



Lipid droplets in activated mast cells – a significant source of triglyceride-derived arachidonic acid for eicosanoid production

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ABSTRACT

Mast cells are potent effectors of immune reactions and key players in various inflammatory diseases such as atherosclerosis, asthma, and rheumatoid arthritis. The cellular defense response of mast cells represents a unique and powerful system, where external signals can trigger cell activation resulting in a stimulus-specific and highly coordinated release of a plethora of bioactive mediators. The arsenal of mediators encompasses preformed molecules stored in cytoplasmic secretory granules, as well as newly synthesized proteinaceous and lipid mediators. The release of mediators occurs in strict chronological order and requires proper coordination between the endomembrane system and various enzymatic machineries. For the generation of lipid mediators, cytoplasmic lipid droplets have been shown to function as a major intracellular pool of arachidonic acid, the precursor for eicosanoid biosynthesis. Recent studies have revealed that not only phospholipids in mast cell membranes, but also triglycerides in mast cell lipid droplets are a substrate source for eicosanoid formation. The present review summarizes current knowledge about mast cell lipid droplet biology, and discusses expansions and challenges of traditional mechanistic models for eicosanoid production.

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1. Introduction

Classically, mast cells have been considered to be “allergy cells” that play a causative role in allergic disorders (Galli and Tsai, 2012; Rivera and Gilfillan, 2006) and as major effectors in host defense against parasites (Rodríguez and Wilson, 2014). However, multifaceted studies outside the traditional mast cell research areas have provided new evidence for a much broader functional spectrum of these cells. They play important roles under various pathophysiological conditions, including atherosclerosis, diet-induced obesity, diabetes, and many other inflammatory diseases

(Bot et al., 2015; Kovanen, 2007; Xu and Shi, 2012). Another traditional research focus is their exceptional capability to store preformed biologically active mediators within secretory granules and to trigger their release upon cellular activation. The synthesis and release of eicosanoids has been extensively studied especially in connection with phospholipids serving as a source of their precursor, the arachidonic acid. Many questions about where and how lipids are formed and metabolized within mast cells are still unanswered. There is growing evidence that lipid droplets represent a dynamic compartment with lipid regulatory functions in mast cells. Here, we want to review the current literature about mast cell lipid metabolism, and in particular, discuss the roles of mast cell lipid droplets as well as the impact of certain fatty acids on mast cell functions.

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2. Human mast cells

2.1. Origin, development, and morphology

Mast cells, first described in 1878 by Paul Ehrlich as aniline-positive cells in connective tissue, are now known to derive from hematopoietic stem cells in the bone marrow (Kitamura et al., 1978). The majority of hematopoietic cells exit the bone marrow in a mature and recognizable state, whereas mast cells are released as progenitor cells that circulate in the blood and finally mature from mast cell-committed progenitors when entering tissues (Maaninka et al., 2013). Committed mast cell progenitors express specific surface markers including CD34, CD13, and CD117, but lack the expression of the high affinity IgE receptor (FcεRI).

Tissue-homing of mast cells is mediated by different molecules including KIT ligand (also termed mast cell growth factor or stem cell factor (SCF)) (Galli et al., 1995; Nilsson et al., 1994), eotaxin (Juremalm and Nilsson, 2005), and integrins. The phenotype of mature mast cells is determined by growth factors and cytokines of the tissue microenvironment in a strictly regulated process (reviewed in Dahlin and Hallgren (2015)). Among all mast cell maturation factors, the most essential molecule for their differentiation is the KIT ligand. KIT ligand is secreted by various tissue cells, such as fibroblasts and endothelial cells (Miyamoto et al., 1997), and binds to the mast cell surface receptor CD117 (c-kit) (Lennartsson and Rönnstrand, 2012). Besides the KIT ligand, T-cell-derived interleukin 3 and interleukin 9 play important roles in the regulation of mast cell growth and differentiation (Razin et al., 1984; Williams et al., 1990). Once mature, the mast cells reside in various body tissues and survive there for months or even years (Westerberg et al., 2015). The unusual morphological features of resident mast cells have caught researchers' attention for a century. The most comprehensive studies on the ultrastructural morphology of human mast cells have been performed by Dvorak (2005a, 2005b, 2002, 1992). Generally, mature human mast cells contain a single, large nucleus and harbor in their cytoplasm membrane-bound secretory granules that are filled with pre-formed, highly active biological molecules (Wernersson and Pejler, 2014). Mast cells are able to respond to danger signals by releasing a fraction of their secretory granules in an exocytotic process termed 'degranulation', thereby influencing their cellular environment (Choi and Abraham, 2015). Their long-lived nature allows mast cells to regenerate new granules and to undergo several cycles of degranulation (Westerberg et al., 2015).

The secretory granules display different ultrastructural patterns with scroll, regular crystalline, and particulate patterns being the most prominent ones. Distinct combinations of patterns can occur in a single mast cell and reflect the high complexity of their granules (Dvorak, 1995). In addition to the typical granules, mast cells contain unique non-membrane-bound lipid-rich structures, originally called 'lipid bodies' (reviewed in Dvorak (1992)), later also termed lipid droplets (Fig. 1) (Dichlberger et al., 2011). In this review, we use the term lipid droplets (LDs). The presence, absence, or number of LDs adds another dimension to the phenotypic heterogeneity of human mast cells (Dvorak, 1995). Subsequent to their discovery, structures similar or identical to the LDs have been termed fat bodies, spherosomes, or in adipocytes, 'adiposomes' (Liu et al., 2004; Smirnova et al., 2006; Zechner et al., 2009), which confusingly, is the designation chosen in some reports for adipocyte-secreted microvesicles (Aoki et al., 2007; Müller et al., 2009).

2.2. Phenotypic plasticity and activation

The distinct expression pattern of neutral proteases, the major components of granules in mast cells of different tissues, has been

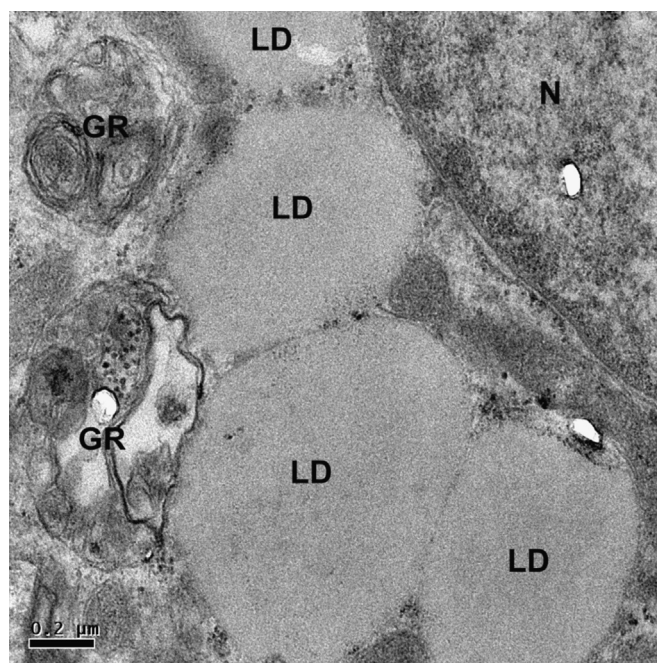


Fig. 1. Electron microscopic image of lipid droplets in human mast cells. This electron micrograph shows an embedded and sectioned mature non-stimulated human mast cell generated by incubating peripheral blood progenitors for 13 weeks under conditions optimal for mast cell differentiation. The mature mast cell contains several round-shaped cytoplasmic lipid droplets (LD) lining up around the nucleus (N). Typical secretory granules (GR) show mainly scroll and particulate ultrastructural patterns. For detailed methods, see Dichlberger et al. (2011). The image was taken by Katarina Hattula at the Institute of Biotechnology, Biocenter Finland EM Unit, University of Helsinki, Finland.

used to phenotypically characterize and distinguish human mast cell populations. The first evidence for the existence of different human mast cell types arose from specific tissue immunostainings performed to detect the enzymes tryptase and chymase (Irani et al., 1989). Two different mast cell phenotypes, one containing only tryptase (mast cell_T) and the other containing tryptase and chymase (mast cell_{TC}), were identified, providing the basis for establishing a protease-based classification of mast cell phenotypes (Irani et al., 1986). This classical characterization concept has been modified to accommodate the discovery of chymase only-containing mast cells (Li et al., 1996), the identification of various other proteases, including additional tryptase isoforms (TPSAB1, TPSB2, TPSD1, and TPSG1), carboxypeptidase A3 (CPA3), cathepsin G, and granzyme B (Wernersson and Pejler, 2014), and the fact that mast cells can change their phenotype due to differences in their cellular environment (Galli et al., 2011; Valent et al., 2010). Importantly, a recent study suggests that human mast cells derive from a common mast cell-committed progenitor that carries the potential to express the full set of granule-stored proteases (Maaninka et al., 2013).

A major aspect of this review are the biological consequences of a process termed "mast cell activation", a generalized term for a complex cellular event that can take place in a number of different ways. The ability to distinguish between activating and inhibiting stimuli, as well as the strength and duration of the stimulus, guide the mast cell towards a specific response (Rivera and Gilfillan, 2006). In order to sense and react to different stimuli, mast cells express a wide range of cell surface receptors (reviewed in Xu and Shi (2012)). The major and best known mechanism for mast cell activation is triggered by antigen-specific IgE-bound cross-linking of the high-affinity IgE receptor, FcεRI, by which mast cells execute their pathophysiological roles in IgE-mediated hypersensitivity reactions, i.e. in many allergic conditions such as the allergic

asthma (Galli et al., 2011; Rivera and Gilfillan, 2006). In addition, mast cells can react to external signals via IgG-dependent Fcγ receptors, c-KIT, complement C3a and C5a receptors, toll-like receptors, adenosine receptors, and cytokine receptors (reviewed in Xu and Shi (2012)).

Once receptor-mediated signaling is triggered, mast cells respond with an immediate and coordinated release of biologically active molecules. There is a clear distinction between the release of “primary” or “preformed” mediators stored in secretory granules and that of “secondary” or “*de novo* -synthesized” mediators (Moon et al., 2014). The release of preformed granule-stored mediators occurs via the degranulation process, and is classified into “anaphylactic degranulation” and “piecemeal degranulation” (Dvorak and Kissell, 1991; Moon et al., 2014). The major preformed mast cell-specific granule components are heparin and the proteases tryptase (Glenner and Cohen, 1960), chymase (Benditt and Arase, 1959), and carboxypeptidase A3 (CPA3) (Haas et al., 1979). Mediator release via degranulation is an exceedingly fast and powerful event, since large amounts of the preformed bioactive molecules are secreted within seconds upon cellular activation (Blank et al., 2014). As stated above, upon activation, mast cells also release *de novo*-synthesized compounds, these including lipid mediators, cytokines, chemokines, and interferons (reviewed in Blank et al. (2014), Boyce (2007), Galli et al. (2005), Moon et al. (2014) and Theoharides et al. (2007)).

With regard to the roles of fatty acids, mast cell activation triggers the release of acutely *de novo*-synthesized lipid mediators, whose synthesis requires regulated provision of the appropriate precursors. Our insights into the generation of these lipid mediators and the mechanisms for their secretion are quite limited. Furthermore, due to the generally very short lifespan of lipid mediators, it can be assumed that not all of them have been identified yet. Among the known lipid mediators of mast cells, eicosanoids play the most prominent role. The common precursor molecule for eicosanoids is arachidonic acid (AA; 5,8,11,14 – eicosatetraenoic acid). Three major pathways of eicosanoid biosynthesis are established, in which free AA is oxidized either by i) cyclooxygenases 1 and/or 2 (COX-1 and/or COX-2) to form prostaglandins (PGs), ii) lipoxygenases (LOs) to generate hydroperoxyeicosatetraenoic acids (HPETEs) and subsequently leukotrienes (LTs), or iii) cytochrome P450 epoxygenase and ω-hydroxylase to produce epoxyeicosatrienoic acids (EETs) and hydroxyeicosatetraenoic acids (HETEs), respectively (Funk, 2001; Lundstrom et al., 2013). A recent study in murine mast cells provided comprehensive data about the phenotype-specific production and release of lipid mediators originating from the three major pathways of eicosanoid biosynthesis (Lundstrom et al., 2013). Upon activation, mast cells rapidly generate three major eicosanoid molecules from AA: prostaglandin D2 (PGD2) and the leukotrienes B4 (LTB4) and C4 (LTC4), all of them contributing to the pathophysiology of allergic and inflammatory diseases (Boyce, 2007; Van den Elsen et al., 2013). Similar to histamine, PGs and LTs can cause acute-phase symptoms due to vasodilation, increased vascular permeability, and bronchoconstriction. Notably, the mast cell-derived LTs are often much more powerful in their actions than histamine. Thus, airway responsiveness to LTC4 is approximately 1000-fold higher than to histamine in asthmatic subjects (irrespective of aspirin tolerance) (Christie et al., 1993). On the other hand, when compared with histamine, the vascular effects of PGD2 and its metabolite 9 α, 11 β-PGF2 in human skin are only minor (Beasley et al., 1988). Dahlen et al. (1981) in their seminal work elegantly demonstrated major differences between histamine and leukotrienes, and differences even among various LTs, as triggers of acute inflammatory response in arterioles and postcapillary venules in golden hamsters. Thus, while arteriolar constriction was triggered by LTC4 and LTD4, but not by histamine

or LTB4, plasma leakage from postcapillary venules was triggered by histamine, LTC4 and LTD4, the latter ones being, respectively, 5000 and 1000 times more potent than histamine, while LTB4 was without effect. Finally, of the studied three leukotrienes, only LTB4 induced leukocyte adhesion to the endothelial surface of postcapillary venules (Dahlén et al., 1981). Thus, the mast cell-derived lipid mediators are essential in the coordination of the recruitment of various immune cells to the sites of inflammation (Theoharides et al., 2007). In the cardiovascular system, the LTs have potent effects on the heart, and, regarding coronary artery disease, LTC4 and LTD4 induce contractions in atherosclerotic, but not in healthy, human coronary arteries (Allen et al., 1998). Moreover, the extent of myocardial ischemia/reperfusion injury depends on LTC4- and LTD4-dependent activation of the endothelial cysteinyl leukotriene 2 receptor, which increases leakage of coronary endothelium and induces cardiomyocyte apoptosis (Moos and Funk, 2008; PoECKel and Funk, 2010). Since tissue mast cells locate close to the endothelial cells both in microvessels and large vessels, and, once activated, they extremely rapidly secrete histamine and LTs, the mast cells truly represent a rapid-reaction innate immune force of the body. The importance of LTs in the pathophysiology of asthma is discussed by Sampson and coworkers, and the impact of PUFAs in allergic diseases by Willemsen and coworkers in this issue of the European Journal of Pharmacology.

3. Mast cell lipid droplets

3.1. Discovery of lipid droplets in mast cells

In 1983, Ann Dvorak published a study about the presence of LDs (“lipid bodies”, as she called them) in human lung mast cells (Dvorak et al., 1983). She first revealed that cytoplasmic LDs are a major storage site for AA in human mast cells, and also concluded that they might regulate cellular functions in various pathophysiological processes (Dvorak et al., 1983). Most what we know now about mast cell LDs is derived from comprehensive electron microscopic studies. The presence of LDs has been reported in mast cells from various different human tissues (Table 1). Whereas human skin mast cells seem to possess only few LDs, mast cells from human lung or gut have been demonstrated to contain numerous LDs in their cytoplasm (reviewed in Dvorak (1992)). Surprisingly, besides the studies of Dvorak et al. only fragmentary novel information about the lipid and protein composition of mast cell LDs has been gained.

3.2. Biological features of mast cell lipid droplets

Lipid droplets have been studied most extensively in adipocytes and in macrophage foam cells. In these and other cells, like mast cells, neutrophils, and eosinophils, LDs contain a large range of lipid species and are associated with an ever growing number of

Table 1

Human tissues in which mast cells have been reported to contain lipid droplets.

Human tissue	Reference
Colon	(Beil et al., 1995)
Heart	(Patella et al., 1995)
Lung	(Dvorak et al., 2003; Hammel et al., 1985)
Nasal mucosa	(Enerbäck et al., 1986; Henderson and Chi, 1992)
Rectum	(Pulimood et al., 1998)
Skin	(Dvorak and Kissell, 1991; Dvorak et al., 1991)
Small intestine	(Qadri et al., 2004)
Synovium	(De Paulis et al., 1996)
Uterus	(Massey et al., 1991)

newly discovered regulatory proteins and enzymes (reviewed in e.g., Beller et al. (2010); Brasaemle (2007); Dichlberger et al. (2013); Goodman (2008); Olofsson et al. (2009); Walther and Farese (2012; 2009)). Lipid droplets are modeled as simple structures analogous to lipoprotein particles, consisting of a core of lipid esters and a surface lipid monolayer harboring resident and exchangeable specific proteins. In mast cells, as in adipocytes, the main component of the LD neutral lipid core is triglycerides (TGs), while in macrophages/foam cells, the core consists mainly of cholesteryl esters (CEs). In addition, diglycerides (DGs), retinylesters (Blaner et al., 2009; Testerink et al., 2012; Walther and Farese, 2012), and ether lipids have been reported to be core components of LDs in different cell types (Bartz et al., 2007; Chilton and Connell, 1988). The LD surface monolayer is viewed as consisting of phospholipids (PLs), free cholesterol, and lysophospholipids. However, recent biophysical studies of circulating lipoproteins, notably of low-density lipoprotein (LDL) particles (Kumar et al., 2011) and/or very low-density lipoprotein (VLDL) (Mikl et al., 2011) have shown that apolipoprotein (apo) B-100, the resident apolipoprotein of these particles, likely interacts in a dynamic fashion not only with the molecules of the surface phospholipid layer, but also with components of the neutral lipid core, as originally suggested by aid of computer models (Hevonoja et al., 2000). Due to the conformational flexibility of apoB-100, this property permits dynamic adaptation to the different sizes of VLDL and LDL and thus, stabilization of the particles (Mikl et al., 2011). In analogy, the LD-associated PAT proteins (described below) may interact with either or both the surface and core domains of these organelles, which can change their lipid composition rapidly. Some investigators perceive LDs as being built of curled endoplasmic reticulum (ER) with associated ribosomes immersed in TGs/CEs covered by a phospholipid monolayer that in fact may be the ER's cytoplasmic leaflet (Robenek et al., 2009; Wan et al., 2007). This multitude of models likely reflects the large degree of inhomogeneities in size and contents of LDs, their wide range of functions, and their adaptation to rapidly changing metabolic conditions occurring in LD-containing cell types (Olofsson et al., 2011). However, our knowledge about the lipid components of LDs, the LD proteome facilitating and/or regulating the assembly and degradation of LDs, and the utilization of specific components of LDs is far from complete.

The metabolic functions of LDs comprise anabolic reactions such as fatty acid synthesis and activation, biosynthesis of sterols and fatty acyl ester molecules, and catabolic reactions, which include hydrolysis, mobilization, and further metabolism of the various components, particularly the fatty acids of PLs, of steryl esters, and of TGs. Proteins identified as actors in this dynamic environment include the so-called PAT proteins perilipin, adipophilin, TIP-47, S3-12, and OXPAT (see Kimmel et al., 2010) for a suggested nomenclature of LD proteins); certain Rab proteins (Azouz et al., 2012; Murphy et al., 2009) and caveolins (Fujimoto et al., 2001; Le Lay et al., 2009; Murphy et al., 2009; Umlauf et al., 2004); a large number of lipogenic and lipolytic enzymes and of lipid transport proteins; and, finally, kinases and phosphatases acting on lipids and proteins (reviewed, e.g. in Beller et al., 2010; Walther and Farese, 2009). Several proteins classically defined as residents of other cellular membranes and/or organelles have been proposed or demonstrated to be components of LDs. Thus, LDs may have tight associations and interactions with mitochondria, ER domains, endosomes, and peroxisomes (Brasaemle et al., 2004; Goodman, 2008; Wan et al., 2007; Zehmer et al., 2009). Such interactions would enable LDs to perform a multitude of functions traditionally assigned to other individual subcellular organelles. As essential organelles for storage and mobilization of lipids, LDs must be able to rapidly change their protein and lipid composition in response to extracellular cues without compromising their functionality.

Understanding the classical function of LDs, i.e., the accumulation of lipids for storage via *influx*, has been the starting point for delineating an integrated view of LDs as important organelles that assist intracellular protein and lipid transport, not the least in order to mobilize and regulate the *efflux* of lipids (Ducharme and Bickel, 2008; Olofsson et al., 2009; Walther and Farese, 2009). The rapid turnover of key LD components can perfectly fulfill the requirements for the generation of secretory signaling molecules, i.e. lipid mediators, via enzymatic conversion of stored precursors. Since mast cells produce and secrete powerful lipid mediators derived from certain fatty acids (Boyce, 2007), they are a suitable system to elucidate details of the involvement of LDs and fatty acids in this complex process. An important class of lipid mediators is the eicosanoids, which are the main focus of the article by Bozza et al. in this issue of the European Journal of Pharmacology. As will be outlined below, identification of the relevant sources for the fatty acids to be funnelled into the initial reactions of the biosynthetic pathways targeted at lipid mediator formation will be a central task when attempting to define the key elements of LD biology.

3.3. Sources and sites of lipid mediator synthesis in mast cells

Most studies on LDs with human primary cells have been performed with isolated and purified blood leukocytes, such as the neutrophils, eosinophils, and basophils (reviewed in Bozza et al. (2009), Melo et al. (2011) and Weller et al. (1999)). Studies on mast cell biology require either the isolation of mast cells from a tissue, or the preparation of circulating mast cell progenitors and their subsequent maturation in culture, since the circulating blood does not contain bona-fide mast cells. Thus, the early work on LDs in human mast cells was limited to mast cells isolated from human lungs, a rich source of mast cells (Dvorak et al., 1984; Granata et al., 2009; Peters et al., 1985; Schleimer et al., 1986; Triggiani et al., 1995). Later, only few observations and/or studies about LDs in mast cells derived from other sources have been reported including mast cells derived from peripheral or cord blood CD34⁺ progenitors (Dichlberger et al., 2011; Minai-Fleminger et al., 2010), from murine bone marrow progenitors (Greineisen et al., 2012), or alternatively from cell lines including the human mast cell line LAD-2 (Dichlberger et al., 2011) or rat basophil-like cell line RBL-2H3 (Greineisen et al., 2012). However, it remains to be seen whether observations made in the cell lines actually represent the characteristics of mature tissue mast cells in the respective species. Therefore, here we will focus, whenever possible, on studies employing well-defined non-transformed mast cell populations generated in vitro from progenitor cells, and employing cells of the myeloblastic lineage (basophils, neutrophils, eosinophils) isolated from blood as mature cells (Dichlberger et al., 2011; Minai-Fleminger et al., 2010). In the following sections, the metabolic and biological features of the different sources of fatty acids used for lipid mediator synthesis are highlighted.

3.3.1. Phospholipids as source of fatty acids for lipid mediator formation

Any cell harboring LDs must be able to react to extracellular cues in a more or less rapid fashion. Since both the neutral core lipids and the phospholipids in the peripheral layer of LDs provide fatty acids for many different demanding processes, the fatty acid pool must be efficiently replenished by re-esterification/re-acylation reactions. In fact, fatty acids likely recycle between the PLs and TGs in order to facilitate cellular lipid homeostasis. To this end, PL molecules originating from the LDs and/or from cellular membranes and TG molecules are resynthesized by acyltransferase-catalyzed reactions utilizing PL- and TG-derived fatty acids, respectively. While this metabolic concept is widely accepted, the

biophysical problem of how lipolytic enzymes access their substrates within LDs remains essentially unsolved. To access their hydrophobic substrates, either the enzymes must translocate their catalytic portion through the PL monolayer, or a core lipid domain must emerge on the surface of the monolayer to become accessible to the catalytic site. The access of lipases to the LD core appears to be regulated, at least in part, by PAT proteins (see Section 3.2.). For instance, perilipin phosphorylation may be crucial for allowing hormone-sensitive lipase (HSL) to gain access to its substrates (Sztalryd et al., 2003).

Clearly, a wide variety of phospholipases are involved in the catabolism of PLs. Importantly, AA is mobilized by the action of various cytosolic and secretory enzymes belonging to the superfamily of the phospholipase A2 (PLA2) enzymes (Schaloske and Dennis, 2006), which hydrolyze the *sn*-2 fatty acyl bond of membrane glycerophospholipids. Important sources for arachidonate production are phosphatidylcholine (PC), phosphatidylethanolamine (PE) (Kennerly, 1990; Yamada et al., 1987), and phosphatidylinositol/-serine (PI/PS) (Garcia-Gil and Siraganian, 1986), the nature of the stimulus determining the predominant PL pool utilized (Garcia-Gil and Siraganian, 1986). In addition, PL species, such as phosphatidic acid (PA) (Kitatani et al., 2000) and lysophospholipids (reviewed in Goetzl and An (1998)), and also monoglycerides (MGs) derived from phosphatidylinositol (PI), which are minor components of membranes, have also been proposed to serve as AA donors in IgE receptor-mediated activation of mast cells (Kennerly, 1987). Cytosolic PLA2 (cPLA2 α , also termed group IV PLA2) liberates AA from the ER, phagosomal membrane, nuclear envelope, but not from the plasma membrane (Bozza et al., 2011). However, although association of cPLA2 with LDs has been amply demonstrated, it has yet to be proven directly that lipolysis of PL molecules by this enzyme in a surface lipid monolayer, like that of LDs, is indeed occurring. Interestingly, secretory types of phospholipase A2 (sPLA2) have been reported to be active also intracellularly, i.e., in the course of their secretion (Mounier et al., 2004). Since these enzymes also act on the PL monolayer of lipoprotein particles, it is possible, by inference, that not only cPLA2s, but also sPLA2s, can act on the single PL leaflet of LDs (Öörni and Kovanen, 2009).

Regarding the relevant pathways for AA liberation in mast cells, the broad spectrum of enzymes with PLA2 activity that has been discovered (Murakami et al., 2011) makes it difficult to unambiguously identify any particular PLA2 enzyme(s) as regulator (s) of the mast cell response involving AA. For example, while one of the three cPLA2s of mast cells shows a preference for several PL species bearing AA in the *sn*-2 position (Murakami et al., 1992), the suggested substrates preferred by certain cPLA2s were PI and PC (Chock et al., 1991). Furthermore, the PA produced by hydrolysis of PLs by phospholipase D appears to be the major, but not exclusive, substrate for the enzyme cPLA2 α (Kitatani et al., 2000). Granata et al. (2009) reviewed studies on PLA2s released at low levels from immunomodulatory cells that include human lung mast cells. Interestingly, the sPLA2s released from macrophages may also activate mast cells and induce their degranulation in a paracrine fashion (Granata et al., 2009). Early studies by Fonteh et al. (1994) reporting the release of sPLA2 activity by activated murine bone marrow-derived mast cells (BMMCs) were subsequently extended by showing that murine BMMCs express all members of the group II subfamily of sPLA2s (Enomoto et al., 2000). However, it is doubtful whether sPLA2s can mobilize AA from the plasma membrane of mast cells, in agreement with the above-mentioned proposal by Bozza et al. (2011).

3.3.2. Triglycerides as source of fatty acids for lipid mediator formation in human mast cells

The evidence reviewed above is based on the dogma that PLs are by far the predominant, if not sole, source of AA for eicosanoid

generation. However, this view may need to be modified in the light of several findings related to LD biology in mast cells described below. Since the population of LDs in mast cells contains much less TGs than that in adipocytes and hepatocytes, mast cell LDs can safely be assumed to function in processes distinct from cellular and/or systemic TG metabolism related to storage and supply of energy. Such alternative processes likely include intracellular pathways of lipid signaling and the release of lipid mediators. Indeed, as suggested by recent work from our laboratory (Dichlberger et al., 2011), the TGs of LDs may contribute to the pool of AA utilized for mast cell lipid mediator secretion. The study leading to this novel concept was facilitated by the establishment of a protocol for the generation of human mast cells under well-defined conditions in the absence of serum (Lappalainen et al., 2007). The results are also in accordance with the finding that human cord blood-derived mast cells contain LDs after *in vitro* differentiation and cultivation in the presence of 10% fetal bovine serum (Andersen et al., 2008; Minai-Fleminger et al., 2010). The serum-free protocol produces exclusively connective tissue-type mast cells, i.e., mast cells that contain both tryptase and chymase in their granules (mast cell_{TC}). Interestingly, differentiation and maturation of the mast cells was accompanied by a steady increase in the number and size of LDs (Dichlberger et al., 2011). When these mast cells were incubated with unsaturated fatty acids, but not with saturated fatty acids, the formation of LDs was accelerated, and incubation of mast cells with AA led to its efficient incorporation into the TG-pool of the mast cells. The AA-rich TGs were shown to be the predominant lipid class in the LDs of mast cells. In fact, tandem mass spectrometry of mast cells LDs revealed that, among the TG molecules containing arachidonyl residues, a unique species existed in which arachidonyl residues were present in all three positions (Dichlberger et al., 2011). Furthermore, following stimulus-induced activation of the mast cells, secretion of cytoplasmic granules was not associated with a release of LDs, nor was the appearance of LDs altered (Dichlberger et al., 2011). Thus, an axis connecting extracellular AA with its uptake and subsequent delivery to the TG pool of LDs in human mast cells exists.

3.4. Regulation of eicosanoid production by adipose triglyceride lipase (ATGL)

The presence of eicosanoid-forming enzymes in LDs would allow for the focal formation of eicosanoids within LDs and the utilization of the substantial amounts of AA in the TG-pool of the LDs (Dichlberger et al., 2011). If so, how is the release of AA from the LD-TG pool coordinated with the utilization of AA derived from the cellular PL pools? The current view is that acylglycerol-hydrolyzing lipases first liberate AA from TGs for incorporation into PLs, from which PLA2s subsequently release the acid for conversion to eicosanoids. A viable alternative possibility is that the mast cell activation process, leading to rapid production and release of eicosanoids, could be considerably accelerated by bypassing the above-described intermediate step. Thus, the AA released from TGs could be directly used as a substrate for the COX/LO enzymes (Fig. 2). The most obvious candidate lipases for acting on the acylglycerols for direct release of arachidonate from TGs, DGs, and MGs are the rather ubiquitously expressed enzymes adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase (MGL), respectively. In fact, ATGL has already been shown beyond doubt to have a crucial role in initiating the lipolytic cascade of TGs in adipocyte LDs (Zechner et al., 2012; Zimmermann et al., 2004).

The mode of fatty acid liberation from LDs by ATGL may vary widely among different cell types. Detailed studies in mouse white adipose tissue on the stereoselectivity of ATGL, HSL, and diacylglycerol-acyltransferase 2 (DGAT2) suggested a “3-pool” model for cellular DGs (Eichmann et al., 2012). Interestingly, ATGL alone

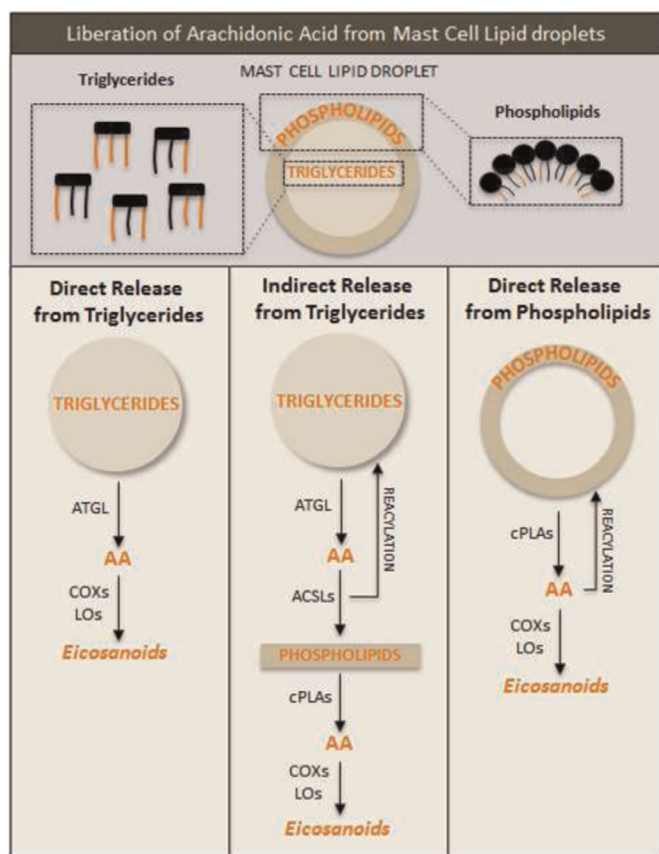


Fig. 2. Model of different pathways of arachidonic acid liberation for eicosanoid biosynthesis in human mast cells. Lipid droplets (LDs) represent potential compartments of lipid mediator biosynthesis in human mast cells. The precursor fatty acid for eicosanoids, arachidonic acid (AA) (illustrated in orange), can be esterified into triglycerides (TGs) or into phospholipids (top panel). By being able to esterify up to three arachidonyl moieties on the TG backbone, TG-rich mast cell LDs have a high capacity of incorporating AA into the TGs (Dichlberger et al., 2011). Upon mast cell activation, AA can be liberated from the LDs via three putative pathways, including either direct or indirect routes of the substrate. First, TGs are hydrolyzed by adipose triglyceride lipase (ATGL) and the liberated AA is channeled from the TG pool directly towards COXs/LOs pathways for the generation of eicosanoids (left lower panel). Second, AA liberated from the TG pool by the action of ATGL, can serve as a substrate for ACSLs to be re-acylated into TGs and/or incorporated into membrane phospholipids. Then, AA can be made available by the action of cytosolic phospholipase (cPLA) for downstream pathways of eicosanoid generation (middle lower panel). Third, AA is directly released from the *sn*-2 position of phospholipids by cPLA, thus being used directly from the phospholipid pool for eicosanoid production (right lower panel).

specifically generates *sn*-1,3 DGs, whereas in the presence of its co-activator CGI-58, it also produces *sn*-2,3 DGs. Moreover, when DGAT2 is associated with LDs, it prefers ATGL-derived *sn*-1,3 DGs for re-esterification into TGs. Thus, at least in adipocytes, ATGL-derived *sn*-1,3 and *sn*-2,3 DGs could be either substrates for HSL-mediated catabolism or for DGAT2-mediated TG synthesis, but do not serve directly as substrates for PL biosynthesis or protein kinase C (PKC) activation (Eichmann et al., 2012). Smirnova et al. (2006) modulated the level of ATGL in HeLa cells by RNA interference or by overexpression of wild-type and a mutated form of GFP-labeled ATGL, respectively. Lipid droplet size was inversely correlated with the ATGL activity present in HeLa cells, indicating that the enzyme, as in adipocytes, likely is a key enzyme for LD catabolism in many cell types. Interestingly, ATGL is a member of a family of lipases with high homology to serine esterases including the PLA2s (Smirnova et al., 2006).

The observed intracellular accumulation of TGs in various tissues and peripheral blood leukocytes in patients with neutral lipid

storage disease (NLSD), which is caused by a loss-of-function mutation in ATGL (Campagna et al., 2008; Kobayashi et al., 2008) suggests that ATGL could be present in human mast cells and have a functional role in TG metabolism in these cells as well. Actually, the above-presented speculation of ATGL as a candidate lipase for the release of arachidonate and its direct use for eicosanoid synthesis prompted us to focus on the possible role of this enzyme as a regulator of eicosanoid production in activated human mast cells (Dichlberger et al., 2014).

We first compared the acute phase-release of PGD₂ in ATGL-silenced and control mast cells upon IgE-dependent activation; indeed, the rapid release of PGD₂ by the ATGL-silenced mast cells was clearly reduced compared to that by mast cells expressing ATGL. In addition, silencing of ATGL also influenced the synthesis of eicosanoids generated via the leukotriene pathway, since activated ATGL-silenced mast cells released dramatically less LTC₄ than activated control cells. Interestingly, silencing of cPLA2 in these mast cells reduced the release of eicosanoids to the same extent as silencing ATGL. It can be concluded that ATGL expression significantly contributes to the immediate generation of lipid mediators via both the prostaglandin and the leukotriene pathways in human mast cells, demonstrating the regulatory and quantitative importance of ATGL in the hydrolysis of the large LD pool of TGs for eicosanoid production (Dichlberger et al., 2014; Schreiber and Zechner, 2014).

3.5. Regulation of eicosanoid production by long chain acyl-CoA synthases

Lipid mediator generation can be regulated at various different levels, such as through the availability of the substrate(s) or required enzymes, as well as the site of mediator generation within different cellular compartments (Bozza et al., 2011). The molecular mechanisms regulating the availability of lipid mediator precursor fatty acids involve their cellular uptake from different sources and their remodeling into and release from different intracellular pools such as PLs (see Section 3.3.1.) and TGs (see Section 3.3.2.), respectively. When mast cells are exposed to high concentrations of unesterified AA, e.g. upon migration of the cells into sites of inflammation, the TG-pool is considered to act as a physiologically important buffer to maintain low intracellular levels of unesterified AA and to prevent excessive production of proinflammatory lipid mediators (Triggiani et al., 1995).

An important additional way of regulating the provision of any fatty acid is the formation of an activated intermediate. In order to be utilized by the cell, AA, like all other fatty acids, needs to undergo an activation process by esterification with coenzyme A. These esterification reactions are catalyzed by acyl-CoA synthetases (ACSLs). The ACSL family members are classified according to their specificity for fatty acids and differ in their distribution among tissues and cell types, and in their subcellular localization (Soupe and Kuypers, 2008). We have found that among the five isoforms of long chain ACSLs (ACSLs) identified in mammals, ACSL3 and ACSL4 (isoform1, v1) are predominantly expressed in mature human mast cells (Fig. 3A), and that ACSL3 partially localized to the cytoplasm and around LDs, whereas ACSL4 was located solely in the plasma membrane (unpublished data).

There is strong evidence that the downstream fate of the generated acyl-CoA product depends on its subcellular localization and on the ACSL isoenzyme, which had catalyzed its synthesis (Li et al., 2010). Exposure of human mast cells to exogenous AA induces generation of AA-enriched LDs, in which the AA appears specifically incorporated into the TG pool (Dichlberger et al., 2011). Under these conditions we observed significant increases in mRNA levels for ACSL1, 3, and 4 after 5 min and 30 min of exposure to AA (Fig. 3B), indicating an involvement of these enzymes in AA

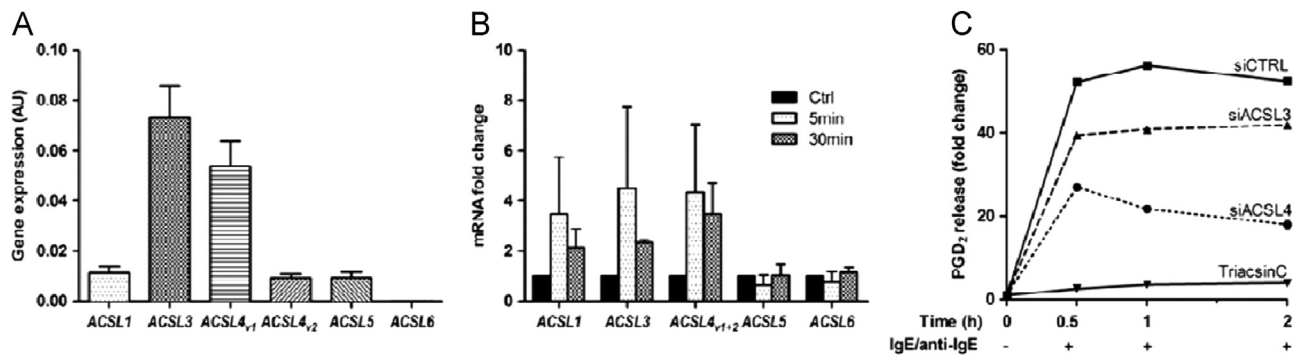


Fig. 3. Long chain acyl-Co synthases regulate arachidonic acid metabolism and eicosanoid generation in human mast cells. (A) Gene expression levels of long chain acyl-Co synthases (ACSL) were determined by quantitative RT-PCR in human peripheral blood-derived mast cells. Data are shown as arbitrary units (AU) ($n=3-6$). (B) ACSL gene expression in response to addition of arachidonic acid (final concentration, 400 μ M) after 5 and 30 min. Data are shown as fold changes compared to untreated cells (Ctrl; 0 min, set to 1) ($n=2$). (C) Release of PGD₂ from ACSL3- and ACSL4-silenced as well as Triacsin C-treated mast cells after IgE/anti-IgE stimulation. Media were collected at the indicated time points after activation, PGD₂ concentrations were measured with a PGD₂mox enzyme immunoassay, and are shown as fold increase compared to untreated mast cells ($n=2$). For detailed methodology, see Dichlberger et al. (2011).

activation. These findings are in line with studies in human fibroblasts and rat hepatocytes (Igal et al., 1997; Muoio et al., 2000) showing that ACSL1, 3, and 4 channel acyl-CoAs to *de novo* lipid synthesis. In smooth muscle cells, ACSL4 was reported to mediate the incorporation of AA into PE and PI (Golej et al., 2011), while in hepatic stellate cells this enzyme was required for esterification of polyunsaturated fatty acids (PUFAs) into TGs (Tuohetahuntala et al., 2015). In both of these reports and in a study from human breast cancer cells (Maloberti et al., 2010), genetic or pharmacological inhibition of ACSL4 was shown to result in a markedly reduced release of prostaglandins. Similarly, in our hands, inhibition of ACSL3 and ACSL4 activity in immunologically activated human mast cells by siRNA-mediated knockdown or addition of the pharmacological ACSL inhibitor Triacsin C resulted in a marked inhibition of PGD₂ release (Fig. 3C). Taken together, these observations reveal an important regulatory role of the acylation/reacylation pathway mediated by ACSLs 3 and 4 in human mast cells, with a significant impact on eicosanoid generation in these cells.

The observed dominance of the LD TG-derived AA as a precursor of lipid mediators is a highly challenging finding, since, thus far, phospholipids of the cellular plasma membrane, and more recently also the membrane phospholipids of various cellular compartments, such as those in the nuclear membrane, have been described as a sources of AA for eicosanoid biosynthesis (Ghosh et al., 2006), as was described in Section 3.3.1. Although our studies (Dichlberger et al., 2014) support the hypothesis that ATGL plays an important role as a TG hydrolase by providing AA for eicosanoid biosynthesis in human mast cells, it is not yet entirely clear whether AA released from TGs can be used for eicosanoid generation directly, indirectly with an "intermediate stop" in PLs, or in both ways (Fig. 2). Bypassing the transfer of AA from TGs to PLs and its subsequent liberation by cPLA2, i.e. the direct utilization of TG-derived AA, would significantly accelerate the provision of the substrate for eicosanoid-forming enzymes, which allows the ultra-rapid release of eicosanoids from mast cells upon their activation.

We have come to realize that major functions of LDs in inflammatory cells differ fundamentally from the classical energy-providing role of LDs in adipocytes. In particular, LDs accumulate in various immune cells during inflammatory and infectious conditions (reviewed in Ghosh et al. (2006)), and LDs in certain immune cells have been identified as sites of eicosanoid biosynthesis (Araújo-Santos et al., 2010; Fujishima et al., 1999). Previous speculations that AA-containing TGs might also serve as a source of AA for the generation of lipid mediators (discussed in Dichlberger et al. (2013), Dvorak et al. (1983), Ghosh et al. (2006) and Triggiani et al. (1995)) have clearly been strengthened by the revelation that

functional ATGL in activated human mast cells is required for the generation of PGD₂ and LTC₄ (Dichlberger et al., 2014). The identification of the regulatory role of ATGL in eicosanoid production by mast cells will facilitate studies towards quantification of the relative contributions to eicosanoid production of the substrate AA derived from the three candidate pathways, i.e., directly or indirectly from TGs, or directly from PLs (Fig. 2).

4. Immunomodulation of mast cell functions by fatty acids

Complex lipids are the major components of cell membranes, and their fatty acid composition modulates membrane properties at many different levels, i.e. membrane fluidity and permeability (Ibarguren et al., 2014). Cell membranes contain structurally and functionally distinct domains consisting of a liquid-ordered phase, termed lipid rafts, which are enriched in sphingolipids and cholesterol, and of a liquid-disordered phase that mainly consists of choline phospholipids (Simons and Gerl, 2010). Lipid rafts represent regulatory platforms that are essential for cellular trafficking, signaling and sorting (Simons and Gerl, 2010). Dietary fatty acids are incorporated in cellular membranes, thus linking nutrition, inflammation and immunity (Wendell et al., 2014). An increased ratio of *n*-6:*n*-3 PUFAs in Western diets has been suggested to play an important role in the development of mast cell-associated inflammatory conditions like allergic diseases (Hagenlocher and Lorentz, 2015). Interestingly, mast cell mediators have been found to be primarily responsible for the clinical signs of canine atopic dermatitis, which, together with cultured canine mast cells, has turned out to be a useful model to study the effect of PUFAs on mast cell function in vivo and in vitro (Schumann et al., 2014). By using the canine mastocytoma cell line C2, the influence of *n*-3 and *n*-6 PUFAs on the release and activity of different mast cell mediators has been studied (Gueck et al., 2003). Neither *n*-3 nor *n*-6 PUFA supplementation showed any effect on the activity of chymase. In contrast, tryptase activity was reduced or increased, when the cells were incubated with *n*-3 PUFAs or *n*-6 PUFAs, respectively. Besides that, *n*-3 PUFA treatment reduced the release of PGE₂ and histamine from mastoparan-stimulated C2 cells (Gueck et al., 2003). Moreover, incubation of the C2 cells with various PUFAs was found to modulate the lipid composition of the membrane microdomains, including lipid rafts (Basiouni et al., 2012). In mast cells, lipid rafts seem to be engaged in IgE receptor (FcεRI) signaling, where the Src tyrosine kinase Lyn-mediated phosphorylation of FcεRI occurs in a cholesterol-dependent manner, thus highlighting the importance of these cholesterol-enriched membrane domains (Davey et al., 2008). Based on the

currently available information, at least parts of the anti-inflammatory and anti-allergic effects of *n*-3 PUFAs can be attributed to their mast cell-modulating properties, which may result in suppressed signal transduction and its associated decrease in the release of proinflammatory mediators.

Mast cells are involved also in the pathogenesis of human atopic eczema (AE) (Liu et al., 2011). Indeed, increased mast cell numbers with an elevated activation status are present in the atopic skin lesions where they control the recruitment and function of immune cells in skin inflammation, including keratinocytes and T cells. As demonstrated in an allergy-induced dermatitis model in mice, treatment with PUFAs, including AA and DHA, reduced skin inflammation (Weise et al., 2013). The number of dermal mast cells was reduced in comparison to the untreated control group, although mast cell degranulation status was unchanged.

The effect of PUFAs (AA, Eicosapentaenoic acid; EPA, Docosahexaenoic acid; DHA) on mast cell activation has been demonstrated in the human mast cell lines LAD-2 and HMC-1 (Van den Elsen et al., 2013). Although none of the PUFAs had any effect on the degranulation process in LAD-2 cells, AA treatment increased the release of PGD₂ and TNF- α upon calcium ionophore-induced activation in HMC-1 cells, whereas EPA and DHA blocked the secretion of IL-4 and IL-13 by these cells. PUFAs have been shown to regulate the activity and trafficking of phospholipase D isoform 1 (PLD1) in activated canine C2 mast cells (Basiouni et al., 2013). Among all PUFAs analyzed, including α -linolenic acid (LNA), EPA, DHA, linoleic acid (LA) and AA, only AA failed to prevent the translocation of PLD1 from intracellular vesicular structures to the plasma membrane. EPA as a substrate for cyclooxygenases (COXs) is a competitive inhibitor of AA metabolism. As demonstrated in human mast cells, EPA treatment inhibits the production of PGD₂ primarily via the COX-2 pathway upon IgE-dependent activation (Obata et al., 1999). Although, several studies demonstrate the immunomodulatory impact of PUFA supplementation on mast cell function (reviewed in Wang and Kulka (2015)), the regulatory pathways still need to be elucidated.

Regarding cytoplasmic LDs, their fatty acid composition likely reflects the external availability of certain fatty acids. Interestingly, the stimulatory effect of different fatty acids on LD biogenesis has been shown to be cell type-specific. In 3T3-L1 cells, *n*-3 PUFAs reduced LD formation, whereas stearic acid (SA) did not have any effect on LD size (Barber et al., 2013). In contrast, only unsaturated, but not saturated fatty acids, were able to induce LD formation in neutrophils, eosinophils and monocytes/macrophages (Bozza et al., 2009). Also in human mast cells, only unsaturated fatty acids, including oleic acid (OA) and AA, but not saturated fatty acids, such as SA, were able to stimulate LD generation (Dichlberger et al., 2011). Besides OA and AA, also DHA treatment induced formation of LDs in the human mast cells, and, in addition, resulted in complete inhibition of PGD₂ release upon mast cell activation (unpublished). Overall, there is growing evidence, that an increase in the ratio of *n*-6 to *n*-3 PUFAs has a significant impact on mast cell biology.

5. Conclusions and perspectives

Mast cells have been assigned a pathophysiological role in various diseases for decades, but revelation of the underlying molecular mechanisms is still young, leaving them mostly unexplored. Early on, studying mast cell biology was a big challenge due to fact that mast cell numbers are quite low compared to other types of cells in the relevant tissues. Indeed, obtaining reasonable amounts of material for experimental approaches was a severe limitation, which only changed upon the development of novel

protocols for proliferating and differentiating mast cell progenitors into mature functional cells in vitro. The research history on mast cell LDs highlights the importance of in vitro-generated mature functional cells for mechanistic studies. Already being discovered in the early 1980s, LDs of human tissue mast cells were only characterized by electron microscopy. By using in vitro-generated mast cells, we were able to broaden the molecular characterization and function of their LDs as dynamic cellular compartments of eicosanoid biosynthesis. The fact that human mast cells contain LDs composed of AA-enriched triglycerides, together with the identification of triglyceride-hydrolyzing enzyme(s) in these cells, revealed neutral lipids as a source of AA for eicosanoid generation. This novel finding challenges the traditional concept of AA being liberated merely by the cytosolic phospholipase from membrane phospholipids. It is important to note that the triglycerides in LDs likely contain, besides the *n*-6 PUFA AA, also the *n*-3 PUFAs EPA and DHA, which, provided that ATGL liberates them from the triglycerides, also could serve as precursors for lipid mediator generation. Several studies have shown the strong impact of diverse fatty acids, in particular various PUFAs, on mast cell function with a potential impact on mast cell-related diseases (Wang and Kulka, 2015). Therefore, investigating pro- and anti-inflammatory lipid signaling molecules in mast cells and other immune cells is an important, and potentially a rewarding, challenge for future studies.

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