Trends in Immunology

CellPress

Review

Antigen Receptor Nanoclusters: Small Units with Big Functions

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Adaptive lymphocytes express highly variable antigen receptors, allowing them to recognize a large variety of proteins, for example, derived from pathogens and tumor cells. Despite decades of investigations, the signaling mechanisms of these receptors are still incompletely understood. Super-resolution imaging studies revealed that antigen receptors, their coreceptors, and even some downstream signaling molecules tend to form dynamic nanometers-sized self-clusters in quiescent cells. Antigen stimulation induces the coalescence of these nanoclusters to form membrane proximal signalosomes that can mediate efficient signal transduction. In this review, we discuss the dynamic structures of T cell receptor and B cell receptor nanoclusters, the driving forces behind this spatial reorganization, as well as their potential relevance in the modulation of lymphocyte activation and function.

Trends

Super-resolution imaging studies reveal that antigen receptors form nanoclusters in quiescent lymphocytes.

Antigen stimulation induces the spatial reorganization of nanoclusters, resulting in the coalescence of antigen receptors and signaling proteins to mediate efficient signal transduction.

Homotypic interactions and interactions with the cytoskeleton and membrane lipids all play an important role in the regulation of antigen receptor clustering.

Modulating antigen receptor clustering directly affects lymphocyte signaling and function.

Antigen Receptors

Lymphocytes use surface-expressed antigen receptors to survey and capture antigens. Upon the recognition of cognate antigens, lymphocytes may undergo activation, proliferation, and differentiation, accounting for the establishment of immune responses against invading pathogens and tumor cells. Antigen receptors in both B and T lymphocytes lack tyrosine kinase activity, thus the activation signaling is initiated by the phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) within the cytoplasmic domain of the associated signaling proteins, which are Igα:Igβ in the B cell receptors (BCRs) [1] and CD3εδ, εγ, and ζζ in the T cell receptors (TCRs) [2] (Figure 1). Over the past decades, many biochemical and genetic studies have identified the key components of the ITAM-derived signaling pathways in lymphocytes, and these have been reviewed previously [3–6]. However, how antigen binding triggers transmembrane (TM) signaling of antigen receptor is still not fully understood. Over the past decades, fluorescence imaging techniques have been developed and applied to directly visualize the behavior changes of antigen receptors upon antigen binding in lymphocytes. Initially, immunologists used confocal fluorescence microscopy to image the formation of TCR/BCR aggregates on the plasma membrane upon antigen binding. Antigen receptors cluster at the interface between lymphocytes and antigen-presenting cells, forming the central supramolecular complex (cSMAC) [1,7–10]. Moreover, soluble multivalent antigen induced a drastic aggregation of BCRs, leading to patching and capping during the initiation of B cell activation [1]. Subsequently, the development of high-resolution high-speed total internal reflection fluorescence microscopy enabled the observation of microscopic clusters (microclusters) of antigen receptors and signaling molecules upon antigen binding, which were believed to be the most fundamental structure on the plasma membrane in the initiation of lymphocyte signaling [11–16] (Figure 2). Recently, substantial progress has been made in this fast-growing field with the help of advanced super-resolution imaging techniques. Especially,

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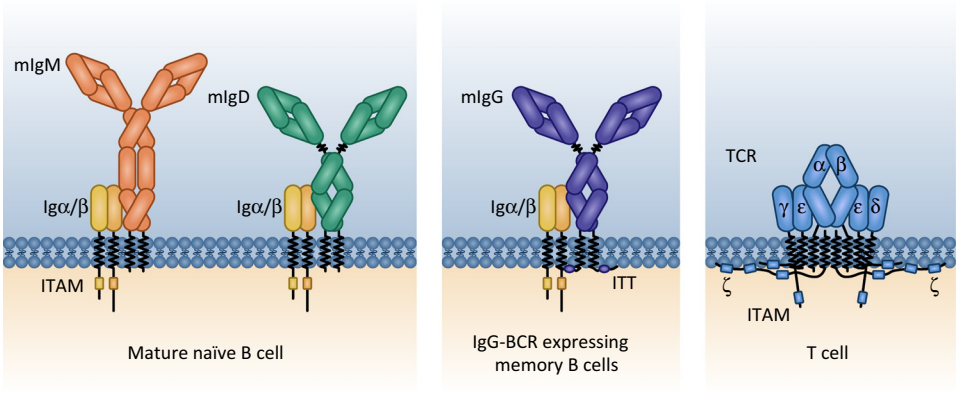

Figure 1. Schematic Presentation of Antigen Receptors. The B cell receptor (BCR) is composed of a membrane-bound immunoglobulin, associated with a covalently linked Igα and Igβ heterodimer. The αβ T cell receptor (TCR) is a heterodimer of α and β subunits, associated with CD3εδ, εγ, and ζζ subunits. In mature naïve B cells, both IgM-BCR and IgD-BCR are expressed on the cell surface simultaneously, while in IgG-switched memory B cells, isotype-switched IgG-BCR is the only type of antigen receptor that is presented on the cell surface. The immunoreceptor tyrosine-based activation motif (ITAM) is responsible for the initiation of activation signaling after the engagement between antigen and antigen receptors. ITAMs are only present in the cytoplasmic domain of signaling proteins, that is, Igα and Igβ in BCR and CD3ε, δ, γ, and ζ in TCR. In quiescent cells, the cytoplasmic domains of membrane IgG heavy chain, which contain immunoreceptor tail tyrosine (ITT) motifs, ionically interact with the acidic phospholipids in the inner leaflet of the plasma membrane. Similarly, the cytoplasmic domains of CD3ε and CD3ζ containing ITAMs are also bound to membrane via ionic protein–lipid interactions.

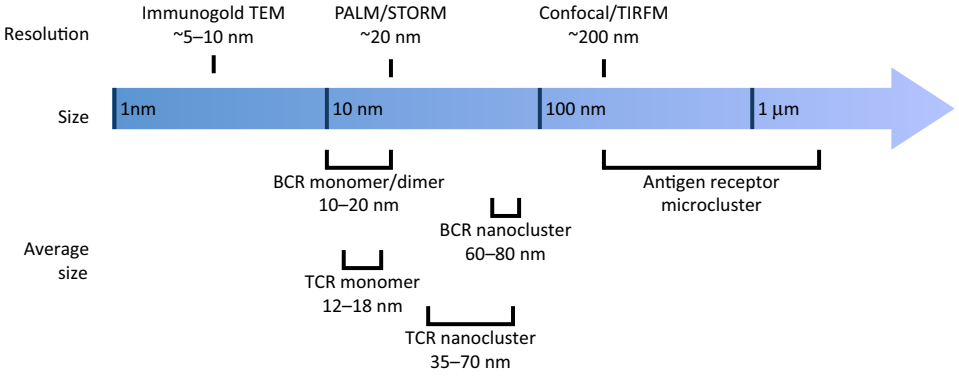

Figure 2. Average Sizes of Various Forms of Antigen Receptors. With the help of the development of advanced imaging techniques, visualizations of various forms of antigen receptors could be obtained with proper imaging methods referring to their own resolution limits. The estimated lateral size of T cell receptor (TCR) or B cell receptor (BCR) monomer is between 5 and 20 nm. When packaged into nanoclusters in quiescent cells, these antigen receptor nanoclusters could have a size of several tens of nanometers, and can be up to 300 nm. In activated cells, antigen receptors are concatenated to form microclusters, whose size would be hundreds of nanometers, and even reaches level of micrometers with the growth of the microclusters in response to antigen stimulation. Abbreviations: PALM, photoactivated localization microscopy; STORM, stochastic optical reconstruction microscopy; TEM, transmission electron microscopy; TIRFM, total internal reflection fluorescence microscopy.

resolutions of tens of nanometer level have enabled the visualization of the fine structure of antigen receptor nanoclusters in both quiescent and activated lymphocytes (Figure 2), dramatically furthering our understanding of lymphocyte signaling. In this review, we focus on the dynamic structures of antigen receptor nanoclusters and discuss the factors that mediate their unique self-aggregation within the membrane bilayer.

Glossary

photoactivated localization microscopy and stochastic optical reconstruction microscopy: abbreviated as PALM and STORM, respectively. Both PALM and STORM can break through the diffraction barrier. Both achieve nanoscale resolution by stochastically activating only a few fluorescent proteins in each frame to avoid the overlapping of fluorescent spots. The center of mass of each spot is then computationally determined to precisely locate each fluorophore. The collection of thousands of frames to activate all the possible fluorophores allows the reconstruction of a high-resolution image. PALM is originally designed to utilize photoactivatable fluorescent proteins, while STORM utilized photoswitchable dyes in the imaging experiment.