CHAPTER 13

Visualization of Cell-Cell Interaction Contacts—Synapses and Kinapses

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Abstract

T-cell activation requires interactions of T-cell antigen receptors (TCR) and peptides presented by major histocompatibility complex molecules (MHCp) in an adhesive junction between the T-cell and antigen-presenting cell (APC). Stable junctions with bull's eye supramolecular activation clusters (SMACs) have been defined as immunological synapses. The term synapse works in this case because it joins roots for "same" and "fasten," which could be translated as "fasten in the same place." These structures maintain T-cell-APC interaction and allow directed secretion. We have proposed that SMACs are not really clusters, but are analogous to higher order membrane-cytoskeleton zones involved in amoeboid locomotion including a substrate testing lamellipodium, an adhesive lamella and anti-adhesive uropod. Since T-cells can also integrate signaling during locomotion over antigen presenting cells, it is important to consider adhesive junctions maintained as cells move past each other. This combination of movement (kine-) and fastening (-apse) can be described as a kinapse or moving junction. Synapses and kinapses operate in different stages of T-cell priming. Optimal effector functions may also depend upon cyclical use of synapses and kinapses. Visualization of these structures in vitro and in vivo presents many distinct challenges that will be discussed in this chapter.

Introduction

The partnership between dendritic cells (DC) and T-lymphocytes (T-cells) defends the body against microbes, parasites, abnormal cells and environmental toxins that breach the barrier function of skin and epithelial surfaces.12 Diverse tools including those of biochemistry, cell biology, genetics and imaging have been employed to understand the mechanistic basis of this partnership. In recent years, imaging approaches have become increasingly useful as molecular technologies for labeling cells and proteins and imaging hardware and software have improved. In vitro imaging led to the initial definition of the immunological synapse (IS, or synapse) based on the organization of polarity and adhesion molecules to fasten (-apse) the T-cells to the same (syn-) antigen presenting cells (APCs) or place.38 Advances in near-field in vitro imaging have led to the description of TCR microclusters that sustain signaling in the periphery of synapses.911 Introduction of two-photon laser-scanning microscopy and methods for long-term in vivo observation have led to a basic understanding of the dynamics of T-cell-APC interactions in the living lymph node and the effects of antigen, which leads to signal integration via both short and long-lived T-cell-APC contacts.12-14 The long-lived interactions can be defined as synapses since they fasten the T-cell to

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the same APC. The short-lived interactions both early and late in the process appear to be the product of combining motility and cell-cell communication as a continuous kinetic process. The T-cells move while maintaining extensive contact with the APC. This dynamic interaction, for which there is no convenient descriptor, could be described as a kinapse—combining roots indicating movement (kine- ) and fastening (-apse) at the same time. A challenge for in vitro molecular imaging is to provide insight into how T-cells integrate signals from synapses and kinapses. It is likely that TCR microclusters will be common structures in this process.

One of the basic biological questions in immunology is what distinguishes T-cell responses to DC that lead to tolerance or priming. One concept is that the outcome of antigen presentation depends upon the activation status of the DC. Immature DCs patrol the tissue spaces and boundaries of the body and gather antigenic structures, both self and foreign. Induced or spontaneous maturation of DC triggers their migration to the lymph node and concurrent processing of antigens to generate peptides that bind to major histocompatibility complex molecules (MHCp) that are then presented at the cell surface. DC migrate to the lymph node via the lymphatics and then migrate in the parenchyma and join DC networks in the T-cell zones where they encounter many T-cells. The level of costimulatory molecules expressed by the DC is determined by the level of cytokines-like TNF produced in response to various endogenous or exogenous activators of innate immunity. This level of innate stimulation appears to control whether the antigen-dependent T-DC interactions lead to tolerance or priming of an immune response over a period of 5–7 days. While some have argued that tolerance induction does not involve synapses in vitro or in vivo, we have found that the TCR-MHCp interactions alone control in vitro synapse formation and that T-cells do synapse with DC during tolerance induction in vivo. T-cells also synapse with DC during induction of oral tolerance.

Once T-cells are primed they may take on a number of fates. They may become memory cells that continue to recirculate, exit the secondary lymphoid tissues altogether to sites of inflammation, remain in T-cells zones to help CD8+ T-cell responses, or move to follicles within the lymph node to help B-cells. It has been demonstrated that effector CD8+ T-cells are active in killing targets within lymph nodes. The manner in which these fates are established is poorly understood, but may involve processes such as asymmetric cell division set up by synapses or different cytokine milieus encountered by daughter cells as the migrate. Memory T-cells have been shown to accumulate in the bone marrow and to interact with bone marrow DC during secondary stimulation.

Peripheral tissue scanning by DC is only one mode of innate immune surveillance of tissues. Two striking examples are the surveillance of the brain by the dynamic processes of microglial cells and the active patrolling of liver sinusoides by natural killer T-cells, an innate-like T-cell. In this review we will summarize a new view of sustained T-cells activation through the synapses and kinapses. Then, how the synapse and kinapse work together in T-cell tolerance and immune surveillance will be discussed. Throughout the chapter the various visualization methods that are employed will be described and critiqued with respect to potential and limitations.

New Model for Sustained Signaling through the Synapse

Studies on the synapse bring together three parallel lines of experimentation in immunology through high-resolution fluorescence microscopy: TCR signal transduction, T-cell adhesion and polarity mechanisms. TCR signaling is based on a tyrosine kinase cascades that leads to rapid activation of phospholipase Cγ. The key tyrosine kinases are Lck, which initiates phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) in the cytoplasmic domain of the TCR, ZAP-70, which is recruited to phosphorylated ITAMs and phosphorylates LAT and ITK, which phosphorylates phospholipase Cγ that is recruited to phosphorylated LAT. Phospholipase Cγ activation leads to generation of inositol-1,4,5-triphosphate, leading to Ca2+ mobilization and diacylglycerol leading to activation of protein kinase C and Ras exchange factors. The triggering of the cascade is based on recruitment of Lck-associated coreceptors to the TCR and on TCR oligomer formation (see also Chapters 6 and 11).