits actin polymerization, the cyclical force could compensate the Ca^{2+} signals.[148] But how TCR exploits force fluctuations to convert into biochemical signals is unknown. Mechanical-induced dynamic catch bonds of TCR-pMHC interactions may be easier to buffer force fluctuations than slip bonds when the bonds experience fluctuating forces.[141] The detailed molecular mechanism needs to be further explored. We thought that mechanical regulation under cyclic forces may differ from constant force regulation, which may enable the cell to fine-tune its mechanotransduction through membrane receptors. Here, we propose two possible explanations for how membrane receptors buffer force fluctuations. First, force fluctuations may accelerate catch bonds formation and increase the number of the long-lived bond lifetimes, helping membrane receptors to filter the noise interference and amplifying the amount of signal the cell can receive.[19,21,65] Second, force fluctuations may greatly enhance the rebinding and unbinding rate of receptor-ligand binding by inducing more open conformation, accelerating the process of kinetic proofreading and leading to a more stable mechano-feedback loop to reinforce the receptor-ligand interactions.[35,36,149] Thus, the regulatory mechanisms by which biomechanical factors (cycling force) regulate membrane receptors signaling transduction are worthy of further exploration, and we speculate these mechanisms could be shared by other mechanical-sensing membrane receptors (e.g. integrins, NKG2D, and PD-1).

Conclusions and future directions

Over the past decade, a conceptual framework of physical–chemical regulation of membrane receptor dynamics has emerged to address fundamental scientific questions of viral invasion and immune defense. These findings provide novel mechanisms by which mechanical cues modulate the conformation of membrane receptors or ligands, determining the receptor-ligand binding kinetics and signaling transduction. These dynamic mechanisms will very likely inspire non-canonical thoughts for developing novel clinically relevant immunotherapeutic treatment [e.g. immunotherapeutic antibodies, or TCR-T]. However, multiple outstanding questions remain to be answered:

1. What are the oscillating patterns of forces on receptor-ligand bonds under physiological conditions?
2. Whether and how viral exploit oscillatory force to boost viral invasion?
3. How do force fluctuations dynamically modulate the catch-bond behavior of receptor-ligand interactions?
4. Whether dynamic mechanical stimulation can accelerate the process of kinetic proofreading?

CRediT authorship contribution statement

Rui Qin: Formal analysis, Writing - original draft, Writing - review & editing. **Chenyi An:** Funding acquisition, Supervision, Writing - review & editing. **Wei Chen:** Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing - review & editing.

DATA AVAILABILITY

No data was used for the research described in the article.

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Declaration of Interest

Authors declare that they have no competing interests.

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