*Current perspectives*

# Insights into primary immune deficiency from quantitative microscopy

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**Recent advances in genomics-based technology have resulted in an increase in our understanding of the molecular basis of many primary immune deficiencies. Along with this increased knowledge comes an increased responsibility to understand the underlying mechanism of disease, and thus increasingly sophisticated technologies are being used to investigate the cell biology of human immune deficiencies. One such technology, which has itself undergone a recent explosion in innovation, is that of high-resolution microscopy and image analysis. These advances complement innovative studies that have previously shed light on critical cell biological processes that are perturbed by single-gene mutations in primary immune deficiency. Here we highlight advances made specifically in the following cell biological processes: (1) cytoskeletal-related processes; (2) cell signaling; (3) intercellular trafficking; and (4) cellular host defense. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)**

***Key words:*** *Primary immune deficiency, microscopy, cell biology, cytoskeleton, host defense*

*Abbreviations used*

| | |
|---|---|
| AP3: | Adaptor protein 3 |
| Arp2/3: | Actin-related proteins 2 and 3 |
| CDC42: | Cell division cycle 42 |
| CGD: | Chronic granulomatous disease |
| CHS: | Chediak-Higashi syndrome |
| CTL: | Cytotoxic T lymphocyte |
| DC: | Dendritic cell |
| DOCK8: | Dedicator of cytokinesis 8 |
| FHL: | Familial hemophagocytic lymphohistiocytosis |
| HPS2: | Hermansky-Pudlak syndrome type 2 |
| IS: | Immunologic synapse |
| LAD-I: | Leukocyte adhesion deficiency type 1 |
| LFA-1: | Lymphocyte function–associated antigen 1 |
| LYST: | Lysosomal trafficking regulator |
| MAGT1: | Magnesium transporter 1 |
| MTOC: | Microtubule organizing center |
| NADPH: | Nicotinamide adenine dinucleotide phosphate |
| NET: | Neutrophil extracellular trap |
| NF-κB: | Nuclear factor κB |
| NIK: | NF-κB–inducing kinase |
| NK: | Natural killer |
| PID: | Primary immune deficiency |
| ROS: | Reactive oxygen species |
| SNARE: | Sensitive factor attachment protein receptor |
| TCR: | T-cell receptor |
| TEM: | Transmission electron microscopy |
| WAS: | Wiskott-Aldrich syndrome |
| WASp: | Wiskott-Aldrich syndrome protein |

In recent years, the field of primary immune deficiency (PID) has benefited tremendously from the explosion in technology resulting from the advent of relatively inexpensive and increasingly sophisticated sequencing techniques. To date, more than 5000 mutations have been identified leading to 248 unique PIDs (http://web16.kazusa.or.jp/rapid/). With the discovery of the genetic basis of these immunologic diseases comes the opportunity to use samples from these naturally occurring deficiency "models" to address important questions in cell biology and at the same time gain true insight into the way human immunity works on a mechanistic level. In the best case these insights can help improve both the diagnosis and treatment of these often rare, devastating, and complex diseases.

In parallel with genetic technology, the field of optical imaging has undergone a rapid revolution in the past 10 years through use of highly quantitative microscopy techniques. This has led to an extraordinary period of growth in the field of cell biology, as well as objective answers to many questions previously considered purely theoretical. A variety of optical microscopy approaches have enabled improvements in molecular localization, optical sectioning, 3-dimensional reconstruction, live cell imaging and even 4-dimensional reconstructions (Table I).[1-38] One of these technical advances, superresolution imaging, is particularly noteworthy because it has dramatically decreased the size of resolvable objects within cells from previous limits of about 200 nm to new reaches of even single-digit nanometer levels through the use of optics. Recognition for this powerful advance was provided through the 2014 Nobel Prize in Chemistry and Physics.

The application of highly quantitative imaging, objective image analysis, and, more recently, superresolution approaches to immune cell function has resulted in an increased understanding of the molecular basis of immunity, which has in turn led to a greater understanding of the diseases that occur as a result of perturbations of the immune system. Quantitative microscopy, when thoughtfully and carefully applied, provides detailed and powerful information about the subcellular localization of proteins of interest with relatively minimal biological sample input, making it a valuable tool for those seeking to study rare

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**TABLE I.** High-resolution imaging techniques used in cell biology studies of PIDs, including references to use in PID

| Name | Description | PID references |
|---|---|---|
| Confocal microscopy | Use of spatially aligned pinhole to improve resolution of fluorescence microscopy through exclusion of out-of-focus light | 4-6, 8, 9, 12, 13, 15-22, 24-34, 36, 37 |
| Superresolution microscopy | Use of optics or mathematic algorithms to achieve resolution below the diffraction limit of light | 14, 15 |
| Fluorescence wide field | Use of whole-specimen illumination to visualize structures labeled with fluorophores | 10, 11, 35 |
| TEM | Use of a focused beam of electrons passed through an ultrathin specimen to illuminate structural elements | 1-3, 7, 15, 23, 25, 27, 34, 37, 38 |
| Total internal reflection microscopy | Use of an evanescent wave to selectively illuminate membrane proximal 150 nm of the cell | 24 |
| Imaging flow cytometry | Combination of conventional flow cytometry with fluorescence microscopy to assess both population-based phenotype and protein localization | |
| Correlative light electron microscopy | Use of light microscopy of fixed cells, followed by imaging of structures by means of electron microscopy | 24 |

human disorders, often in children from whom samples can be scarce.

Here we review the application of highly quantitative imaging to the field of PIDs, with a specific focus on 4 areas that have particularly benefited from this increased resolution, namely mutations that affect (1) cytoskeletal-related processes, (2) cell signaling, (3) intercellular trafficking, and (4) cellular host defense.

## DEFECTS IN CYTOSKELETON-RELATED PROCESSES

The structural framework contained within the cell cortex is made up of filaments of cytoskeletal actin, also known as F-actin. Each cell contains robust F-actin rearrangement machinery that is required to rapidly change shape, generate force, reorient signaling complexes, and coordinate migration. The nucleation of F-actin is the process in which the building of an actin filament is begun and is critical for immune cell migration and effector function.[39] As such, mutations affecting the cytoskeleton have a profound effect and lead to significant disease.[39] In particular, the regulated control of F-actin nucleation and remodeling is required for the formation and function of the immunologic synapse (IS; Fig 1, *A*). The IS can be broadly described as a highly specialized signaling platform formed between 2 immune cells and was originally described between a $CD4^+$ T cell and an antigen-presenting cell. Arguably one of the best-characterized types of IS, however, might be the so-called "lytic synapse," which leads to the directed secretion of specialized secretory lysosomes or lytic granules. Both cytotoxic T lymphocytes (CTLs) and natural killer (NKs) cells form lytic synapses and seem to share many common mechanisms of synapse formation. Lytic synapse function occurs in 3 stages (recognition, effector, and termination), which can be further broken down into discrete steps.[40] Central to an increasingly appreciated number of these steps is the highly regulated function of the cytoskeleton (Table II),[39,41,42] particularly F-actin nucleation, remodeling, and deconstruction. The central role of F-actin in immune cell function and its large-scale perturbation in the context of immune deficiency have been well reviewed elsewhere.[39]

The study of PIDs affecting the regulation of F-actin have led to critical insight into the cytoskeletal processes of immune cells. Here we focus specifically on those PIDs that have driven discoveries enabled by the use of quantitative microscopy. Of these, perhaps the best characterized and studied is Wiskott-Aldrich syndrome (WAS). More than 300 distinct mutations in Wiskott-Aldrich syndrome protein (WASp) have been described, leading to classical WAS, X-linked thrombocytopenia, or X-linked neutropenia.[43] WAS as a heritable condition was first described in 1954 and is recognized clinically by the characteristic triad of thrombocytopenia, neutropenia, and immune deficiency.[43] The WASp gene was identified in 1994 and led to the subsequent characterization of a large related family of proteins of the WASp family: N-WASp, WAVE, SCAR, WASF1-3, WASH, WHAMM, and JMY.[44] WASp (and its homologues) activates the actin-related proteins 2 and 3 (Arp2/3) complex to promote branched F-actin nucleation. This allows for a new filament of actin to be built at an angle to an existing filament, which provides tremendous structural integrity akin to interlocking girders in a building. Although actin nucleation is just one of many processes regulating F-actin dynamics (others include elongation, capping, severing, and linear nucleation), it is a critical component of migration, adhesion, and signaling. Because of this and WASp's ubiquitous expression in hematopoietic cells, WAS affects multiple immune cell types and functions.[43]

WAS was one of the first PIDs to be studied from a cell biology perspective using higher-resolution microscopy. Scanning electron microscopy was initially used to study lymphocytes from patients with WAS[1] and semiquantitatively score the presence and relative size of microvillus projections on the cell surface. The morphologic abnormalities observed in patients' lymphocytes were the first indication that cytoskeletal structural alterations were contributing to the loss of immune function, although at the time, the mechanism was not clear. Subsequent studies also described the "bald" phenotype of WAS lymphocytes[2,3,45] through electron microscopy with regard to microvillus projections. Certain studies have suggested that WASp deficiency does not alter lymphocyte microvilli frequency or length, but these have often been done under activating conditions that could potentially access pathways bypassing WASp deficiency.[1,3,45] Furthermore, the effects seen in cells from patients with WAS can be variable, including some "baldness" but also altered and heterogeneous morphology, especially in T-cell lines. B cells