

Mechanisms of Formation and Function of Eosinophil Lipid Bodies: Inducible Intracellular Sites Involved in Arachidonic Acid Metabolism

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Lipid bodies, inducible lipid-rich cytoplasmic inclusions, are characteristically abundant in cells associated with inflammation, including eosinophils. Here we reviewed the formation and function of lipid bodies in human eosinophils. We now have evidence that the formation of lipid bodies is not attributable to adverse mechanisms, but is centrally mediated by specific signal transduction pathways. Arachidonic acid and other cis fatty acids by an NSAID-inhibitable process, diglycerides, and PAF by a 5-lipoxygenase dependent pathway are potent stimulators of lipid body induction. Lipid body formation develops rapidly by processes that involve PKC, PLC, and de novo mRNA and protein synthesis. These structures clearly serve as repositories of arachidonyl-phospholipids and are more than inert depots. Specific enzymes, including cytosolic phospholipase A₂, MAP kinases, lipoxygenases and cyclooxygenases, associate with lipid bodies. Lipid bodies appear to be dynamic, organelle-like structures involved in intracellular pathways of lipid mobilization and metabolism. Indeed, increases in lipid body numbers correlated with enhanced production of both lipoxygenase- and cyclooxygenase-derived eicosanoids. We hypothesize that lipid bodies are distinct inducible sites for generating eicosanoids as paracrine mediators with varied activities in inflammation. The capacity of lipid body formation to be specifically and rapidly induced in leukocytes enhances eicosanoid mediator formation, and conversely pharmacologic inhibition of lipid body induction represents a potential novel and specific target for anti-inflammatory therapy.

Key words: lipid bodies - eosinophils - eicosanoids - inflammation

Eicosanoids is the term used to describe a group of biologically active oxygenated fatty acids derived from the enzymatic metabolism of arachidonic acid. Eicosanoids function as paracrine mediators of inflammation as well as intracellular signals. Eicosanoids play major roles in inflammatory responses and have been implicated in the pathogenesis of many inflammatory diseases, including asthma, endotoxic shock, psoriasis, rheumatoid arthritis and inflammatory bowel disease (Henderson 1994, Serhan 1994). The synthesis of eicosanoids is catalyzed by lipoxygenases (LO) leading to the synthesis of leukotrienes (LT),

HETEs and lipoxins; and prostaglandin endoperoxide H synthases, also known as cyclooxygenase (COX) that metabolizes the arachidonic acid into prostaglandins (PG) and thromboxanes (Samuelsson et al. 1987, Smith et al. 1990, Sigal 1991). Although the enzymatic pathways for eicosanoid formation are well understood, the intracellular sites of action of these enzymes and the cellular sources of arachidonic acid remain less clear. The arachidonic acid is released from cellular arachidonyl-phospholipids, which may reside in various membranes, by the actions of phospholipases (Needleman et al. 1986, Lin et al. 1992). While translocation from cytosol to membranes may facilitate interactions of cytosolic enzymes with membrane-bound arachidonate, there is increasing evidence that specific compartmentalization of eicosanoid formation within cells may relate to the different autocrine and paracrine functions of eicosanoids (Serhan 1996, Smith et al. 1996). In addition to membranes, a novel putative site for paracrine eicosanoid production within inflammatory cells are lipid bodies. In this review

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we discuss the metabolic pathways for lipid body formation and the utilization of lipid body-derived arachidonate for eicosanoid synthesis.

MECHANISMS OF LIPID BODY FORMATION IN HUMAN EOSINOPHILS

Lipid bodies, although small in number, are normal constituent of leukocytes, including eosinophils. Interestingly, lipid bodies characteristically increase both in size and number *in vivo* in cells associated with inflammation; including leukocytes from joints of patients with inflammatory arthritis (Coimbra & Lopes-Vaz 1971, Weinstein 1980, Schlesinger et al. 1982), the airways of patients with acute respiratory distress syndrome (Triggiani et al. 1995) and casein- or lipopoly-saccharide-elicited guinea pig peritoneal exudates (Robinson et al. 1982). In eosinophils, increased lipid body numbers have been observed in patients with hypereosinophilic syndrome (HES) (Solley et al. 1976, Weller et al. 1991a), in biopsies from Crohn's disease (Beil et al. 1995), and blood of antigen challenged asthmatic patients (Weller, unpublished observations).

Lipid bodies, identical to lipid bodies in leukocytes *in vivo* as evidenced both by light and electron microscopy, can also be rapidly (within 15-60 min) elicited *in vitro* after stimulation of cells with *cis*-unsaturated fatty acids, PAF or PKC activators (Weller et al. 1989, 1991b, Bozza et al. 1996a,b). Although incubation of leukocytes with exogenous phospholipids and fatty acids provides a source of lipids for incorporation into newly formed lipid bodies, mechanisms other than simple availability of lipid precursors seem to be involved in lipid body formation. First, specific unsaturated fatty acids, in a stereochemically restricted manner, can elicit *de novo* formation of lipid bodies in human leukocytes, whereas fully saturated fatty acids are not active (Weller et al. 1989, 1991b). Moreover, PAF but not its precursor and metabolite, lyso-PAF, that shares the lipid structure of PAF but has no receptor agonistic activity (for review see Braquet et al. 1987), have the capacity to induce lipid body formation (Bozza et al. 1996a, 1997). In addition, PAF stimulated eosinophil lipid body formation was dose-dependent (10^{-8} - 10^{-6} M), with significant lipid body induction at nanomolar PAF concentrations, consistent with a receptor-dependent phenomenon (Bozza et al. 1997). In agreement, PAF-induced lipid body formation was dose-dependently inhibited by the PAF receptor antagonist WEB 2086. Moreover, G protein coupled PAF receptor signaling is pertussis toxin sensitive (Murphy 1994), and pertussis toxin significantly inhibited PAF-induced lipid body formation in granulocytes (Bozza et al. 1996a, 1997). Interestingly, other leukocyte agonists, including

LTB₄, C5a, FMLP and IL-8, that also signal through G protein coupled receptors failed to induce lipid body formation in neutrophils (Bozza et al. 1996a).

The downstream intracellular signaling pathways involved in lipid body formation were further evaluated. It has been demonstrated that rather than a toxic reaction of the cells, lipid body formation in leukocytes is a tightly controlled cellular response that involves the activation of different signaling pathways. A role for PKC mediation of lipid body formation has been suggested in studies using specific inhibitors (Weller et al. 1991b, Bozza et al. 1996a). The PKC inhibitors staurosporine and H-7 significantly inhibited the lipid body formation induced by PAF and *cis* fatty acids (Weller et al. 1991b, Bozza et al. 1996a). In addition, a similar degree of inhibition of lipid body formation was obtained by the treatment of leukocytes with two highly selective PKC inhibitors, chelerythrine, that interacts with the catalytic domain, and calphostin C that acts on the regulatory domain of PKC (Bozza et al., 1996a, 1997). Accordingly, it was previously demonstrated that direct PKC activators including 1-oleoyl-2-acetyl-glycerol and two active phorbol esters (phorbol myristate acetate and phorbol 12, 13 dibutyrate), but not an inactive phorbol, effectively induced lipid body formation in human neutrophils (Weller et al. 1991b). Pretreatment of cells with two specific phospholipase C (PLC) inhibitors, U-73122 (Yule & Williams 1992) and D609, a phosphatidylcholine-PLC specific inhibitor (Shutze et al. 1992), drastically inhibited the stimulated induction of lipid bodies (Bozza et al. 1996a). Thus indicating that in addition to PKC activation, induction of leukocyte lipid bodies also requires the activation of PLC. In order to analyze whether new protein synthesis is required during the induction of lipid bodies in eosinophils, inhibitors of transcription (actinomycin D) and translation (cycloheximide) were evaluated. Pretreatment of eosinophils with either actinomycin D or cycloheximide significantly inhibited lipid body formation induced by PAF or *cis* fatty acids, thus indicating that the stimulated induction of lipid bodies depends on new protein synthesis and its likely that specific early response genes are activated during the process of lipid body formation (Bozza et al. 1996a,b, 1997). The formation of structurally distinct lipid bodies reflects mechanisms whereby proteins and lipids coalesce in a deliberate, highly regulated manner. Lipid body can result from mobilization and reorganization of endogenously-derived lipids as well as incorporating exogenous lipid. Thus, although lipid bodies are not conventional membrane-bound organelles, they appear to represent specialized intracellular whose induced formation is centrally related to activating mechanisms within the cells.

LOCALIZATION OF EICOSANOID-FORMING ENZYMES TO HUMAN EOSINOPHIL LIPID BODIES

Lipid bodies are lipid-rich cytoplasmic inclusions that are candidates to play a major role in the formation of eicosanoid mediators during inflammation. Lipid bodies can be sites of esterified arachidonate localization in cells including neutrophils and eosinophils (Weller et al. 1989, 1991a). In human eosinophils lipid bodies have been shown to incorporate [³H]-arachidonic acid into specific phospholipid classes by electron microscopic autoradiography and biochemical analysis of purified lipid bodies (Weller et al. 1991a). If lipid bodies are to have roles in eicosanoid mediator formation, then the arachidonic acid present in those lipid-rich structures must be liberated by phospholipases, and the free arachidonate must have access to eicosanoid-forming enzymes. Accordingly, the co-compartmentalization of cytosolic phospholipase A₂ (cPLA₂) and mitogen-activated protein (MAP) kinases, the upstream enzymes involved in arachidonic acid liberation (Clark et al. 1990, Leslie 1991, Lin et al. 1993), within lipid bodies has been recently described (Yu et al. 1997 in press). Immunocytochemistry from whole cell and immunoblotting of proteins from isolated lipid bodies (free of cytosol and other organelles) established that cPLA₂ as well as the MAP kinases, ERK1, ERK2, p85 and p38, were localized at lipid bodies. In addition, isolated lipid body fractions contained cPLA₂ enzymatic activity and released arachidonic acid from *sn*-2-position of phosphatidylcholine (Yu et al. 1997).

The two major enzymes, 5-LO and COX, involved in the enzymatic conversion of arachidonic acid into eicosanoids within eosinophils were also shown to localize within lipid bodies. By using immunocytochemistry at the light level and ultrastructural postembedding immunogold, cyclooxygenase has been reported to localize at eosinophil lipid bodies, both in naturally formed lipid bodies in eosinophils from HES patients and in PAF-induced lipid bodies (Dvorak et al. 1992, 1994, Bozza et al. 1997). Previous studies on the intracellular localization of 5-LO have shown that 5-LO localization is cell type-specific and also varies according to the activation state of the cell (Rouzer & Kargman 1988, Reid et al. 1990, Woods et al. 1993, 1995, Brock et al. 1994). 5-LO was shown to localize within the nuclear environment (nuclear membrane and euchromatin) of alveolar macrophages and basophilic leukemia cells (Brock et al. 1994, Woods et al. 1995), whereas 5-LO was found to be predominantly cytosolic in human neutrophils (Brock et al. 1994) and resting peritoneal macrophages (Peters-Golden & McNish 1993). In addition to the nuclear environment, we have recently demonstrated the compartmentaliza-

tion of the key enzyme for leukotriene production, 5-LO, within human eosinophil lipid bodies (Bozza et al. 1997). Interestingly, immuno-reactivity for 5-LO was also detected in lipid bodies from enucleated human eosinophils (Bozza et al. 1997). Human eosinophils upon stimulation preferentially produce LTC₄ as their 5-LO product (Weller et al. 1983). LTC₄-synthase is the terminal LT-forming enzyme and is present selectively in eosinophils, basophils and mast cells (MacGlashan et al. 1982, 1986, Weller et al., 1983). Similarly to that observed for 5-LO, LTC₄-synthase was shown to co-localize within eosinophil lipid bodies (Bozza et al. 1997). Together our findings support a role for lipid bodies to function as specific sites for eicosanoid formation. The compartmentalization of arachidonate substrate (Weller et al. 1989, 1991), cPLA₂ (Yu et al. 1997), and eicosanoid-forming enzymes (Dvorak et al. 1992, 1994, Bozza et al. 1997) provides in one locale an efficient mean to regulate arachidonate release and directly couple it with the enzymes to form eicosanoids.

INVOLVEMENT OF LIPID BODIES IN ENHANCED GENERATION OF EICOSANOIDS BY EOSINOPHILS

Because eosinophil lipid bodies are sites of intracellular localization of eicosanoid-forming enzymes (Dvorak et al. 1994, Bozza et al. 1997) and also stores of the eicosanoid precursor arachidonic acid (Weller et al. 1989, 1991a), we analyzed if increases in lipid body numbers in eosinophils would correlate with increased LTC₄ and PGE₂ production by human eosinophils. Indeed, stimuli known to prime leukocytes to induce eicosanoid generation, including PKC activators, arachidonate and PAF (Bauldry et al. 1988, 1991, Stewart et al. 1990, Bozza et al. 1996a, b), are also active in stimulating lipid body formation (Weller et al. 1991b, Bozza et al. 1996a, b, 1997). Accordingly, we observed a significant correlation between PAF-induced lipid body formation and enhanced generation of both LO- and COX-derived eicosanoids in human neutrophils and eosinophils (Bozza et al. 1996a, 1997). A similar enhancement in eicosanoid production by eosinophils was also observed after lipid body induction by *cis*-fatty acids (Bozza et al. 1996b). Analogously, agents that inhibited lipid body formation also resulted in inhibited priming for eicosanoid production. Pretreatment of granulocytes with the protein synthesis inhibitors actinomycin D or cycloheximide inhibited not only PAF-induced lipid body formation, but also priming for LTC₄ and PGE₂ release by eosinophils, under conditions where they failed to inhibit calcium ionophore-induced LTC₄ and PGE₂ in cells not prestimulated with PAF (Bozza et al. 1996a, 1997). In order to evaluate the role of cytoplasmic lipid bodies in enhanced eicosanoid

formation independently of the nuclear-pool of eicosanoid-forming enzymes and lipids, eosinophil enucleated cytoplasts were studied. As observed with intact eosinophils, PAF-induced a dose-dependent increase in the number of lipid bodies in eosinophil cytoplasts. Likewise, PAF-induced lipid body formation in nuclei-free cytoplast strongly correlated with increased LTC₄ and PGE₂ production following submaximal stimulation with A23187 (Bozza et al. 1997).

Collectively these findings indicate that lipid bodies are cytoplasmic, nuclei independent, sites for eicosanoid-forming enzyme localization and eicosanoid production. Taken together with the evidences indicating the nucleus environment as important pools for eicosanoid metabolism (Serhan 1996, Smith et al. 1996), our findings raise intriguing possibilities that different sites for eicosanoid production might exist in a cell leading to the production of eicosanoids for autocrine release within the nuclei acting as regulators of transcription or external release as paracrine inflammatory mediators.

INHIBITION OF LIPID BODY INDUCTION

In light of the putative role that lipid bodies may play in the development of inflammation, the effect of anti-inflammatory drugs on lipid body formation was studied. Lipid body induction by PAF required 5-LO activity, and was inhibited by the FLAP antagonist, MK 886, and the 5-LO inhibitor, zileuton, but not by COX inhibitors. Corroborating the dependency of PAF-induced lipid body formation on 5-LO, PAF failed to increase lipid body numbers in cells obtained from 5-LO knockout mice. Likewise, the capacity of PAF to elicit lipid body formation *in vivo* was shown to be 5-LO dependent in studies with an acute model of inflammation in mice. The intrathoracic injection of PAF significantly increased the number of lipid bodies in pleural resident macrophages and in the neutrophils that migrated to the pleural cavity from wild-type mice, but failed to stimulate macrophages or neutrophils from 5-LO knockout mice. These findings indicate the requisite role for 5-LO activity in the *in vitro* and *in vivo* induction of leukocyte lipid bodies by PAF (Bozza et al. 1996a).

In contrast, lipid body formation induced by *cis*-unsaturated fatty acids is independent of 5-LO activation, but is inhibited by non-steroidal anti-inflammatory drugs including aspirin, indomethacin and sodium salicylate (Bozza et al. 1996b). Interestingly, the non-cyclooxygenase inhibitor, sodium salicylate, was as potent as aspirin in inhibiting the lipid body formation elicited by *cis*-fatty acids, thus suggesting that the inhibitory effect of aspirin in lipid body formation is independent of COX inhibition. In ad-

dition, *cis*-fatty acids-induced lipid body formation in cells from COX-1 and COX-2 deficient mice was not impaired; and NSAIDs, including aspirin, sodium salicylate, indomethacin and NS-398, inhibited arachidonic acid-induced lipid body formation equally in macrophages from wild-type, and COX-1 or COX-2 deficient mice (Bozza et al. 1996b). Other evidence also suggests COX-2 does not participate in lipid body formation. COX-2 induction takes 2 - 48 h (Lee et al. 1992, Reddy & Herschman 1994, Smith et al. 1994), whereas lipid body formation occurs within 1 h (Weller et al. 1991b, Bozza et al. 1996a, b, 1997). Furthermore, dexamethasone, a potent inhibitor of COX-2 expression (Kujubu & Herschman 1992), did not block lipid body formation (Bozza et al. unpublished observations). Thus, COX-2 is unlikely to have a role in the induction of lipid bodies, and since this induction can still occur in COX-1 deficient macrophages neither COX is essential in this process.

In view of the ability of aspirin to inhibit lipid body formation both *in vivo* and *in vitro* (Reginato et al. 1985, Bozza et al. 1996b), we evaluated whether the inhibitory effect of aspirin on lipid body formation would have an effect on *cis*-fatty acid-induced priming for enhanced production of both COX and 5-LO metabolites. Pretreatment of leukocytes with aspirin significantly inhibited PGE₂ production by neutrophils and eosinophils in either vehicle- or *cis*-fatty acid-stimulated leukocytes, but such an effect might be based on the known ability of aspirin to inhibit COX (Bozza et al. 1996b). It has been well documented that aspirin is devoid of any direct inhibitory effects upon lipoxygenases in leukocytes and platelets (Cerletti et al. 1983, Punnonen & Uotila 1984, Cerletti 1985). However, aspirin and aspirin-like drugs were shown to indirectly regulate lipoxygenase activity in different systems (Siegel et al. 1980, Paajanen et al. 1982, Villanueva et al. 1993). In our studies, aspirin significantly inhibited *cis*-fatty acid-induced priming for enhanced LTB₄ production by neutrophils and LTC₄ production by eosinophils. Moreover, the effect of aspirin on inhibiting priming for enhanced eicosanoid production was correlated with its ability to inhibit *cis*-fatty acid-induced lipid body formation (Bozza et al. 1996b). Those results suggest that the capacity of aspirin to inhibit the induction of lipid bodies may enable aspirin to inhibit formation of both cyclooxygenase and lipoxygenase pathway derived eicosanoids and help to elucidate its range of anti-inflammatory activities.

CONCLUDING REMARKS

In the present study we review the evidence for a role of lipid bodies as specific inducible sites of eicosanoid mediator formation. Of special interest

for human diseases, those structures are naturally occurring *in vivo* in a variety of inflammatory pathological conditions, many of which are also known to have enhanced generation of arachidonic acid metabolites, such as leukotrienes and prostaglandins.

In conclusion, we have been identifying cytoplasmic inclusions, termed lipid bodies, as having previously unrecognized roles in the paracrine formation of eicosanoid mediators of inflammation. Lipid bodies are specialized intracellular domains for eicosanoid-forming enzyme localization, whose formation is specifically inducible, dependent upon transcriptional activation of early response genes and highly regulated by intracellular signaling pathways. Moreover, the capacity of inhibitors of lipid body formation to block eicosanoid generation identifies a novel target for anti-inflammatory drug action.

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