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New weapons against inflammation: dual inhibitors of phospholipase A_2 and transglutaminase

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Despite decades of research, corticosteroids and NSAIDs remain the main pharmacological weapons to control inflammation in the clinic. Unfortunately, these drugs have significant side effects, especially when used chronically. Consequently, there is tremendous interest in the development of novel, safer, and more effective anti-inflammatory drugs. Most anti-inflammatory agents directly or indirectly inhibit the formation or the effects of arachidonic acid metabolites collectively known as eicosanoids (prostaglandins, leukotrienes, thromboxanes, endoperoxides, and other mediators) (Table 1). It has long been recognized that inhibition of phospholipase A_2–catalyzed (PLA_2-catalyzed) arachidonic acid release from cell-membrane glycerophospholipids could potentially block the synthesis of all eicosanoids. The vast PLA_2 enzyme family includes cellular isoforms involved in signal transduction, such as three cellular isoforms of PLA_2 ($cPLA_2$s), and ten secretory isoforms of PLA_2 ($sPLA_2$s) (1). Various $sPLA_2$ isoforms participate in digestive physiology, antimicrobial defense, and inflammation. The $cPLA_2$s, and $sPLA_2$s IIA and V, play key roles in arachidonic-acid release during acute inflammation (1). Two families of endogenous proteins include members whose synthesis and/or secretion are induced by glucocorticoids in the lung that exhibit anti-inflammatory activity in experimental models. These are the lipocortins, or annexins (2), and the secretoglobins, whose prototype is uteroglobin (3). These families include proteins with distinct and pleiotropic biological properties. Lipocortins I and V, as well as rabbit and human uteroglobin, have anti-inflammatory properties that can be explained, at least in part, by their ability to inhibit $sPLA_2$. Human uteroglobin or "Clara Cell 10 kDa protein" is currently in clinical development for the prevention of airway inflammation in neonatal lung disease. The mechanism of $sPLA_2$ inhibition by lipocortins and uteroglobin remains controversial and may depend on the assay system. However, a 9–amino acid sequence that is highly conserved in uteroglobin and the anti-inflammatory lipocortins I and V was identified as early as 1988 (4). Synthetic peptides corresponding to this shared sequence exhibit striking anti-inflammatory activity in vivo and inhibit $sPLA_2$ in vitro. Mutagenesis data show that this sequence is necessary for $sPLA_2$ inhibition by uteroglobin (5). Peptides derived from

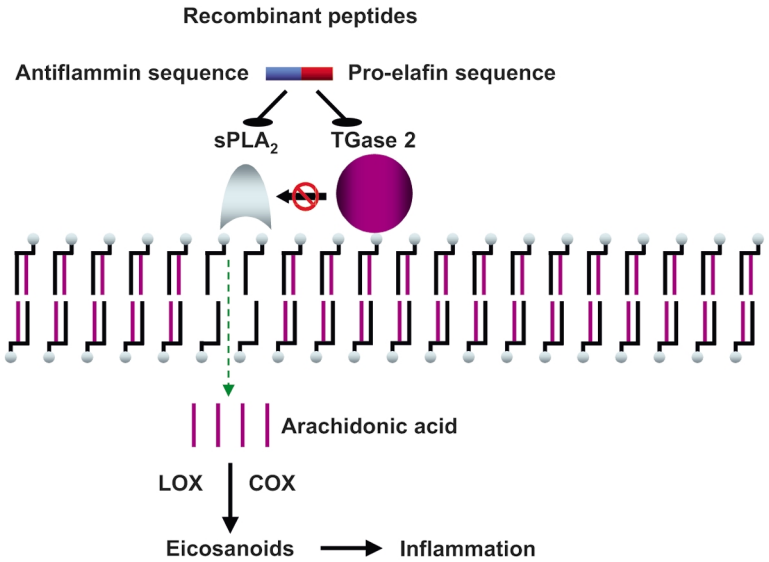

Figure 1
$sPLA_2$s hydrolyze the ester bond at the *sn*-2 position of membrane glycerophospholipids, generating free arachidonic acid. This acid is metabolized in a complex series of reactions involving COX or lipoxigenases (LOX), generating pro-inflammatory eicosanoids. TGase-catalyzed post-translational modifications activate $sPLA_2$, potentially increasing eicosanoid production during acute inflammation. The new recombinant peptides contain a pro-elafin sequence that inhibits TGase and an antiflammin sequence that inhibits $sPLA_2$. Thus they prevent TGase-induced $sPLA_2$ activation.

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Nonstandard abbreviations used: phospholipase A_2 (PLA_2); cellular isoform of PLA_2 ($cPLA_2$); secretory isoform of PLA_2 ($sPLA_2$); transglutaminase (TGase).

Table 1
Mediators and inhibitors of eicosanoid synthesis and inflammation

Molecule	Biological action	Inhibited by:
Eicosanoids		
Prostaglandins Leukotrienes Thromoxanes Endoperoxides Lipoxins	Metabolic products of arachidonic acid. Roles in inflammation, fever, blood pressure regulation, blood clotting, reproductive processes, tissue growth, tumorigenesis; regulate the sleep/wake cycle. Lipoxins may inhibit the effects of other eicosanoids.	Corticosteroids; NSAIDS. Corticosteroids inhibit eicosanoid production by multiple mechanisms. Many NSAIDs inhibit COX 1, 2, or 3 with variable specificity. Some COX inhibitors also inhibit LOX at high plasma concentrations. Specific LOX inhibitors are being actively tested.
PLA_2		
$cPLA_2$ (3 isoforms, intracellular, require μM Ca^{2+})	Release arachidonic acid for eicosanoid synthesis during signal transduction and acute inflammation. $cPLA_2$s can induce $sPLA_2$ IIA gene expression via LOX.	Corticosteroids; lipocortin I inhibits $cPLA_2$ in vitro.
$sPLA_2$ (10 isoforms, secretory, require mM Ca^{2+})	Mediate acute inflammation, digestion, and antimicrobial defense. $sPLA_2$ isoform IB can activate $cPLA_2$ via a membrane receptor. Isoforms IIA and V release arachidonic acid during inflammation.	Lipocortins I and V; uteroglobin; antiflammins
$iPLA_2$ (3 isoforms, intracellular, Ca-independent)	Less specific; participate in phospholipid remodeling. May have a role in arachidonic acid release in some cases.	No available drugs
TGases		
TGase 2[A] TGase 1 Coagulation factor XIIIa	Crosslink proteins during development, extracellular matrix remodeling, tissue repair, skin corneogenesis, blood coagulation, and apoptosis. Crosslink diamines and polyamines to proteins. TGase 2 also acts as a G-protein. Activate $sPLA_2$s by crosslinking or polyamination.	No available drugs. Uteroglobin is a TGase substrate.

LOX, lipoxigenases. [A]Also known as tissue TGase.

uteroglobin and lipocortins are collectively known as antiflammins, now recognized as one of the most potent classes of anti-inflammatory agents identified to date (6). The elegant work by Sohn et al. (7) appearing in this issue of the *JCI* builds on that early discovery and on the observation that some $sPLA_2$s are further activated by post-translational modifications catalyzed by transglutaminases (TGases). Transglutaminases are multifunctional enzymes that form isopeptide bonds between specific lysine and glutamine residues of substrate proteins or crosslink polyamines to glutamine residues (8). Cordella-Miele et al. showed that the TGase-catalyzed formation of an intramolecular isopeptide bond within $sPLA_2$s (9) or the polyamination of $sPLA_2$ (10) enhances the activity of $sPLA_2$s. Basing their work on these findings, Sohn et al. designed a novel series of chimeric peptides that include a fragment of pro-elafin (a TGase substrate in keratinocytes), and the conserved core of antiflammins (the sequence KVLD corresponding to uteroglobin residues 43–46). These new peptides inhibit $sPLA_2$ and TGase activity, and the TGase-catalyzed post-translational activation of $sPLA_2$ (Figure 1). Interestingly, the authors show that even the original antiflammins inhibit TGase, though not as efficiently as the new chimeric peptides. Uteroglobin is a well-known TGase substrate and Lys 43 a likely acyl acceptor (11). The chimeric peptides exhibit dramatic in vivo anti-inflammatory activity in a clinically relevant model of allergic inflammation: ragweed pollen–induced allergic conjunctivitis in guinea pigs. Inhibition of $sPLA_2$ and TGase activity was documented in tissue extracts from treated animals, and in vivo anti-inflammatory activity correlated with in vitro inhibitory potency on $sPLA_2$ and TGase. Chimeric peptide R2 was as potent as topical steroid or antihistamine drops, based on clinical inflammation scores, and was even more effective in reducing eosinophil infiltration. These findings have potentially great therapeutic relevance if one considers the number of patients who are chronically treated with antihistamines or steroids for seasonal allergies.

Future directions

The findings of Sohn et al. (7) establish that peptides or recombinant proteins that inhibit TGases and $sPLA_2$ or their peptidomimetic derivatives are highly attractive candidates for clinical development as anti-inflammatory agents. The potential applications of these molecules could go well beyond the realm of seasonal allergies. However, several important questions remain open. First, the precise in vivo mechanism of action of the new chimeric peptides, or for that matter the original antiflammins, remains unclear. Would a pure inhibitor of TGase that does not inhibit $sPLA_2$ be as effective? Are the effects of the new peptides abolished by arachidonic acid, as is the case for antiflammins? Are there additional in vivo mechanisms that were not explored by the relatively simple in vitro assays used in this and other studies? Antiflammins modulate leukocyte adhesion proteins (12), and uteroglobin binds fibronectin (13). Could the new peptides inhibit leukocyte migration via either or both of these mechanisms? Further mechanistic studies are necessary. Nonetheless, it is fair to say that a promising new class of anti-inflammatory agents may soon be added to our therapeutic arsenal.

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