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► To cite this version:

Pierre Bongrand, En Cai, Kyle Marchuk, Peter Beemiller, Casey Beppler, et al.. How can T cells perform ligand analysis during a second-scale contact ?. 2017, 10.1126/science.aal3118 . inserm-03440289

HAL Id: inserm-03440289

<https://www.hal.inserm.fr/inserm-03440289>

Submitted on 22 Nov 2021

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Visualizing dynamic microvillar search and stabilization during ligand detection by T cells

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Science 12 May 2017:

Vol. 356, Issue 6338, eaal3118

DOI: 10.1126/science.aal3118

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- RE: How can T cells perform ligand analysis during a second-scale contact ?
 - [Pierre Bongrand](#), emeritus professor (immunology, medical school), Aix-Marseille Université, INSERM, CNRS
(11 July 2017)

How can T cells perform ligand analysis during a second-scale contact ?

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One sentence summary :

Cai et al. (1) showed that T lymphocytes could scan large surface areas very rapidly with highly dynamic microvilli ; there remains to understand how a tiny spot can be accurately analyzed within a few seconds.

Abstract :

Immunologists have been puzzled for decades by the extraordinary efficiency with which T lymphocytes scan antigen presenting cells to detect foreign material. Recently, Cai et al. (1) showed that T cells could survey an extensive contact area with highly dynamic microvilli touching small areas during a few seconds. There remains to understand what pieces of information can be obtained during such a sort period of time. The best way to address this problem might consist of performing ultrasensitive force measurements with high spatial and temporal accuracy in the contact area. This approach might yield useful information about the general strategy used by living cells to analyze their environment.

Manuscript text :

T lymphocytes can detect foreign material with extraordinary sensitivity, speed and specificity. They can detect nearly a single foreign peptide (p) bound to major histocompatibility complex (MHC) on an antigen-presenting cell (APC) (2) . Detection can be performed within a few minutes (3), matching the lifetime of an encounter between a randomly migrating T cell and an APC in vivo (4). T cells can discriminate between interaction lifetimes of 5s versus 10s (5), and specificity is an absolute requirement to discriminate between numerous self peptides and a few foreign molecules. All these achievements are close to the limitations set by physical laws (6) for at least two reasons :

Firstly, how can cells associate sensitivity and speed ? If a T lymphocyte touches an APC with the tip of a microvillus and examines pMHCs diffusing through the contact region, general diffusion laws imply that several hours would be needed for all surface-bound pMHCs to pass randomly through the contact zone (7). A simple, although potentially expensive way of overcoming this difficulty would consist of increasing the contact zone by cell spreading on the surface before any detection event, however, much experimental evidence shows that spreading does not precede but rather follows antigen detection (8,9). A major advance was recently provided by Cai et al. (1) : they monitored the initial contact between T cells and antigen-bearing surfaces with high spatial and temporal resolution. They found that T cells searched surfaces with highly dynamic microvilli that could survey an extensive contact zone within 1 minute, with a dwell time of a few seconds for each contact. They emphasized that this dwell time was comparable to the threshold lifetime allowing to discriminate between activating

(agonist) and non activating pMHCs. This finding nicely completed the previous report that T cell receptors were concentrated on the tip of microvilli (10), thus strongly suggesting that antigen scanning involved highly dynamic microvilli scanning individual APCs through transient contacts with a duration on the order of 3.5 – 6 s each (1). This estimate is consistent with the earlier finding that T cells microvilli could detect a ligand on a surface within less than 13.7s, as evidenced by a retraction event (11). The authors estimated the average contact time between an microvillus and a neutral surface at 8.6 s, in close agreement with the estimate by Cai et al. (1).

Secondly, there remains to understand how cells could simultaneously achieve speed and specificity. Since discrimination was long reported to be correlated to the lifetime of TCR-pMHC interaction (5,12) and the initial contact time between T cell microvilli and APCs is on the order of the threshold lifetime between activating and non-activating ligands, it would be tempting to conclude that pMHC quality is evaluated by determining how long a TCR-pMHC bond persisted during microvillus-to-APC contact. However, this cannot be the whole story : first, accurate bond lifetime measurements may require much more time than natural bond duration : Indeed, bond rupture is a random event and a single observation is not informative enough to allow a quantitative estimate of natural bond lifetime (6). Secondly, it is not easy to understand how a T cell would “know” that a TCR is engaged on the tip of a microvillus contacting a surface, since T cell activation is not likely to be triggered by a mere conformational change of the T cell receptor (TCR) (6). Recently, several reports supported the view that antigen detection might be mediated by the application of a force on the TCR : shortly after the report that the TCR acted as a mechanotransducer (13, 14), Zhu and colleagues used an ultrasensitive tool known as the biomembrane force probe to show that the TCR could trigger a definite rise of intracellular calcium after being subjected to sequential transient pulling events with an intensity on the order of 10 pN and a total duration of several seconds, during a total period of time of 1 minute (15). Interestingly, it was soon shown with DNA-based nanoparticle tension sensors that T cells indeed transmitted forces on the order of 10 pN during their contact with antigen bearing surfaces (16). These data might seem to resolve the speed-and-specificity paradox (17), since it was concluded (16) that the 10 pN force might strongly enhance the lifetime of TCR-pMHC bonds formed with agonist (i.e. activating) ligands, a property of so-called “catch bonds”, in contrast with so-called “slip bonds”, the lifetime of which is reduced by forces (18).

However, more information is needed to assess the validity and generality of the model suggested by these results. Firstly, it is important to assess the significance of signaling events such as calcium rises, that are often used as reporters of T cell activation. As mentioned above, T cell microvilli were found to detect an antigen within about 13.7s (11), and this is on the order of the dwell time of a microvillus on a contact spot (1, 11). However, protrusion dynamics was found to be independent of calcium signaling (10), et calcium signaling triggered by TCR stimulation seemed correlated to a 1-minute integration period (15). Thus, a calcium rise cannot be a valid reporter of local agonist detection on the tip of a dynamic microvillus. This emphasizes the need to discriminate between signaling events that are involved in the dynamics

of microvilli and those mediating T cell activation. This is by no means trivial since activation is a multistep process and the definition of activation triggering is somewhat arbitrary. It may be suggested that a significant event such as T cell arrest (18) or decision to spread on a surface (8, 9) is a more convincing reporter of a T cell decision than a transient calcium rise. Another important point is that a calcium rise may occur as a local event (19) that might not be indicative of a cell decision, and published data are not always sufficient to discriminate between a local calcium rise versus a whole cell response. Secondly, we need a better knowledge of cell protrusion dynamics : how do the tips of microvilli behave during the apparent contact ? do they push towards the APC, or do they pull at the bond ? Interestingly, quantitative fluorescence measurements suggested that the tips of T cell microvilli spontaneously displayed high frequency fluctuations of several tens of nanometer amplitude (20). It is therefore attractive to speculate that the tips of microvilli exert rapidly fluctuating forces on bonds, and a single microvillus could perform multiple sequential lifetime measurements on bonds formed with the same TCR ligand, thus assessing a combination of bond lifetime and binding kinetics. This mechanism would account for the possible importance of ligand rebinding during T-cell/APC interaction (21).

These results strongly emphasize the need to achieve simultaneous real time monitoring of the force exerted by a T cell on a surface with high sensitivity (on the piconewton range) and spatial resolution (microvillus thickness is on the order of several tens of nanometer). This is a tough challenge due to the thermal fluctuations in the biological milieu, the measure of which is on the order of 4 pN.nm, and signal processing will be required to take full advantage of currently available tools such as new optical methods (1) and ultrasensitive force measurement based on biomembrane force probes (15), substratum-embedded fluorescent microspheres (22) or tunable DNA strands (16). However, such a combination should be most rewarding : force measurements may provide the best way to monitor contact between fuzzy structures such as cell membranes that have no clearcut boundary at the nanometer scale .

Unraveling the scanning strategy of T cells, involving initial highly localized events localized on the tips of microvilli, rapidly followed by a cell-size decision such as migration arrest or spreading, would thus allow us to assess the significance of signaling events. Dissecting a well-defined cell process with subsecond and submicrometer resolution is certainly a powerful means of discovering causal relationships within complex regulation networks in association with high throughput analysis methods (23), which is a current challenge for immunologists and cell biologists.

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Competing Interests: None declared.

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