INTRODUCTION

Despite intensive investigation, the mechanisms of T cell receptor (TCR)-mediated signal generation remain poorly understood. It was suggested that the unique challenge set on TCR recognition might impose the simultaneous involvement of several triggering mechanisms including aggregation, conformational changes, and segregation (van der Merwe and Dushek, 2011). Here we review recent evidence showing that dynamic membrane phenomena influence signal generation at the TCR/antigen presenting cell (APC) interface. Indeed, these phenomena influence at the same time the species of molecules encountering each other and the physical conditions of molecular encounters, each of which are key components of signaling. Here, we shall discuss how membrane-confined molecules have known involvement in T cell activation, we shall focus exclusively on the TCR.

Recent evidence provided new insight into the effect of the membrane environment on TCR/pMHC interaction

Since TCR/pMHC interactions occur between surface-attached molecules, i.e., under two-dimensional (2D) conditions, it is difficult to relate the T cell response to the physical properties of the TCR/pMHC interaction measured in solution (Matsui et al., 1999; Akcasu et al., 2010), i.e., under 3D conditions. Indeed (Bongrand, 1999), 2D and 3D conditions differ for several reasons: First, the kinetics of bond formation between surface-attached molecules depends on the distance between surfaces, lateral motility of receptors, and size and flexibility of interacting molecules. Second, bond dissociation between surface-attached ligands and receptors depends on the relative motion of surfaces and applied forces. Third, the formation of multivalent attachments between surface-bound ligands and receptors depends on aforementioned parameters, surface roughness and ligand density, thus obscuring the link between affinity and avidity. Over the last 15 years, many investigators have devised new ways of monitoring bond formation and dissociation between surface-attached molecules. Devices have included laminar flow chambers (Kaplanski et al., 1995; Alon et al., 1995) and atomic force microscopes (Florin et al., 1993). Biomembrane force probes were introduced to dramatically improve the atomic force microscopes: the cantilever is replaced with a soft vesicle that acts as a tunable spring, thus increasing the dynamic range of measurements (Merkel et al., 1999).

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Despite intensive investigation, the mechanisms of T cell receptor (TCR)-mediated signal generation remain poorly understood. Here we review various dynamic processes at the cell membrane that might critically control this signaling. Firstly, we summarize recent reports providing new information on the sensitivity of TCR/ligand interaction to the membrane environment and particularly to applied forces. Secondly, we review recent evidence that forces and displacements are continuously generated at cell surfaces. Thirdly, we summarize recent experimental evidence demonstrating the capacity of forces to generate signals. Lastly, we provide a quantitative model to exemplify the capacity of dynamic processes to modulate TCR properties such as specificity that were previously difficult to explain with conventional models. It is concluded that the described dynamic processes must be integrated into current models of TCR signaling.

Keywords: T lymphocyte activation, T cell receptor, dynamics, forces, mechanotransduction, membrane curvature, rafts, antigen presenting cell
Adherent cells exert a continual traction on underlying surfaces (Harris et al., 1980). In addition, many cell types including dissociation rates measured for each pMHC. These findings are association rate, but no difference could be found between the correlated. FORCES RUNNING PARALLEL TO THE CELL MEMBRANE (LONGITUDINAL FORCES)

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Adherent cells exert a continual traction on underlying surfaces (Harris et al., 1980). In addition, many cell types including leukocytes were found to generate lateral oscillations of several tens of nanometer amplitude and between 0.2 and 30 Hz frequency (Krol et al., 1990). Furthermore, many cell types, including lymphocytes, spread on adherent surfaces displayed sequential waves of membrane protrusion and retraction on their periphery with a timescale of several tens of seconds (Doherty et al., 2006). Finally, measuring of the forces generated by cultured neurons with optical tweezers revealed the presence of alternate pulling and pushing forces to the order of 50 pN with a total cycle duration of several seconds (Shahapure et al., 2010).

TCRs CAN GENERATE SIGNALING CASCADES IN RESPONSE TO FORCES

Intracellular calcium rise is a hallmark of T cell activation. It was recently reported that a tangential force but not a normal force of 50 pN applied on T cells through anti-CD3-coated magnetic beads could induce a calcium rise within a few minutes, and this effect was ascribed to a quaternary conformational change induced by...
forces (Kim et al., 2009). In other experiments, when T cells were bound to APCs through an engineered CD3 ligand, no calcium rise was observed in static conditions, whereas mild hydrodynamic forces triggered a calcium rise within minutes (Li et al., 2010). However, hydrodynamic forces did not induce any calcium rise in T cells tethered through CD28 receptors rather than CD3.

Thus, TCR can act as a mechanotransducer, i.e., it can generate signaling cascades in response to forces. While this role of TCRs is clear, the precise molecular mechanisms involved remain poorly understood. An in-depth review of the mechanotransduction mechanisms that were recently demonstrated in a number of cell models other than immune cells is therefore warranted to assess the possible relevance of such mechanisms to T cells.

**GENERAL MECHANISMS OF MECHANOTRANSDUCTION**

For the sake of clarity, we shall discuss separately (i) some mechanisms potentially involved in TCR mechanotransduction, and (ii) the expected dependence of these mechanisms on the dynamics of lipid organization in the cell membrane.

**GENERAL MECHANISMS FOR MECHANOTRANSDUCTION**

Membrane ionic channels may act as force transducers (Chatellie, 2009).

However, while TCR-mediated T lymphocyte activation involves an early calcium rise, most evidence suggests that the opening of calcium channels is a secondary event in the signaling cascade (Smith-Garvin et al., 2009).

**Force-induced conformational changes**

A force-induced conformational change of membrane-associated proteins might generate new docking sites. As previously emphasized (Piersier et al., 2009), a force F acting on a protein can induce a conformational change if this would generate a displacement d such that $F \cdot d$ is substantially higher than $k_B T$, where $k_B$ is Boltzmann's constant and $T$ is the absolute temperature. Since $k_B T$ is about 4 pN × nanometer, a force of 40 pN could induce a significant conformational shift provided it involved a deformation of at least 0.1 nm. Interestingly, integrins may undergo deformations that are in the order of 1 nm (Salas et al., 2004). Also, a force of a few piconewtons applied on talin exposed docking sites allowing vinculin binding (De Ru et al., 2009) also (Ehlicher et al., 2011), forces were found to influence the connection of actin to integrins through the actin binding protein filamin.

**Force-induced displacement of a receptor relative to the membrane**

A force might move membrane molecules relative to the lipid bilayer, thus masking or unmasking docking sites for cytoplasmic molecules. Indeed, a force of 20 pN might suffice to uproot a membrane-embedded molecule (Bell, 1978). It was recently suggested that the interaction of the CD3/TCR complex with membrane bilayers could result in the sequestration of some key tyrosine residues that might be exposed after ligand binding (Ma et al., 2008; Xu et al., 2008).

**Signal generation following a change of membrane curvature**

Sucking lymphocytes into a micropipette with an inner diameter in the order of a few micrometers and a pressure of a few tens of Pascals thereby generating a force of several hundreds of piconewtons (Foa et al., 1988; Tözeren et al., 1989) might generate a protrusion with a radius of curvature in the order of a micrometer. Also, the application of a pulling force of about 25 pN to a fibroblast could generate the formation of a membrane tube of about 200 nm radius (Dai and Sheetz, 1995; Bauchler and Sheetz, 1999). Thus, the transverse forces described in Section “Forces and Movements Perpendicular to the Cell Membrane (Transverse Movements)” might change the membrane curvature and generate nanometer-scale protrusions. This might trigger a signal through two non-exclusive mechanisms: (i) Curvature-sensitive proteins might be recruited into a localized area and nucleate a signaling scaffold (Peter et al., 2004; Saitosi et al., 2006). (ii) If traction forces are exerted through cell membrane adhesion receptors, these receptors may be gathered into the contact area, resulting in a local change in protein composition. All these mechanisms are depicted in Figure 1.

Importantly, all these mechanisms can be strongly influenced by the nanometer-scale organization of membrane lipids. We shall now briefly discuss this point.

**MECHANOTRANSDUCTION IS MODULATED BY THE HIGHLY DYNAMIC PLASMA MEMBRANE**

The cell receptor/pMHC interaction is likely strongly influenced by the membrane organization and dynamics. It is thus legitimate to ask whether membrane domains such as lipid rafts could play a role in the TCR triggering mechanism. After more than one decade of intensive investigation and debate, current views converge on the notion that lipid rafts exist in the cell membrane as fluctuating molecular assemblies/domains with typical sizes of less than 100 nm (Lingwood and Simons, 2010; He and Marguet, 2011). It has been suggested that in the resting state TCRs might partition into raft nanodomains (He and Marguet, 2008; Simons and Gerl, 2010). In such a scenario, raft nanodomains could be involved in TCR triggering in several ways.

Docking sites generated by forces or enzyme processing will preferentially interact with molecules localized within the same nanodomains as the TCR (Lingwood et al., 2008). In a recent study using single-molecule near-field scanning optical microscopy it was shown that raftophilic proteins such as CD95 or LFA-1, but not the non-raft protein CD71, were recruited to regions proximal (<150 nm) to CTxB-GM1 raft nanodomains without physical intermixing (van Zanten et al., 2010). Therefore, TCR partitioning into rafts could facilitate its encounter with Lck (Zhang et al., 2011), since the latter is most likely also a raftophilic protein.

Conformational changes induced by forces and/or ligand binding may be influenced by the local composition of the membrane. Indeed, through promoting selective lipid–protein interactions, raft nanodomains could enhance certain conformations/spatial orientations of TCR/CD3 complexes that might either positively or negatively regulate receptor activity as was demonstrated on other models. Coskun et al. (2011) recently reported that cholesterol-rich Id/Lo, but not cholesterol-poor Id membrane
He and Bongrand | TCR triggering and membrane dynamics

**FIGURE 1** | Mechanisms for mechanotransduction. Five potential mechanisms for mechanotransduction are depicted. (A) A local force (red arrow) may generate a protrusion or a tether, resulting in the recruitment of curvature-sensitive molecules (green disks; Suetsugu et al., 2006). (B) Increased membrane tension (red arrows) may result in the opening of mechanosensitive channels (Chalfie, 2009). (C) A separating force (red arrow) exerted on attached cells may result in the concentration of adhesion receptors into a smaller area (Tözeren et al., 1989). (D) A force applied to a protein may result in a conformational change and exposure of docking sites shown as green rectangles (Del Rio et al., 2009). (E) A pulling or pushing force (red arrow) may alter the position of a protein with respect to the plasma membrane, resulting in the exposure of docking sites shown as pink rectangles (Xu et al., 2008).

Environments enhanced the GM3 ganglioside-dependent inactivation of EGFR autophosphorylation. In the case of TCR, acidic phospholipids including several phosphoinositides were shown to bind to the cytoplasmic domain of CD3ε and CD3ζ, and were proposed to regulate the access of ITAMs by Lck (Xu et al., 2008; Deford-Watts et al., 2009, 2011). Raft nanodomains could be involved in such a binding mechanism since it has been shown that they strongly contributed to the membrane recruitment of pleckstrin homology (PH) domain-containing proteins by the phosphatidylinositol-3,4,5-trisphosphate (Lasserre et al., 2008).

The recruitment of curvature-sensitive molecules should be influenced by local nanodomains

Finally, experimental evidence suggests that membrane curvature is controlled by its constituent molecules, and conversely, curvature could participate in organizing membrane proteins and lipids (Groves, 2007).

In conclusion, available evidence suggests that dynamic phenomena do influence TCR signaling. This may strongly influence the performance (i.e., sensitivity and specificity) of TCR recognition.

**A SIMPLE MODEL ILLUSTRATES THE NEED TO INCORPORATE DYNAMIC PROCESSES TO BE ABLE TO EXPLAIN THE PERFORMANCE OF TCR-MEDIATED SIGNALING**

The kinetic proofreading mechanism was suggested to account for the extraordinary specificity of TCR-mediated ligand recognition: how can a TCR robustly discriminate between ligands that bind with fairly comparable kinetics and affinity (McKeithan, 1995)? We shall address this question by comparing the information provided by TCR engagement under static and dynamic conditions.

The T cell decision to become activated after encountering a pMHC is at least partly linked to the lifetime of the TCR/pMHC interaction (Matsui et al., 1994). How could a T cell discriminate between two pMHCs (1) and (2) that its TCR binds with dissociation rates of respectively, say, $k_1 = 0.5$ and $k_2 = 2 \times 10^{-7}$? Suppose the criterion used is whether a bond survives for at least time $t$. The probability that pMHCs (1) and (2) will meet this criterion are respectively $P_1 = \exp(-k_1 t)$ and $P_2 = \exp(-k_2 t)$. If we require that $P_1/P_2$ be higher than, say, 100 to ensure specificity, we obtain:

$$P_1/P_2 = \exp([k_2 - k_1]t) > 100; \quad t > \ln(100)/(k_2 - k_1) = 3 \text{ s}.$$  (1)

This emphasizes two limitations of the discrimination procedure: firstly, the decision takes at least 3 s, in line with the kinetic proofreading mechanism, secondly, the probability that the pMHC with the lowest dissociation rate $k_1$ be bound for at least 3 s is $\exp(-k_1 \times 3) = 0.223$. Thus, the detection sensitivity is only 22%.

Sensitivity might be improved without decreasing the specificity by performing two determinations and starting activation if at least one of the binding events lasts for more than time $t$. This gives:

$$P_1/P_2 = \frac{2\exp(-k_1 t) - \exp(-2k_1 t)}{2\exp(-k_2 t) - \exp(-2k_2 t)}.$$  (2)
The minimum time required for a specificity ratio $P_r/P_i$ of 100 is now 3.14 s, and measurement time is at least 2 $\times$ 3.14 = 6.28 s. The detection sensitivity is $2 \times 10^{-15}$ M. Thus, specificity and sensitivity can both be increased by increasing the number of measurements. However, this also increases the measurement time, which impairs recognition speed. This might be avoided by pulling at bonds, thus increasing the dissociation rate in accordance with Bell's law (Robert et al., 2012). This thus demonstrates how forces could help increase detection efficiency.

CONCLUSION
Recent results support the conclusion that (i) the region of initial contact between T cells and APCs is highly dynamic and thus generates forces; (ii) these forces influence TCR/PMHC binding and dissociation as well as signal generation; and (iii) these forces may strongly influence the performance of TCR recognition. Since these mechanisms are not exclusive of previously suggested triggering mechanisms (van der Merwe and Dushek, 2011), TCR triggering might involve a combination of these and previously suggested mechanisms. Predicting the outcome of a T cell/APC encounter will thus require quantitative modeling to account for a combination of multiple mechanisms.

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REFERENCES
Alkon, D., Bade, O., Zhang, H., Shindler, K., Chernomordik, V., Csombo, D., and van der Merwe, P. A. (2010). Dependence of T cell antigen recognition on T cell receptor-peptide MHC confirmation time. Immunology 52, 163–174.
Alon, R., Hammer, D. A., and Springer, T. A. (1995). Lifetime of the P-selectin-carbohydrate bond and its response to tonesk force in hydrodynamic flow. Nature 377, 359–362.
Bell, G. I. (1978). Models for the specific association rate in accordance with Bell's law. J. Phys. Chem. 80, 561–573.
Bernard, A. M., Lévy, P. E., Wulfing, C., Albanesi, J. P., Love, P. E., Wulffing, C., Doxey, T. C., and Tschochner, T. (2009). The CD3 zeta subunit contains a phosphatidylinositol-binding motif that is required for the stable accumulation of TCR-CB complex at the immunological synapse. J. Immunol. 184, 6839–6847.
Dobereiner, H. G., Dubin-Thaler, B. J., Magre, J. M., Belkaya, S., Johnson, B. A., Bachtold, N., Kao, L.-S., Tao, M.-H., Lieber, R., and Dushek, O. (2010). Cutaneous T cell autoreactivity in a laminar shear flow. J. Immunol. 184, 5959–5963.
Fuchs, C., Miyagawa, H., Furutani, J.-H., Weis, D. A., and Stossel, T. P. (2011). Mechanical strain in actin networks regulates FAK/GAP and integrin binding to fibronectin. Nature 470, 289–292.
Herm, E. L., Meyr, V. T., and Gauth, H. (1998). Adhesion forces between individual ligand-receptor pairs. Science 280, 415–417.
He, H. T., and Marguet, D. (2008). T cell antigen receptor triggering and lipid raft: a matter of space and time scales. Taking Point on the involvement of lipid rafts in T-cell activation. EMBO J. 29, 525–530.
He, H. T., and Marguet, D. (2011). Detecting nanodomains in living cell membrane by fluorescence correlation spectroscopy. Annu. Rev. Phys. Chem. 62, 417–438.
Hillenbrand, W., and Soriano, R. M. (2014). Unilaminar, tonic interaction and cohesion of fluid membranes. Nuovo Cimento D 35, 137–151.
Huang, J., Ziemnia, V., Liu, R., Edwards, L. J., Jiang, N., Evavold, B. B., and Zhu, C. (2010). Storching single tail red molecular activates vinculin binding. Science 323, 639–640.
Hoffman, J. L., Dhade-Thubil, B. I., He, H. T., and Marguet, D. (2008). Adhesion forces between T cell receptor and lipid raft: a matter of space and time scales. Taking Point on the involvement of lipid rafts in T-cell activation. EMBO J. 29, 525–530.
Horn, E. L., Meyr, V. T., and Gauth, H. (1998). Adhesion forces between individual ligand-receptor pairs. Science 280, 415–417.
Huang, J., Ziemnia, V., Liu, R., Edwards, L. J., Jiang, N., Evavold, B. B., and Zhu, C. (2010). Storching single tail red molecular activates vinculin binding. Science 323, 639–640.
Horn, E. L., Meyr, V. T., and Gauth, H. (1998). Adhesion forces between individual ligand-receptor pairs. Science 280, 415–417.
Ivy, C., Chen, B.-M., Wu, P.-C., Cheng, T.-L., Lin, S.-L., Lo, M.-H., Liou, A., and Roifman, S. R. (2010). Cutting edge: mechanical forces leading to T cells immobilized via the TCR complex can trigger TCR signaling. J. Immunol. 184, 3599–3605.
Kaplanis, G., Farrant, C., Toos, O., Pieters, A., Bendeix, A.-M., Alken, M.-C., Kaplanis, S., and Bourgand, E. (2001). Cutaneous T cell autoreactivity in a laminar shear flow. J. Immunol. 164, 1922–1935.
Kim, S. T., Takeuchi, K., Sun, Z.-Y., Toru, M., Castro, E. C., Fahmy, A., and Lang, M. J. (2009). The T cell receptor is an anistropic mechanosensor. J. Biol. Chem. 284, 31026–31037.
Koel, A. Y., Grunfeld, M. G., Levin, S. V., and Smigelrdinis, A. D. (1996). Local mechanical oscillations of the cell surface within the range 0.2–30 Hz. Eur. Biophys. J. 19, 95–99.
Lanser, R., Guo, X., Crole�ondra, F., Haman, Y., Hazuch, O., Bertrand, A. M., Souza, S. M., Leine, P. F., Brünig, H. O., Ofek, H., Borsch, G., Nemes, J. A., Peronneau, R., Marguet, D., and He, H. T. (2008). T cell receptor triggering and lipid raft: a matter of space and time scales. Taking Point on the involvement of lipid rafts in T-cell activation. EMBIO J. 29, 525–530.
Lyu, C., Chen, B.-M., Wu, P.-C., Cheng, T.-L., Lin, S.-L., Lo, M.-H., Liou, A., and Roifman, S. R. (2010). Cutting edge: mechanical forces leading to T cells immobilized via the TCR complex can trigger TCR signaling. J. Immunol. 184, 3599–3605.
Lymph, D., Riau, J., Schwieler, P., and Simons, K. (2008). Plasma membrane are poised for activation of raft phase coalescence at physiological temperature. Proc. Natl. Acad. Sci. U.S.A. 105, 10005–10010.
Lymph, D., and Simons, K. (2011). Lipid rafts as a membrane-organizing principle. Science 327, 46–50.
Mue, Z., Immer, P. A., an Finkel, T. H. (2008). The receptor deformation model of TCR triggering. J. Immunol. 182, 1002–1008.
Munch, K., Benfica, J. J., Stoffel, F., Roep, P. A., and Dubos, M. M. (1994). Kinetics of T cell receptor binding to peptide/MHC complex: correlation of the dissociation rate with T cell responsiveness. Proc. Natl. Acad. Sci. U.S.A. 91, 12862–12866.
Merkel, R., Nassoy, P., Leung, A., McKeithan, T. W. (1995). Kinetic He and Bongrand TCR triggering and membrane dynamics. Nature 377, 50–53.

Nogales, P. A., Kraeva, T. B., Khan, A., Retchussium, H. H., and Calabret, M. D. (1996). Polarity of T cell shape, motility and sensitivity to antigen. Immunity 4, 421–430.

Pelling, A. E., Voriat, S. F., Chu, C. P. K., Nicholls, B. M., Hemsley, A. L., Mason, C., and Horton, M. A. (2007). Mapping correlated membrane pulsations and fluctuations in human cells. J. Mol. Recognit. 20, 467–475.

Peters, B. J., Kent, H. M., Mill, I. G., Calvillo, L., Boyer, C., and Bongrand, P. (2008). How membrane undulations help cells tiptoe on adhesive surfaces before sticking. Proc. Natl. Acad. Sci. U.S.A. 105, 15437–15442.

Peters, A., Monnet-Curti, V., Benoliel, A.-M., and Bongrand, P. (2009). Force generation in lamellipodia is a probabilistic process with fast growth and retraction events. Biophys. J. 98, 597–608.

Richter, K., and Evans, E. (1999). Force spectroscopy. ligand bonds explored with dynamic force spectroscopy. Nature 397, 50–53.

Ritchie, K., and Evans, E. (1999). Force measurements of TCR/pMHC recognition at T cell surface. Proc. Natl. Acad. Sci. U.S.A. 96, 11935–11939. doi: 10.1073/pnas.96.22.11935

Smith-Garvin, J. E., Koretzky, G. A., Simons, K., and Gerl, M. J. (2010). Revising biomembrane BAR structure. Nature 467–475.

Springer, T. A. (2004). Rolling adhesion, and insights. Science 303, 495–499.

Suetsugu, S., Maruyama, K., Sakamoto, A., Haraue-Suetsugu, K., Seto, A., Oikawa T., Motolna, C., Shirotani, M., Takayama, T., and Yokoyama, S. (2010). The RAC-binding domain of IRSp53 mediates membrane deformation. J. Biol. Chem. 281, 35347–35356.

Suetsugu, S., Tözeren, A., Sung, K. L. P., and Chen, S. (1999). Theoretical and experimental study on cross-bridge migration during cell disaggregation. Biophys. J. 75, 479–487.

Valitutti, S., Müller, S., Cell, M., Padovani, E., and Lanteri-Becuchois, A. (1995). Serial triggering of many T-cell receptors by a few peptide-MHC complexes. Nature 375, 146–151.

van der Merwe, P. A., and Dushek, O. (2011). Mechanics for T cell receptor triggering. Nat. Rev. Immunol. 11, 47–55.

van Zanten, T. S., Gómez, I., Murao, C., Kamijo, R., and Garcia-Parajo, M. F. (2010). Direct mapping of nanoscale communication on intact cell envelopes impedes adhesion. Proc. Natl. Acad. Sci. U.S.A. 107, 15437–15442.

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