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Review Article

Lipid droplets in leukocytes: Organelles linked to inflammatory responses

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ABSTRACT

Studies on lipid droplets (LDs) in leukocytes have attracted attention due to their association with human diseases. In these cells, LDs are rapidly formed in response to inflammatory stimuli or allergic/inflammatory diseases including infections with parasites and bacteria. Leukocyte LDs are linked to the regulation of immune responses by compartmentalization of several proteins and lipids involved in the control and biosynthesis of inflammatory mediators (eicosanoids). In this mini review, we summarize current knowledge on the composition, structure and function of leukocyte LDs, organelles now considered as structural markers of inflammation.

Contents

1. Introduction

Lipid droplets (LDs) are typical organelles present in the cytoplasm of leukocytes such as neutrophils, eosinophils and basophils. In these cells, LDs greatly differ from classic LDs found in adipocytes, in which they are mostly associated with neutral lipid storage and metabolism. In leukocytes, LDs, generally referred as lipid bodies (LBs), act as modulators of immune responses and therefore are closely linked to the functional capabilities of these cells. Particularly, LDs reside in the leukocyte cytoplasm as highly dynamic stations able to change their composition in response to inflammatory events. A plethora of studies have been describing human inflammatory diseases and experimental inflammatory stimuli that elicit LD formation in leukocytes (reviewed in [1–4]).

The association of LDs with inflammation in leukocytes has been demonstrated for nearly 30 years. Pioneer works with human leukocytes demonstrated a role for LDs in the cellular metabolism of arachidonic acid (AA) indicating that these organelles were potentially able to initiate cascades that culminate in the formation of inflammatory mediators (eicosanoids) [5,6]. Following studies in the 1990s fully demonstrated localizations of eicosanoid generating enzymes within leukocytes LDs [7–10], and during the 2000s, in situ synthesis of eicosanoids (prostaglandins and leukotrienes) was documented in these organelles within activated leukocytes and other cells from the immune system (for example, see [11–14]).

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Here, we discuss the role of leukocytes LDs as inflammation-associated organelles as well as their structure and composition within these cells.

2. LDs as critical organelles of leukocytes

In contrast to adipocytes that have a large number of cytoplasmic LDs, resting leukocytes have a small number of LDs. However, during in vivo inflammatory diseases, there is an accentuated and rapid accumulation of LDs within these cells. LDs biogenesis is well documented both in vitro and in vivo in response to inflammatory stimuli and diseases (reviewed in [1,2,4]). For instance, increased LDs numbers are observed during acute respiratory distress syndrome [15] and allergic inflammation [16] in humans. Interestingly, eosinophils from patients with hypereosinophilic syndrome (HES) are naturally activated and show both increased numbers of LDs and enhanced leukotriene C_4 (LTC_4) production [17,18].

Ligand-initiated, receptor-mediated pathways activate intracellular signaling that directs to increased formation of LDs within leukocytes. For example, platelet activating factor (PAF), through its receptor, induces LD formation in neutrophils and eosinophils [9,19] while the chemokines CCL11 and CCL5, acting via CCR3 receptors, stimulate LD formation in eosinophils and basophils [11]. Numerous stimuli, listed in other reviews [2,4] are now recognized to trigger LD formation in leukocytes.

The induction of LD formation by pathogens has also been increasingly documented within leukocytes and other cells from the immune system. Under interaction with a variety of pathogens such as parasites, bacteria, viruses and fungi, LDs accumulate in the host cell cytoplasm, increase in size and undergo ultrastructural alterations (reviewed in [20–22]). A list of pathogens that induce LD biogenesis within leukocytes and other mammalian cells is shown in a previous review [20]. Interestingly, pathogen recognition by toll-like receptor 2 (TLR2) is involved in LD genesis in macrophages [13].

Remarkably, host–pathogen interaction is also able to induce an intriguing association of newly formed LDs with phagosomes, which seems to be a general event found during infections with different pathogens in both humans and experimental models [20]. Overall, it is believed that the LD–phagosome interaction has evolved as a pathogen strategy to survive within the host cells by sequestering mainly host lipids [20].

3. LDs composition and structure in leukocytes

LDs in leukocytes share some features with LDs found in other cells: the presence of a core containing neutral lipids (mainly triacylglycerols and sterol esters) and proteins surrounded by a phospholipid hemimembrane with associated proteins, including the structural protein perilipin 2 (PLIN2/ADRP/adipophilin). PLIN2 is found at LD surface [23] and has been frequently used as marker for leukocyte LDs [13,24–26].

Numerous proteins are found in the internum of leukocyte LDs. Common to LDs from several cells, there are proteins involved in cholesterol and triglyceride metabolism, Rab GTPases, kinases, caveolin and many membrane and endoplasmic reticulum (ER)-associated proteins [27–29]. However, different from LDs found in adipocytes and other cells, leukocyte LDs contain AA, a fatty acid that resides in LDs as molecules esterified with diacylglycerols and phospholipids. Additionally, MRP-14, a protein potentially involved in arachidonate transport, and ribosomal subunit proteins and translation regulatory proteins were revealed by proteomic analyses of LDs purified from human monocytic U937 cells [27]. Under activation, leukocytes LDs compartmentalize eicosanoid-forming enzymes, which will be discussed in more detail below. Lastly, the presence of cytokines within leukocyte LDs was investigated. Tumor necrosis alpha (TNF-α) was detected by immunogold EM within cytoplasmic LDs of eosinophils present in vivo in colonic Crohn's disease biopsies [30], though the role of leukocyte LDs as potential cytokine sites remains to be addressed.

One structural aspect that draws attention while analyzing the protein composition of leukocyte LDs is the presence of membrane inserting proteins within their cores, including enzymes for eicosanoid synthesis. Automated electron tomography, a technique that offers 3D information at very high resolution, i.e., at transmission electron microscopy (TEM) level [31], applied to the study of human eosinophils, revealed that LDs from these cells contain in their cores an intricate system of membranes, organized as a network of tubules which resemble the ER [32]. Therefore, as indicated by other studies from our group [27,33], leukocyte LDs are not homogeneous organelles but may exhibit membranous structures which explains the association of polar proteins within LD cores and may be important to understanding LD biogenesis [32].

Another structural aspect of leukocyte LDs, revealed by TEM, is their varied osmiophilia. For example, in eosinophils, LDs are very electron-dense (Fig. 1) while in neutrophils and monocytic lineage U937 cells these organelles are electron-lucent [27]. During inflammatory responses, the numbers and sizes of LDs remarkably increase, occupying large portions of the leukocyte cytoplasm. Even the organelle osmiophilia can change, especially during infectious diseases, indicating that LDs are active organelles, able to modify their composition/structure in concert with cell activation (reviewed in [2,22]). Alterations in the LD ultrastructure within leukocytes likely reflect the cascade of events involved in the synthesis of inflammatory mediators, as discussed below.

Of note, visualization of leukocyte LDs by light microscopy requires the use of specific methodologies involving the use of fluorescent lipophilic dyes since alcohol-based hematological stains routinely used to study leukocytes, such as May-Grünwald–Giemsa, solubilize LDs. The main techniques currently used to visualize LDs in leukocytes by light microscopy are summarized in previous works [2,34].

4. LDs synthesize inflammatory mediators

LDs from leukocytes compartmentalize and co-localize substrate AA esterified in phospholipids and the entire enzymatic machinery for eicosanoid synthesis. The first step in eicosanoid generation within any cell type is performed by a superfamily of enzymes collectively known as phospholipase (PL)A_2 due to their ability to release fatty acids from sn-2 position of phospholipids [35]. These enzymes are considered key regulators of LD homeostasis, regulating their formation at different levels [35]. Under stimulation, cytosolic phospholipase A_2 ($cPLA_2$) co-localize with LDs from U937 cells [36] and dendritic cells, the resident macrophage population of the central nervous system [37] in parallel to increased LD formation.

Other key enzymes implicated in the biosynthesis of eicosanoids have been found specifically localized within LDs of activated leukocytes such as human eosinophils (Fig. 1). These enzymes include the major AA-converting enzymes 5-lipoxygenase, 15-lipoxygenase, 5-lipoxygenase-activating protein, cyclooxygenase-2 and LTC_4 synthase (reviewed in [4,38]).

Significant correlations between LD formation and enhanced generation of eicosanoids by leukocytes have been observed (reviewed in [9]). Stimuli that induce LD formation within leukocytes also dose-dependently and coordinately enhance eicosanoid

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