J. Biochem. 2011;150(3):233–255 doi:10.1093/jb/mvr088

JB THE JOURNAL OF BIOCHEMISTRY

JB Review

Secreted phospholipase A_2 revisited

Received May 11, 2011; accepted June 14, 2011; published online July 11, 2011

Makoto Murakami*, Yoshitaka Taketomi, Hiroyasu Sato and Kei Yamamoto

Lipid Metabolism Project, Tokyo Metropolitan Institute of Medical Science, 2-1-6 Kamikitazawa, Setagaya-ku, Tokyo 156-8506, Japan

*To whom correspondence should be addressed. Tel: +81 3 5316 3228; Fax: +81 3 5316 3125; email: murakami-mk@igakuken.or.jp

Phospholipase A_2 (PLA_2) catalyses the hydrolysis of the *sn*-2 position of glycerophospholipids to yield fatty acids and lysophospholipids. So far, more than 30 enzymes that possess PLA_2 or related activity have been identified in mammals. About one third of these enzymes belong to the secreted PLA_2 ($sPLA_2$) family, which comprises low molecular weight, Ca^{2+} requiring, secreted enzymes with a His/Asp catalytic dyad. Individual $sPLA_2$s display distinct localizations and enzymatic properties, suggesting their specialized biological roles. However, in contrast to intracellular PLA_2s, whose roles in signal transduction and membrane homoeostasis have been well documented, the biological roles of $sPLA_2$s *in vivo* have remained obscure until recently. Over the past decade, information fuelled by studies employing knockout and transgenic mice as well as specific inhibitors, in combination with lipidomics, has clarified *when* and *where* the different $sPLA_2$ isoforms are expressed, *which* isoforms are involved in *what* types of pathophysiology, and *how* they exhibit their specific functions. In this review, we highlight recent advances in PLA_2 research, focusing mainly on the physiological functions of $sPLA_2$s and their modes of action on 'extracellular' phospholipid targets *versus* lipid mediator production.

Abbreviations: AA, arachidonic acid; ARDS, acute respiratory distress syndrome; $cPLA_2$, cytosolic PLA_2; cysLT, cysteinyl LT; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DRG, dorsal root ganglion; EPA, eicopentaenoic acid; HDL, high-density lipoprotein; $iPLA_2$, Ca^{2+}-independent PLA_2; IRS, inner root sheath; LDL, low-density lipoprotein; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LPS, lipopolysaccharide; LT, leucotriene; LXR, liver X receptor; ORS, outer root sheath; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, prostaglandin; PS, phosphatidylserine; PLA_2, phospholipase A_2; PLA2R1, M-type PLA_2 receptor; PLAI, PLA_2 inhibitory protein; PPAR, peroxisome proliferator-activated receptor; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; $sPLA_2$, secreted PLA_2; StAR, steroidogenic acute regulatory protein; VLDL, very low-density lipoprotein.

Phospholipase A_2 (PLA_2) catalyses the hydrolysis of the *sn*-2 position of glycerophospholipids to yield (mostly unsaturated) fatty acids and lysophospholipids (Fig. 1). In 2002, we published a minireview entitled 'Phospholipase A_2' in the *Journal of Biochemistry*, the major topics of which were limited to the classification and enzymatic properties of most if not all PLA_2 enzymes, with some functional aspects of arachidonic acid (AA) metabolism, based largely on *in vitro* cell culture studies (*1*). Until then, only a few transgenic/knockout mice for PLA_2 enzymes had been available, which included transgenic mice for group IIA secreted PLA_2 ($sPLA_2$-IIA) and knockout mice for group IVA cytosolic $PLA_2\alpha$ ($cPLA_2\alpha$) as well as mouse strains in which the gene for $sPLA_2$-IIA (*Pla2g2a*) is naturally disrupted. In 1995, it was found that mouse strains with a frameshift mutation in the *Pla2g2a* gene (so-called 'natural knockout mice') were more susceptible to colorectal cancer than those having the functional enzyme (*2*). However, this finding would have been inevitably affected by the different genetic backgrounds of the mouse strains used as controls, which show distinct susceptibility to diseases. $sPLA_2$-IIA transgenic (*Pla2g2a*-Tg) mice, which were reported in 1996 as the first mouse strain gene-manipulated for PLA_2 enzymes, displayed skin abnormality manifested by alopecia without inflammation (*3*), and subsequently resistance to bacterial infection (*4*, *5*) and increased lesions of diet-induced atherosclerosis (*6*). However, except for the anti-bacterial defence, which well reflects the *in vitro* ability of this enzyme to kill Gram-positive bacteria at low concentrations (*7*), the physiological significance of the phenotypes observed in *Pla2g2a*-Tg mice has remained a mystery. $cPLA_2\alpha$ knockout (*Pla2g4a*$^{-/-}$) mice, which were first reported in 1997, provided a consistent view of the role of this enzyme in the production of eicosanoids [prostaglandins (PGs) and leucotrienes (LTs)] in reproduction, asthma, cancer and other contexts (*8*–*10*). On the basis of these findings, together with the elaborate regulatory system of $cPLA_2\alpha$ by Ca^{2+} and phosphorylation that can account mostly for signal-coupled AA release (*11*, *12*), $cPLA_2\alpha$, rather than $sPLA_2$s, has attracted the attention of the majority of researchers in this field.

Since then, about a decade has passed, during which great advances have been achieved in the field of PLA_2 research by generation of a number of transgenic and knockout mouse lines, the development of inhibitors fairly specific for individual PLA_2 enzymes, and the identification of human diseases in which particular PLA_2 genes are mutated or show polymorphisms. Transgenic and/or knockout mice for the 5 $sPLA_2$ isoforms (groups IB, IIA, III, V and X) have been

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

Featured Article

© The Authors 2011. Published by Oxford University Press on behalf of the Japanese Biochemical Society. All rights reserved

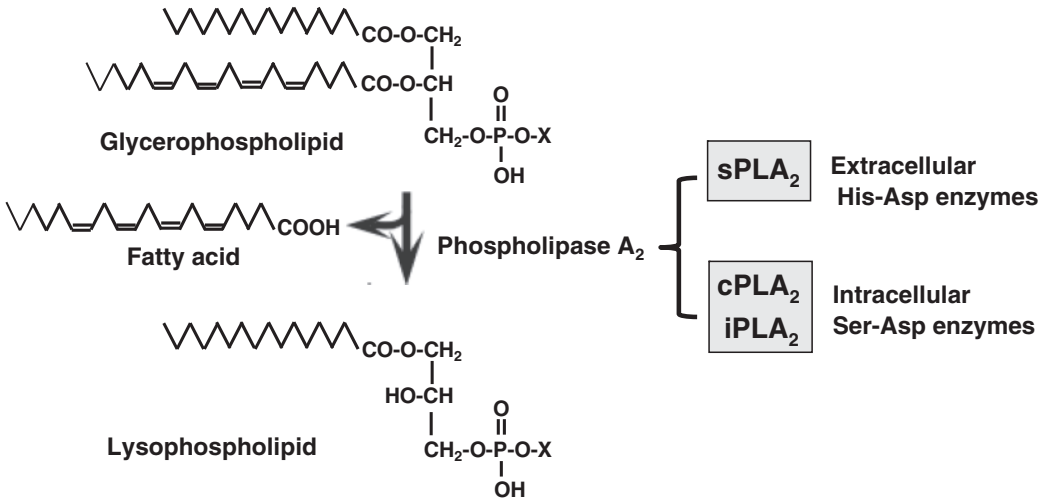

Fig. 1 PLA_2 reaction. PLA_2 enzymes, including extracellular $sPLA_2$s with a His–Asp dyad and intracellular $cPLA_2$s/$iPLA_2$s with a Ser–Asp dyad, hydrolyse the *sn*-2 position of phospholipids to yield fatty acids and lysophospholipids. Several members of the $cPLA_2$ and $iPLA_2$ families also possess PLA_1, lysophospholipase, or triglyceride lipase activity, whereas $sPLA_2$s definitively display PLA_2 activity.

generated, and it has become evident that individual $sPLA_2$s make a distinct contribution to various biological events such as digestion, inflammation, tissue injury, atherosclerosis, host defence, reproduction and skin homoeostasis (*13–15*). Numerous studies using *Pla2g4a*$^{-/-}$ mice have provided a comprehensive view of the contribution of $cPLA_2\alpha$ to various diseases and homoeostasis. In addition, an overall picture of the regulatory mechanisms leading to activation of $cPLA_2\alpha$, as well as its novel role in membrane trafficking, has emerged (*16–19*). Deficiency and/or mutations of Ca^{2+}-independent PLA_2 ($iPLA_2$) members have been found to be associated with various disorders such as neurodegeneration and metabolic syndrome in both mice and humans (*20–27*). In this article, we provide an overview of the functions of $sPLA_2$s, focusing mainly on lessons learned from the use of knockout and/or transgenic mice. In fact, it has become obvious that $sPLA_2$s are involved in many biological processes through multiple mechanisms, and as a consequence they have been returning to the forefront of research. The current classification of $sPLA_2$ enzymes, together with the $cPLA_2$ and $iPLA_2$ families that are not described in the main text, is summarized in Table I. For details of the other PLA_2 groups, please refer to a number of recent excellent reviews (*28–30*).

General aspects of the $sPLA_2$ family

To date, 11 $sPLA_2$s (IB, IIA, IIC, IID, IIE, IIF, III, V, X, XIIA and XIIB) have been identified in mammals (Fig. 2). They are subdivided into three major collections, namely conventional group I/II/V/X $sPLA_2$s and atypical groups III and XII $sPLA_2$s (*13–15*). Conventional $sPLA_2$s are 14–19 kDa secreted enzymes with a highly conserved Ca^{2+}-binding site and a catalytic His–Asp dyad. In addition to these elements, there are six absolutely conserved disulphide bonds and up to two additional unique disulphide bonds, which contribute to the high degree of stability of these enzymes. $sPLA_2$-IB and -X have an N-terminal propeptide that is proteolytically cleaved off, yielding fully active enzymes. The atypical $sPLA_2$s, $sPLA_2$-III and $sPLA_2$-XIIA/XIIB, have poor homology with the I/II/V/X collection of $sPLA_2$s except for the conserved Ca^{2+}-binding loop and catalytic site. For more details of the enzymatic features of individual $sPLA_2$s, please refer to our previous *JB* review in 2002 (*1*).

A database search of $sPLA_2$s in the animal kingdom provides an insight into the evolutional relationships among the isoforms. Of the conventional $sPLA_2$s, $sPLA_2$-IB appears to be the oldest isozyme, whose orthologues are found from nematodes (*e.g. Caenorhabditis elegans*, which has three IB-like genes) to vertebrates, and at a later stage $sPLA_2$-X appeared in amphibians. Beyond the latter stage, evolutionary pressure may have forced the emergence of $sPLA_2$s without an N-terminal propeptide, namely group II $sPLA_2$s: thus, $sPLA_2$-IIA and probably -IID and -IIE, all of which have a standard 'group II' structure, first appeared in snakes, $sPLA_2$-IIC and -IIF, which have a unique extra segment, appeared in birds and $sPLA_2$-V, the simplest and newest isoform, finally appeared in mammals. The group III $sPLA_2$ collection is highly divergent in insects (*e.g.* five genes are found in *Drosophila*), whereas only a single gene for group III is encoded in the vertebrate genome (fish to mammals) and is not present in nematodes. The group XII $sPLA_2$s are found in all vertebrates, but not in invertebrates.

Since $sPLA_2$s are 'secreted' and require mM-order Ca^{2+} for catalysis, their primary target phospholipids should be principally extracellular. $sPLA_2$s can act on cellular membranes in an autocrine or paracrine manner to release not only AA, but also a variety of saturated, monounsaturated and polyunsaturated fatty acids (PUFAs) such as ω-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are precursors of anti-inflammatory lipid mediators. The counterpart product, namely lysophospholipid such as

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

Table I. Classification of the $sPLA_2$, $cPLA_2$ and $iPLA_2$ families.

Families	Gene names	Nomenclatures	Alternative names	Characteristics	Mutations or SNPs in human diseases
$sPLA_2$	*Pla2g1b*	IB	Pancreatic $sPLA_2$	Conventional $sPLA_2$ with group I property	Obesity
	Pla2g2a	IIA	Inflammatory $sPLA_2$	Conventional $sPLA_2$ with group II property	Colon cancer
	Pla2g2c	IIC		Conventional $sPLA_2$ with group II property, absent in humans	
	Pla2g2d	IID		Conventional $sPLA_2$ with group II property	COPD with body weight loss
	Pla2g2e	IIE		Conventional $sPLA_2$ with group II property	
	Pla2g2f	IIF		Conventional $sPLA_2$ with group II property, long C-terminal extension	
	Pla2g5	V		Conventional $sPLA_2$ without groups I and II properties	Type 2 diabetes
	Pla2g10	X		Conventional $sPLA_2$ with both groups I and II properties	
	Pla2g3	III		Atyplical $sPLA_2$, group III collection	Colon cancer, AIDS
	Pla2g12a	XIIA		Atypical $sPLA_2$, group XII collection	
	Pla2g12b	XIIB		Atypical $sPLA_2$, group XII collection, no activity	
$cPLA_2$	*Pla2g4a*	IVA	$cPLA_2\alpha$	C2 domain, AA specific, MAPK phosphorylation	Inherited deficiency with platelet dysfunction
	Pla2g4b	IVB	$cPLA_2\beta$	C2 domain, JimC domain	
	Pla2g4c	IVC	$cPLA_2\gamma$	Prenylation, no C2 domain	
	Pla2g4d	IVD	$cPLA_2\delta$	C2 domain	
	Pla2g4e	IVE	$cPLA_2\varepsilon$	C2 domain	
	Pla2g4f	IVF	$cPLA_2\zeta$	C2 domain	
$iPLA_2$	*Pla2g6*	VIA	$iPLA_2\beta$	Ankyrin repeat	Neurodegenerative diseases
	Pnpla8	VIB	$iPLA_2\gamma$	Mitochondrial and peroxisomal localization signals	
	Pnpla6		$iPLA_2\delta$, NTE	Long N-terminal region with a transmembrane domain	Neurodegenerative diseases
	Pnpla7		NRE	Long N-terminal region with a transmembrane domain	
	Pnpla2		$iPLA_2\zeta$, ATGL	Major triglyceride lipase	Neutral lipid storage disease
	Pnpla3		$iPLA_2\varepsilon$, adiponutrin	Transcylase?	Fatty liver
	Pnpla4		$iPLA_2\eta$, GS2	Retinylester hydrolase? absent in mice	
	Pnpla5		GS2-like		
	Pnpla1			Major splicing variant without a catalytic dyad	

For other PLA_2 families, please see ref (*13*).

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

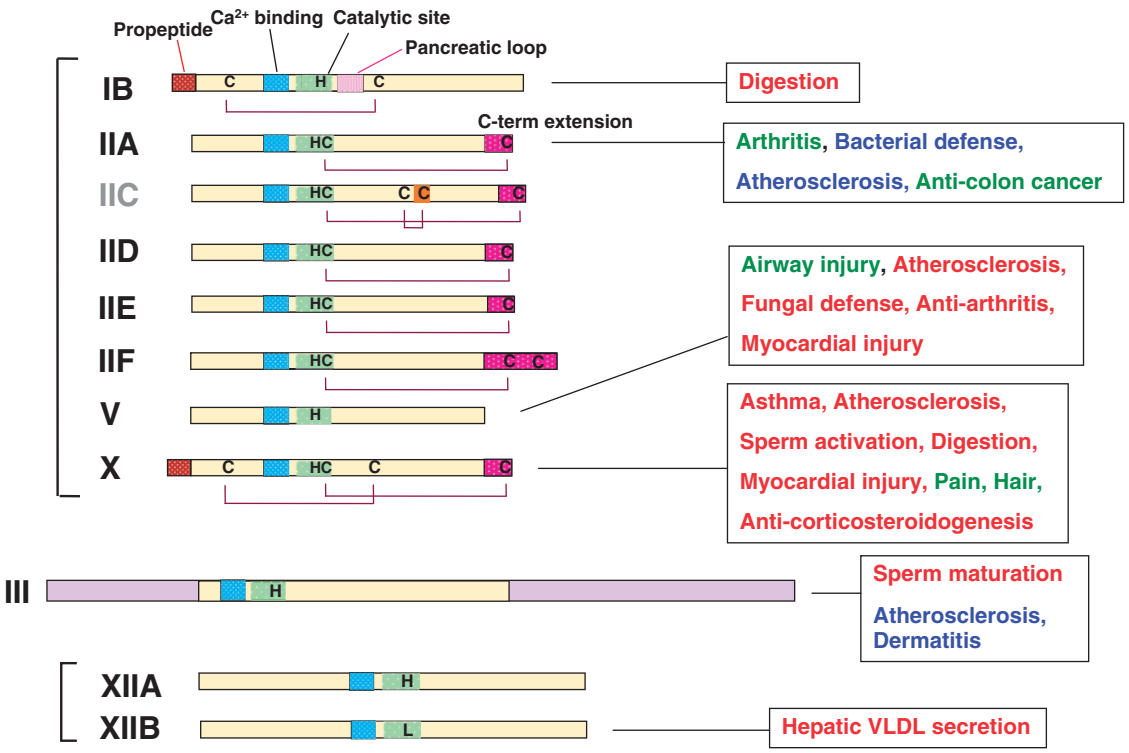

Fig. 2 The $sPLA_2$ family. Mammalian $sPLA_2$s comprise 11 isoforms, which are subdivided into three collections, namely group I/II/V/X (conventional $sPLA_2$s), group III and group XII. All enzymes have a conserved catalytic centre with a His–Asp dyad and a Ca^{2+}-binding loop. $sPLA_2$-IB, a pancreatic $sPLA_2$, is characterized by an N-terminal propeptide whose proteolytic removal gives rise to a functional enzyme, the presence of a Cys11–Cys77 disulphide bond (group I-specific disulphide), and a unique pancreatic loop. The group II subfamily (IIA, IIC, IID, IIE and IIF) is characterized by absence of the propeptide and the presence of Cys49-Cys within the C-terminal extension (group II-specific disulphide). $sPLA_2$-IIC has a unique insertion with an extra disulphide bond, but this isoform is absent in humans (pseudogene). $sPLA_2$-IIF has a longer C-terminal extension, which is Pro-rich. $sPLA_2$-V is evolutionarily close to the group II subfamily, but lacks the group II-specific disulphide and the C-terminal extension. $sPLA_2$-X has both groups I and II properties since it has an N-terminal propeptide and both groups I- and II-specific disulphides. $sPLA_2$-III is unique in that the central $sPLA_2$ domain, which is more similar to bee venom PLA_2 than to group I/II/V/X $sPLA_2$s, is flanked by unique and highly cationic N- and C-terminal domains. The N- and C-terminal domains are removed to produce a mature, $sPLA_2$ domain-only form. The group XII collection contains two isoforms, XIIA and XIIB, whose overall structures (except for the catalytic domain and Ca^{2+}-binding site) do not show any homology with other $sPLA_2$s. The catalytic centre His is replaced by Leu in $sPLA_2$-XIIB, indicating that this enzyme is catalytically inactive. Biological functions assigned to individual isoforms, as assessed by knockouts (red), transgenic (blue) or both (green), are shown in the right boxes.

lysophosphatidylcholine (LPC) and lysophosphatidic acid (LPA), also has various bioactivities. Moreover, $sPLA_2$s can also act on 'non-cellular' phospholipids, such as those in microvesicles, pulmonary surfactant, lipoproteins, microbial membranes and food substances (Fig. 3). Therefore, the physiological functions of a given $sPLA_2$ could reflect a combination of these multiple actions. In keeping with these general views, we will highlight a variety of biological functions of $sPLA_2$ isoforms *in vivo,* particularly in the context of pathophysiology. In many cases, multiple $sPLA_2$s can participate in a given biological process in distinct, redundant, complementary or counter-regulatory ways.

$sPLA_2$ and lipid mediators: *in vitro* and *in vivo*

Numerous *in vitro* studies, mostly on the basis of forcible transfection or exogenous addition of recombinant $sPLA_2$s, have shown that several $sPLA_2$s have the ability to liberate AA from cell membranes, leading to augmented production of eicosanoids (PGs and LTs), with a potency order of X > V > III > IIF > IIA > IB > IID > IIE > XIIA (*31–35*). The high capacity of $sPLA_2$-X, -V and -III to release cellular AA correlates with their high capacity to bind phosphatidylcholine (PC), a major phospholipid in the outer leaflet of the plasma membrane (*33–35*). The cellular AA-releasing function of group II $sPLA_2$s requires co-factors such as heparan sulphate proteoglycans, which help to facilitate their residence in the plasma membrane or internalization, and appropriate pro-inflammatory stimuli, which may induce rearrangement of membrane microdomains leading to exposure of their better substrates, phosphatidylethanolamine (PE) and phosphatidylserine (PS) (*34*, *36*). However, since these *in vitro* studies were often performed using super-physiological amounts of exogenous or overexpressed $sPLA_2$s, it remained uncertain whether or not endogenous $sPLA_2$s could play such a role *in vivo*.

Subsequent studies using mice that are transgenic, or more physiologically knocked out, for individual $sPLA_2$s have revealed that they can mobilize lipid mediators *in vivo*. For instance, transgenic overexpression of $sPLA_2$-III (*Pla2g3*-Tg) or $sPLA_2$-X (*Pla2g10*-Tg) in mice leads to increased PGE_2 production in the

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

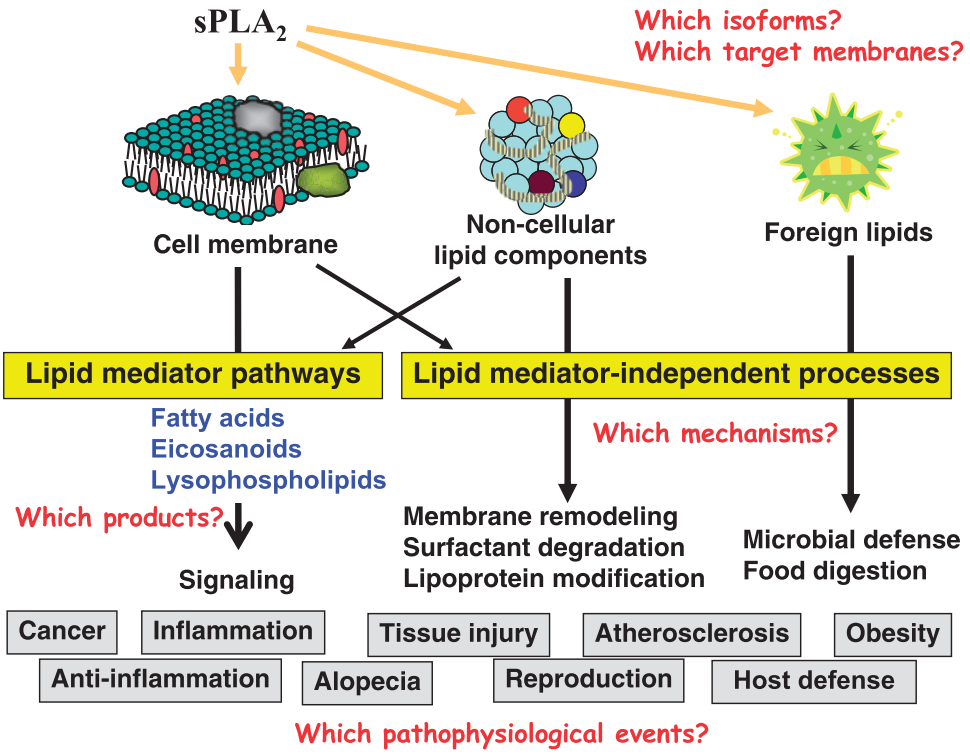

Fig. 3 Schematic diagram of $sPLA_2$ biology. Individual $sPLA_2$ enzymes act not only on cellular membranes, but also on non-cellular phospholipids (*e.g.* surfactant and lipoproteins) and foreign phospholipids (*e.g.* bacterial membranes and dietary phospholipids). Some actions of $sPLA_2$s are mediated by lipid mediator–mediated signalling, while others occur independently of lipid mediators. The physiological functions of a particular $sPLA_2$ could reflect a combination of these varied actions. Thus, in order to gain an overall functional view of $sPLA_2$, it is important to clarify (i) the $sPLA_2$ isoforms that act on (ii) specific target membranes, (iii) the lipid mediators that are produced if 'dependent' upon lipid mediators, (iv) the mechanisms that are responsible if 'independent' of lipid mediators and (v) the pathophysiological events in which individual $sPLA_2$s are involved.

skin (*37*, *38*). Macrophage production of cysteinyl LTs (cysLTs) and PGE_2 (*39*) and mast cell production of PGD_2 (*40*) following zymosan stimulation are partially reduced in mice lacking $sPLA_2$-V (*Pla2g5*$^{-/-}$) compared with replicate control mice. In $sPLA_2$-X-deficient (*Pla2g10*$^{-/-}$) mice, there is a partial reduction of LTB_4 synthesis by neutrophils (*41*). Other examples of altered lipid mediator levels in $sPLA_2$ knockout mice in distinct physiological settings will be described below.

It should be noted that, even though the above results imply that $sPLA_2$s can participate in the lipid mediator-biosynthetic pathways *in vivo*, they do not necessarily mean that these enzymes directly hydrolyse membrane phospholipids to provide AA. Indeed, in several *in vivo* situations, the augmented action of $sPLA_2$s on lipid mediator synthesis depends on the coordinated activation of $cPLA_2\alpha$. One idea is that $sPLA_2$-released lysophospholipids (or other bioactive lipids) may amplify the $cPLA_2\alpha$ activation machinery leading to the burst synthesis of lipid mediators (Fig. 4A), as exemplified by $sPLA_2$-mediated LT production by granulocytes (*42*, *43*) and PGD_2 production by mast cells (*44*). Alternatively, $sPLA_2$s may regulate the bottleneck step of a given disease, and as such, subsequent propagation of the disease results in explosive activation of $cPLA_2\alpha$ that supplies the bulk of lipid mediators within the affected tissue (Fig. 4B). Such coordinated actions of extracellular and intracellular PLA_2s can explain why knockout of either $sPLA_2$ or $cPLA_2\alpha$ culminates in dramatic amelioration of several disease models, such as asthma and arthritis (see below). More importantly, however, many of the phenotypes displayed by $sPLA_2$ gene-manipulated mice described below appear to occur independently of $cPLA_2\alpha$ or lipid mediators (Fig. 4C and D); this again implies complex actions of $sPLA_2$s on distinct phospholipid components outside the cells.

$sPLA_2$ and inflammation

Airway disease

We found that *Pla2g5*-Tg mice, which were the first $sPLA_2$ Tg mice we generated in 2006, died soon after birth because of acute respiratory distress syndrome (ARDS) resulting from aberrant hydrolysis of lung surfactant phospholipids, including dipalmitoyl-PC and -phosphatidylglycerol (*45*). Soon afterwards, similar fatal airway destruction was also reported in macrophage-specific *Pla2g10*-Tg mice (*46*). Pulmonary surfactant is a lipid–protein complex synthesized by alveolar type II epithelial cells, and plays a role in lowering surface tension along the alveolar epithelium, thereby promoting alveolar stability. Destruction of as little as 10–20% of surfactant lipids can cause loss of alveolar stability and impairment of gas exchange, leading to ARDS. ARDS in

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

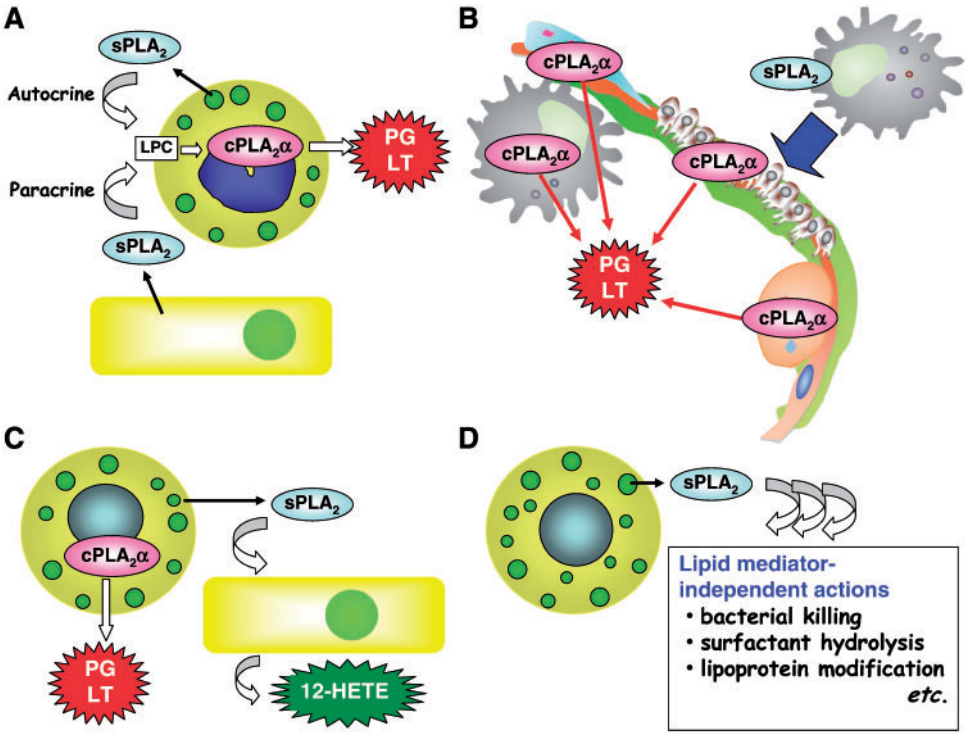

Fig. 4 Various modes of $sPLA_2$ action. (A) During proposed sequential $sPLA_2/cPLA_2\alpha$ cross-talk, LPC or other products released by $sPLA_2$ in an autocrine or paracrine manner may amplify the $cPLA_2\alpha$ activation pathway in the same cell, leading to full propagation of eicosanoid synthesis. An example is the cooperation of $sPLA_2/cPLA_2\alpha$ in eosinophils in bronchial asthma. (B) $sPLA_2$ regulates a certain 'bottleneck' step of the disease in a proximal or distal tissue compartment. Accordingly, suppression of $sPLA_2$ at this step may eventually abrogate subsequent $cPLA_2\alpha$-dependent eicosanoid synthesis linked to the disease. As an example, $sPLA_2$-V in antigen-presenting cells contributes to the sensitization phase (antigen uptake and processing), whereas $cPLA_2\alpha$, which is expressed ubiquitously, contributes to the bulk PG/LT synthesis by various cells in the airway during the effector phase. (C) $cPLA_2\alpha$ and $sPLA_2$ contribute to distinct pools of lipid mediators, probably by acting on different cells. For instance, production of 12-HETE, but not PGs, in the epididymis depends on $sPLA_2$-III. (D) The actions of $sPLA_2$ take place independently of lipid mediators. This includes many examples, *e.g.* anti-bacterial defence by $sPLA_2$-IIA through bacterial membrane degradation, anti-fungal defence by $sPLA_2$-V through macrophage phagocytosis, atherosclerosis by $sPLA_2$-IIA, -III, -V and X through generation of small-dense LDL, alveolar damage by $sPLA_2$-V through surfactant hydrolysis, sperm maturation by $sPLA_2$-III though sperm membrane remodelling, and dietary phospholipid digestion by $sPLA_2$-IB and -X that can be linked to distal adiposity. For details, please see text.

experimental models can be attenuated by administration of a pan-$sPLA_2$ inhibitor, suggesting the contribution of $sPLA_2$(s) to this fatal pulmonary disease (*47*, *48*). Indeed, $sPLA_2$-V is expressed in the bronchial epithelium and alveolar macrophages, and is induced in mouse Th2-dependent asthma models as well as in patients with asthma or severe pneumonia (*49*, *50*). $sPLA_2$-X is also expressed in airway epithelial cells in both mice and humans (*50*, *51*). These two $sPLA_2$s can potently hydrolyse surfactant PC *in vitro* (*52*). Thus, $sPLA_2$-V and -X represent the pathogenic $sPLA_2$s in airway injury, at least in part through the hydrolytic degradation of lung surfactant.

Regardless of the mechanisms involved, the pathological role of these two $sPLA_2$s in airway diseases has rapidly become clearer through subsequent studies using mice with knockout of both enzymes. The first phenotype reported for *Pla2g10*$^{-/-}$ mice in 2007 was remarkable resistance to an antigen-induced asthma model, where the lungs of the mice showing marked attenuation of T cell and eosinophil infiltration, goblet cell metaplasia, thickening of the smooth muscle cell layer, subepithelial fibrosis and Th2 cytokine levels (*53*). In view of the marked reduction of eicosanoid levels in *Pla2g10*$^{-/-}$ lung, this study suggested that the amelioration of the airway phenotype was attributable to reduction of $sPLA_2$-X-mediated eicosanoid synthesis. Considering that mice lacking $cPLA_2\alpha$ (*Pla2g4a*$^{-/-}$) were also protected from this form of induced asthma (*8*), functional cooperation of $sPLA_2$-X and $cPLA_2\alpha$ may be a pathologic feature of asthma. In this scenario, $sPLA_2$-X may be secreted from airway epithelial cells and act in a paracrine manner on recruited leucocytes such as eosinophils, boosting $cPLA_2\alpha$-mediated AA release (Fig. 4A) (*43*). Even if this proves to be the case, it still remains undetermined whether $sPLA_2$-X may play an important role in a specific, as yet unidentified, rate-limiting step for asthma (*e.g.* polarization of alternatively activated macrophages or airway epithelial damage by excessive surfactant hydrolysis), and whether ablation of this regulatory step might subsequently dampen all downstream inflammatory reactions in the airway (Fig. 4B). In fact, the latter mechanism is partly responsible for the pro-asthmatic function of $sPLA_2$-V, as described below.

Soon after the above findings in *Pla2g10*$^{-/-}$ mice, *Pla2g5*$^{-/-}$ mice were also shown to be protected from the same type of antigen-induced asthma (*49*). Likewise, airway hyper-responsiveness in the allergic

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

mice was partially blocked by intratracheal application of anti-$sPLA_2$-V antibody. Conversely, intratracheal administration of $sPLA_2$-V, but not $sPLA_2$-IIA or a catalytically weak $sPLA_2$-V mutant, increased airway resistance and narrowing along with increased leucocyte infiltration (*49*). Bronchoconstriction elicited by $sPLA_2$-V in mice lacking $cPLA_2\alpha$ (*pla2g4*$^{-/-}$) was comparable to that in wild-type mice, suggesting that $sPLA_2$-V exerts its pro-asthmatic action independently of $cPLA_2\alpha$, and thereby of eicosanoids. Similar approaches were conducted in the LPS-induced airway injury model, and this again showed that *Pla2g5*$^{-/-}$ mice were protected from this ARDS-like inflammation (*54*). These data collectively indicate that $sPLA_2$-V is involved in both chronic and acute airway disorders. The fact that intratracheal treatment with $sPLA_2$-V and its neutralizing antibody recapitulated the phenotypes of *Pla2g5*-Tg and *Pla2g5*$^{-/-}$ mice, respectively, supports the speculation that $sPLA_2$-V causes airway inflammation by hydrolysing pulmonary phospholipids, probably surfactants.

Another regulatory step of Th2-driven allergic pulmonary inflammation in which $sPLA_2$-V plays a regulatory role has been identified very recently. Antigen-presenting cells from *Pla2g5*$^{-/-}$ mice showed a delay in allergen uptake and processing and thereby a partial impairment of allergen-induced maturation (*55*). Accordingly, *Pla2g5*$^{-/-}$ mice displayed a reduction of Th2 polarization, thereby resisting subsequent propagation of asthmatic inflammation. This mechanism fits well with the idea illustrated in Fig. 4B. Collectively, $sPLA_2$-V appears to function in two regulatory steps in asthma: (i) in antigen-presenting cells to regulate antigen processing and maturation and thereby the Th2 immune response, and (ii) in airway-resident cells to facilitate subsequent airway inflammation that may involve surfactant degradation.

Arthritis

Since the discovery in 1989 of $sPLA_2$-IIA as an inducible enzyme during inflammation (which is why it is often called 'inflammatory' $sPLA_2$) (*56*, *57*), many studies have focused particularly on the pro-inflammatory role of $sPLA_2$-IIA, mostly in the context of eicosanoid generation. In fact, $sPLA_2$-IIA is highly expressed in synovial cells and chondrocytes in the joints of patients with rheumatoid arthritis, but not those of normal subjects (*56*, *58*), leading to the hypothesis that $sPLA_2$-IIA plays an exacerbating role in this autoimmune disease. However, the contribution of $sPLA_2$-IIA to inflammation *in vivo* has remained obscure until very recently, most likely because the C57BL/6 and 129Sv mouse strains, which are generally used in knockout studies, have a natural frameshift mutation in the *Pla2g2a* gene (*2*), which has made it difficult to assess the functions of this enzyme by classical gene targeting strategies. Exploiting the fact that this mutation does not occur in BALB/c mice, the *Pla2g2a* gene has recently been knocked out in BALB/c mice by breeding them with C57BL/6 mice and then backcrossing with BALB/c mice. Using the *Pla2g2a*$^{-/-}$ BALB/c mice, thus obtained, it has been shown that $sPLA_2$-IIA does play a pro-inflammatory role in a model of arthritis induced by K/BxN autoantibody (*59*). Thus, *Pla2g2a*$^{-/-}$ BALB/c mice displayed a reduced degree of arthritic inflammation in comparison with wild-type BALB/c mice (*59*). Conversely, the same K/BxN autoantibody-induced arthritis was exacerbated in *PLA2G2A*-Tg mice. Thus, these observations have provided confirmation of the pro-inflammatory role of $sPLA_2$-IIA in inflammatory arthritis. However, although the pathology of inflammatory arthritis involves $cPLA_2\alpha$ (*60*) as well as lipid mediators, including PGs and LTs (*61–64*), it has not yet been firmly clarified whether $sPLA_2$-IIA would contribute to the synthesis of these eicosanoids or act through other mechanism(s) in inflamed joints.

As opposed to *Pla2g2a*$^{-/-}$ mice, *Pla2g5*$^{-/-}$ mice showed exacerbation of K/BxN autoantibody-induced arthritis, likely because opsonization-dependent clearance of the pathogenic immune complex by macrophages from inflamed joints was attenuated in the latter (*59*). In support of this, systemic administration of recombinant $sPLA_2$-V protein ameliorated the arthritic response by facilitating phagocytic uptake of the immune complex by macrophages. Thus, $sPLA_2$-V may have an anti-inflammatory, rather than pro-inflammatory, role in particular diseases induced by deposition of immune complexes, such as rheumatoid arthritis. The fact that these two particular $sPLA_2$s, $sPLA_2$-IIA and $sPLA_2$-V, exert promoting and protective effects, respectively, on inflammatory arthritis may explain why clinical attempts to treat patients with rheumatoid arthritis with a pan-$sPLA_2$ inhibitor, which blocks both enzymes, have been unsuccessful (*65*). These observations provide a rationale for testing two new therapeutic approaches for treatment of immune complex-mediated inflammation: (i) the use of an $sPLA_2$-IIA-specific inhibitor and (ii) the use of recombinant $sPLA_2$-V.

On the other hand, $sPLA_2$-V apparently plays a pro-inflammatory role in other models. For instance, the early phase of pro-inflammatory cysLT synthesis in a zymosan-induced peritonitis model was partially reduced in *Pla2g5*$^{-/-}$ mice (*39*). In the air pouch model, *Pla2g5*$^{-/-}$ mice showed reduced recruitment of LPS-induced leucocytes through attenuated expression of adhesion molecules (*66*). These observations, together with its pro-asthmatic role as mentioned above, indicate that $sPLA_2$-V has both pro-inflammatory and anti-inflammatory potential depending on the disease model.

Myocardial injury

Another example of the contributions of two $sPLA_2$s with distinct localizations occurs in the process of myocardial infarction with a similar pro-inflammatory outcome. $sPLA_2$-X is localized in azurophilic granules of neutrophils, but not in cardiomyocytes (*41*, *67*). In a model of myocardial ischaemia/reperfusion, *Pla2g10*$^{-/-}$ mice exhibited a smaller myocardial infarct size and less neutrophil infiltration than did wild-type mice (*41*). This phenotype was recapitulated by adoptive transfer of *Pla2g10*$^{-/-}$ bone marrow cells into wild-type mice, implying the contribution of $sPLA_2$-X in myeloid cells to the myocardial injury. $sPLA_2$-X

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

is released from infiltrating neutrophils at the infarct myocardium and damages cardiomyocytes, probably through LTB_4 production and respiratory burst.

Among various tissues, $sPLA_2$-V shows its highest expression in the heart (*68*), and its expression is elevated in areas of cardiac infarction in human patients (*69*). In the same myocardial ischaemia/reperfusion model, *Pla2g5*$^{-/-}$ mice showed a marked decrease in myocardial infarct size, with lower contents of myocardial LTB_4 and TXA_2 (*70*), eicosanoids with deleterious effects on ischaemic myocardium (*71*). AA release in response to oxidative damage was reduced in *Pla2g5*$^{-/-}$ cardiomyocytes, further supporting the role of myocardial $sPLA_2$-V in this process, possibly in concert with $cPLA_2\alpha$, although the effect of blood cell-borne $sPLA_2$-V was not addressed in that study. These investigations have revealed the pro-inflammatory role of myocardial $sPLA_2$-V and myeloid $sPLA_2$-X in ischaemic heart damage with a common outcome. Interestingly, administration of a pan-$sPLA_2$ inhibitor capable of blocking both $sPLA_2$-V and -X-reduced myocardial infarct size (*41*), suggesting that $sPLA_2$ inhibition may be an effective strategy for treatment of patients with myocardial infarction.

Anti-inflammation

Since $sPLA_2$s can release ω-3 PUFAs such as EPA and DHA, the potential anti-inflammatory role of $sPLA_2$s should also be taken into consideration. Macrophages transfected with $sPLA_2$-X produce more anti-inflammatory IL-10 and less pro-inflammatory TNF-α than do control cells (*46*), suggesting an anti-inflammatory potential of this enzyme. In fact, the alteration of cytokine profiles in $sPLA_2$-X-transfected macrophages raises the possibility that $sPLA_2$-X might facilitate the polarization of macrophages towards an M2 phenotype, a macrophage subset that plays roles in inflammation resolution and tissue repair. This speculation is substantiated by findings suggesting that PUFAs can activate PPARδ, a key transcription factor for M2 polarization of macrophages (*72*, *73*), and that activation of PPARδ by snake venom $sPLA_2$-released PUFAs can switch on the anti-inflammatory programme in endothelial cells (*74*). This is also concordant with the resistance of *Pla2g10*$^{-/-}$ mice to the asthmatic response (*53*), a typical Th2 response in which IL-4/IL-13-driven M2 macrophages play a pro-asthmatic role (*75*).

$sPLA_2$-IID is the closest homologue of $sPLA_2$-IIA and is constitutively expressed in secondary lymphatic organs such as the spleen and lymph nodes (*76*). A recent genome-wide screening of regulatory T (T_{reg})-specific genes has revealed that $sPLA_2$-IID is highly induced in this cell population and is a potent mediator of T_{reg} function (*77*). $sPLA_2$-IID suppressed the proliferation of $CD4^+$ and $CD8^+$ T cells and promoted the differentiation of T_{reg} cells *in vitro*. Moreover, administration of a $sPLA_2$-IID-Fc fusion protein into mice attenuated experimental models of colitis and multiple sclerosis, suggesting its immunosuppressive function. However, because of the limitation imposed by the use of a fusion protein comprising a small $sPLA_2$ molecule conjugated with a large Fc domain, the physiological relevance of the immunosuppressive function of $sPLA_2$-IID awaits a future study employing *Pla2g2d*$^{-/-}$ mice. Interestingly, a polymorphism (G80S) in the human *PLA2G2D* gene is associated with body weight loss in chronic obstructive pulmonary disease (*78*), suggesting the participation of $sPLA_2$-IID in chronic airway inflammation and/or body weight control.

$sPLA_2$ and atherosclerosis

The surfaces of lipoprotein particles, such as low-density lipoprotein (LDL) and high-density lipoprotein (HDL), are surrounded by phospholipids (mainly PC), which serve as a very good 'extracellular' target of several, if not all, $sPLA_2$ isoforms. *In vitro*, the rank order of $sPLA_2$ hydrolytic potency is X > V > III > IIF > IIA > IIE for both LDL and HDL, which appears to roughly correlate with their ability to interact with PC (*79*). Although the activity of $sPLA_2$-IIA on lipoproteins is relatively weak, it can hydrolyze acute phase HDL more efficiently than normal HDL, preferentially attacking PC with oxygenated PUFAs (*80*). Clinically, an elevated plasma $sPLA_2$-IIA level is an independent risk factor for cardiovascular disease (*81*, *82*). LDL particles vary in size and density, and high plasma levels of small-dense LDL particles correlate well with a higher risk of coronary heart disease. A key step for generation of pro-atherogenic small-dense LDL is hypertriglyceridemia, and it is hypothesized that small-dense LDL can penetrate into vessel wall tissue more easily and attach to the extracellular matrix, where the PUFAs in phospholipids on the LDL surface undergo oxidative modification. Several lines of evidence suggest that $sPLA_2$-mediated hydrolysis of lipoproteins also gives rise to a type of small-dense LDL with an increased net negative charge, LPC content and propensity for aggregation, thus contributing to the development of atherosclerosis (*83*, *84*). Indeed, LDL treated with $sPLA_2$-III, -V or -X facilitates the formation of lipid-laden foam cells by macrophages (*79*), a hallmark feature of atherosclerosis. Several $sPLA_2$ isoforms are detectable in atherosclerotic plaques in humans and mice (*79*, *85*).

In 1999, the first experimental evidence for the potential role of $sPLA_2$ in atherosclerosis *in vivo* was obtained from *Pla2g2a*-Tg mice, which developed increased atherosclerotic lesions with slightly reduced plasma HDL levels following intake of a high-cholesterol atherogenic diet (*6*). Adoptive transfer of *Pla2g2a*-Tg bone marrow cells into recipient *Ldlr*$^{-/-}$ mice resulted in a significant increase in atherosclerotic lesions despite the absence of any alteration in lipoprotein composition, suggesting that local LDL modification in the vascular wall is sufficient for macrophage $sPLA_2$-IIA to drive the pro-atherogenic machinery (*86*). Recently, we showed that *Pla2g3*-Tg mice crossed with *ApoE*$^{-/-}$ mice, followed by supplementation with an atherogenic diet, developed more advanced atherosclerotic lesions than did *ApoE*$^{-/-}$ mice, accompanied by marked reductions of LDL and HDL as well as

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

increases in LPC-rich small-dense LDL and the pro-thrombotic TXA_2 (*79*). Interestingly, *Pla2g3*-Tg mice also developed systemic inflammation with increased age (*37*), suggesting that the elevated inflammatory state in the vascular wall may have an additional impact on the promotion of atherosclerosis in these mice. In addition, robust hydrolysis of plasma LDL and HDL was observed in *Pla2g10*-Tg mice (*87*).

Definitive evidence for the contribution of $sPLA_2$s to the development of atherosclerosis has been provided by studies using mice null for $sPLA_2$-V (*88*) and for $sPLA_2$-X (*89*). $Ldlr^{-/-}$ mice subjected to adoptive transfer of *Pla2g5*-transfected bone marrow cells showed increased atherosclerotic lesions with a concomitant elevation of regional collagen deposition, whereas replicate $Ldlr^{-/-}$ mice that underwent adoptive transfer of $Pla2g5^{-/-}$ bone marrow cells showed reduced atherosclerosis (*88*). This result indicates that $sPLA_2$-V in hematopoietic cells (most likely macrophages) does contribute to propagation of atherosclerosis. $sPLA_2$-X deficiency on an $ApoE^{-/-}$ background significantly reduced the incidence of angiotensin II-induced abdominal aortic aneurysm and atherosclerosis, accompanied by a reduction of pro-inflammatory mediators (*89*). Moreover, another study using $Pla2g10^{-/-}$ macrophages has provided another insight into the action of $sPLA_2$-X as a negative regulator of cholesterol efflux from macrophages through alteration of liver X receptor (LXR)-dependent expression of ABC transporters (*90*). Finally, in $ApoE^{-/-}$ mice fed a high-cholesterol diet, a pan-$sPLA_2$ inhibitor that dampens conventional $sPLA_2$s dramatically decreased atherosclerotic lesions, with an increase in plasma HDL (*91*). A phase II double-blind, placebo-controlled trial has demonstrated the efficacy of this inhibitor in patients with cardiovascular disease (*92*). Thus, the use of a $sPLA_2$ inhibitor elicits a promising reduction of biomarkers and effects on surrogate endpoints, encouraging further investigation of whether it can reduce cardiovascular disease events without any off-target toxicity.

$sPLA_2$ and metabolic syndrome

In contrast to the established roles of $sPLA_2$s in cardiovascular diseases as described above, their roles in metabolic syndrome including obesity, type 2 diabetes and insulin resistance have not yet been fully clarified. A recent tagging single-nucleotide polymorphism analysis has revealed an association of the human *PLA2G5*, but not *PLA2G2A*, gene haplotype with LDL and oxidized LDL levels in patients with type 2 diabetes (*93*). Susceptibility of very low-density lipoprotein (VLDL) and LDL to $sPLA_2$-V-promoted lipolysis has been shown to be higher in individuals with type 2 diabetes than in healthy controls (*94*). Thus, although the release of free fatty acids and LPC from diabetic LDL by $sPLA_2$-V may increase the risk of cardiovascular diseases, the above observations suggest that the $sPLA_2$-V-mediated hydrolysis of fat-overloaded, diabetic LDL might have another effect on systemic metabolic disorders by altering the hyperlipidemia state.

Systemic metabolic status is affected by glucocorticoid produced by the adrenal glands, in which $sPLA_2$-X is expressed. $Pla2g10^{-/-}$ mice have significantly higher plasma corticosterone levels than do wild-type mice (*95*). In contrast, overexpression of $sPLA_2$-X significantly reduces steroid production in cultured adrenal cells in a catalytic activity-dependent manner. Expression of steroidogenic acute regulatory protein (StAR), a rate-limiting protein in corticosteroid production, was higher in the adrenals of $Pla2g10^{-/-}$ mice than in wild-type mice, and LXR-dependent StAR promoter activation was attenuated by $sPLA_2$-X-released PUFAs. Thus, adrenal $sPLA_2$-X functions as a negative regulator of corticosteroid synthesis, likely through repression of the LXR–StAR axis. This series of studies also showed that $Pla2g10^{-/-}$ mice had increased obesity, which may be due to inhibition of the LXR-dependent lipogenic programme by $sPLA_2$-X in adipose tissue (*96*).

$sPLA_2$ and digestion

Obesity and associated metabolic syndrome are influenced by various factors, among which gastrointestinal lipid digestion/absorption is a causal factor that can be indirectly linked to adiposity. Phospholipids entering the digestive tract via food and bile comprise the second most abundant lipid class found in the intestinal lumen (*97*). Hydrolysis of phosphoplipids on the surface of lipid emulsion is required prior to digestion of triglycerides, a major lipid nutrient (*98*). The reduced lipid absorption efficiency culminates in protection against diet-induced obesity (*99*). For a long time, $sPLA_2$-IB, a 'pancreatic' $sPLA_2$, has been thought to play a role in digestion of dietary phospholipids. Indeed, under a high-fat/carbohydrate diabetogenic diet, the intestinal production and absorption of LPC, a hydrolytic product of dietary and biliary PC, was markedly reduced in $Pla2g1b^{-/-}$ mice. Accordingly, $Pla2g1b^{-/-}$ mice were protected from diet-induced obesity and hepatic glucose intolerance (*100*, *101*). Moreover, the *PLA2G1B* gene is mapped to an obesity susceptibility locus in humans (*102*). However, on a chow diet, intestinal phospholipid digestion in $Pla2g1b^{-/-}$ mice remained intact (*103*), suggesting that additional lipolytic enzyme(s) in the gut can compensate for the lack of $sPLA_2$-IB.

$sPLA_2$-X is expressed most abundantly in the gastrointestinal tract and reproductive organs (*104*). Consistent with its location, we recently found that gut $sPLA_2$-X represents the second 'digestive' $sPLA_2$ (*104*). Accordingly, reduced gastrointestinal phospholipid digestion in $Pla2g10^{-/-}$ mice receiving a chow diet was eventually linked to delayed onset of a lean phenotype with reduced adiposity, decreased plasma leptin and improved muscle insulin tolerance. Thus, $sPLA_2$-IB and $sPLA_2$-X, the two particular $sPLA_2$s that can be activated through proteolytic removal of the N-terminal propeptide (*1*), may play a compensatory role in phospholipid digestion in the gastroenteric lumen. Since $sPLA_2$-IB is abundantly secreted into the duodenum from the pancreas (*1*), while $sPLA_2$-X is constitutively expressed throughout the

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

gastrointestinal mucosa (*104*), these two 'digestive' $sPLA_2$s may spatiotemporally control the hydrolysis of dietary and biliary phospholipids and thereby absorption of their hydrolytic products, depending on the quantity and quality of dietary and biliary fat input. It is interesting to note that oral application of a pan-$sPLA_2$ inhibitor effectively suppressed diet-induced obesity and diabetes in mice, most probably by preventing the intestinal digestion of dietary and biliary PC by $sPLA_2$-IB and -X (*105*). This result raises the intriguing possibility that pharmacological inhibition of gastrointestinal $sPLA_2$s may be a potentially effective oral therapeutic option for treatment of diet-induced obesity and diabetes.

$sPLA_2$ and anti-microbial defence

The best-known physiologic function of $sPLA_2$-IIA is defence against bacterial infection through hydrolytic degradation of bacterial membrane phospholipids (*106*). Bacterial membranes are rich in PE and phosphatidylglycerol, which are excellent substrates for $sPLA_2$-IIA (*107*). The rank order of potency among $sPLA_2$s against Gram-positive bacteria is IIA > X > V > XIIA > IIE > IB, IIF (*7*). Thus, except for $sPLA_2$-X, which is an acidic enzyme, the bactericidal activity of $sPLA_2$s correlates with positive charges on their molecular surfaces. In fact, $sPLA_2$-IIA effectively kills Gram-positive bacteria and to a lesser extent Gram-negative bacteria *in vitro*, and importantly, *Pla2g2a*-Tg mice show decreased mortality following bacterial infection, associated with improved clearance of bacteria from their organs (*4*, *5*, *108*, *109*).

$sPLA_2$-V is localized in the Golgi and recycling endosomes in quiescent mouse peritoneal macrophages and is recruited to phagosomes upon ingestion of zymosan from the yeast cell wall (*110*). Peritoneal macrophages from *Pla2g5*$^{-/-}$ mice showed marked attenuation of zymosan phagocytosis, an event that was restored by adenoviral expression of $sPLA_2$-V. *Pla2g5*$^{-/-}$ macrophages showed a delay in phagocytosis, phagosome maturation and *Candida albicans* killing, independently of eicosanoid generation (*111*). Moreover, in a mouse model of systemic candidiasis, *Pla2g5*$^{-/-}$ mice showed increased mortality due to an increased fungal burden in multiple organs. Thus, $sPLA_2$-V contributes to the innate immune response against fungi by facilitating phagocytosis and killing. Although the molecular mechanism underlying the $sPLA_2$-V-mediated regulation of phagocytosis remains unknown, it may produce membrane-fusogenic lysophospholipids in membrane microdomains where fusion between the plasma membrane and the phagosome membrane proceeds.

Collectively, $sPLA_2$-IIA is an 'anti-bacterial' $sPLA_2$ that kills invading bacteria by directly degrading bacterial membranes, whereas $sPLA_2$-V works as an 'anti-fungal' $sPLA_2$ by facilitating macrophage phagocytosis of invading fungi. Since bacterial infection can occur anywhere in the body, marked induction of $sPLA_2$-IIA in the circulation as well as in local areas of inflammation or injury can be regarded as an innate defence response for clearance of invading bacteria. Since $sPLA_2$-V is expressed in alveolar macrophages and bronchial epithelial cells in the airways and urinary ducts of the kidney, where fungal infection frequently occurs in immunocompromised patients, the enzyme may contribute to the first line of innate immunity against fungi. In addition, at least *in vitro*, $sPLA_2$-III, -V and -X are capable of suppressing virus entry into host cells in a manner dependent upon the production of LPC from host cell membranes (*112*, *113*). Interestingly, *PLA2G3* gene polymorphisms are strongly associated with development of AIDS (*114*). Several $sPLA_2$s also elicit an anti-malaria action *in vitro*, which depends on hydrolysis of serum lipid components (probably lipoproteins) rather than direct interaction of the $sPLA_2$ with the infected erythrocyte membrane (*115*). Future work using knockout mice will clarify the anti-viral and anti-parasitic roles of $sPLA_2$s *in vivo*.

$sPLA_2$ and cancer

Natural *Pla2g2a*$^{-/-}$ mouse strains (due to a frameshift mutation in the *Pla2g2a* gene; see above) were susceptible to colorectal tumourigenesis (*2*), while introduction of the *Pla2g2a* transgene into C57BL/6 mice strongly suppressed it (*116*), leading to the proposal that $sPLA_2$-IIA has an anti-tumour role. Also in humans, $sPLA_2$-IIA expression in gastric adenocarcinoma is associated with prolonged survival and less frequent metastasis (*117*). These results suggest that $sPLA_2$-IIA confers resistance to gastrointestinal cancer in both mice and humans. There is a positive correlation between *PLA2G2A* expression and the oncogenic Wnt/β-catenin pathway (*118*). Mechanistically, gastrointestinal $sPLA_2$-IIA may alter enterobacterial flora through its anti-bacterial activity (see above), thereby negatively affecting tumour development. Conversely, $sPLA_2$-IIA may play a pro-tumourigenic role in other cancers such as prostate cancer, in which it may serve as a marker of highly proliferative carcinoma cells (*119*, *120*). Moreover, skin-specific *Pla2g2a*-Tg mice showed increased sensitivity to chemical carcinogenesis (*121*). Therefore, the pro- and anti-tumourigenic effects of $sPLA_2$-IIA appear to be tissue specific.

In several types of human cancer, $sPLA_2$-III is distributed in microvascular endothelial cells and tumour cells (*122*). Larger tumour xenografts were formed in nude mice implanted with $sPLA_2$-III-transfected colorectal adenocarcinoma cells than with mock-transfected control cells (*122*). Moreover, $sPLA_2$-III may be a good candidate biomarker for human colon cancer (*123*), and a *PLA2G3* haplotype is correlated with a higher risk of human colorectal cancer (*124*). The pathological relevance of the participation of $sPLA_2$-III in tumourigenesis should be addressed using *Pla2g3*$^{-/-}$ mice in the near future.

$sPLA_2$ and skin

The first phenotype reported in *Pla2g2a*-Tg mice was alopecia with no sign of inflammation (*3*). Although the physiological relevance of this finding was unclear

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

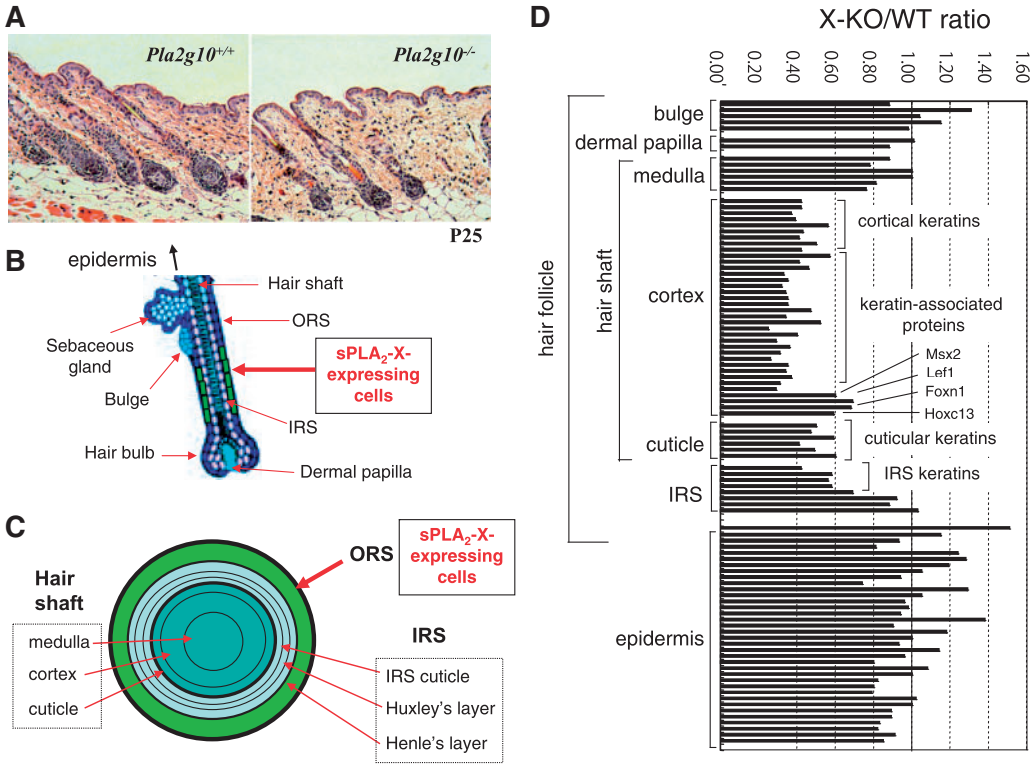

Fig. 5 Hair regulation by sPLA$_2$-X. (A) Histological examination of skin sections from *Pla2g10*$^{+/+}$ and *Pla2g10*$^{-/-}$ mice at P25 revealed a propensity for small hair follicles in the null mice, whereas other skin appendages including epidermis, sebaceous gland and dermis appeared normal. (B) Schematic illustration of a longitudinal section of a hair follicle. Endogenous sPLA$_2$-X is localized in the ORS of hair follicles (shown in green). ORS, outer root sheath; IRS, inner root sheath. (C) Schematic illustration of a cross-section of a hair follicle, which consists of seven layers of cells involving a single uppermost ORS layer, three layers (Henle, Huxley and IRS cuticle) of IRS, and the three innermost layers (cuticle, cortex and medulla) of the hair shaft. sPLA$_2$-X is located in the ORS in the anagen (growing) phase of the hair follicle. (D) Microarray gene profiling of skins from *Pla2g10*$^{+/+}$ and *Pla2g10*$^{-/-}$ mice at P25. Genes associated with hairs, but not those with bulge, dermal papilla and epidermis, are reduced by nearly half in *Pla2g10*$^{-/-}$ mice compared with *Pla2g10*$^{+/+}$ mice, suggesting that the null mice have a hair growth defect.

at that time since endogenous sPLA$_2$-IIA does not exist in mouse skin (*2*), it was suggested that there might be a certain endogenous sPLA$_2$ isoform that participates in regulation of hair growth. Hair follicle morphogenesis is governed by interactions between epidermal keratinocytes committed to hair follicle differentiation and dermal fibroblasts committed to forming the dermal papilla of the developing hair follicles (*125*). These epithelial–mesenchymal interactions culminate in the formation of the hair shaft, which is surrounded by the multi-layered inner root sheath (IRS) and outer root sheath (ORS), the latter being the outermost concentric layer of epithelial cells. Hair follicles undergo repeated cycles of growth (anagen), regression (catagen) and rest (telogen) during their life span. Distinct signalling pathways, including lipids, control hair follicle morphogenesis, postnatal hair growth and hair cycling in a coordinated manner.

We have recently found that sPLA$_2$-X was expressed in the ORS of hair follicles in synchrony with the anagen phase of hair cycling (*126*). *Pla2g10*-Tg mice, like *Pla2g2a*-Tg mice, displayed alopecia, along with epidermal hyperplasia and hair follicle distortion accompanied by reduced expression of genes related to hair development. Proteolytically activated sPLA$_2$-X in *Pla2g10*-Tg mouse skin showed preferential hydrolysis of PE with PUFAs, with concomitant production of PGE$_2$. Importantly, the skin of *Pla2g10*$^{-/-}$ mice had abnormal hair follicles in which a subset of hair genes was noticeably reduced, the ORS was hypoplasic, and melanin granules in the hair shaft medulla were decreased (Fig. 5). Unexpectedly, endogenous sPLA$_2$-X was not coupled with PGE$_2$ synthesis in hair follicles, but rather its deficiency resulted in aberrant cyclooxygenase-2 expression, leading to increased hair follicular PGE$_2$ synthesis and thereby to hair abnormality (*126*, *127*). These results reveal a novel role of sPLA$_2$-X in hair homoeostasis within a highly restricted and specialized skin compartment, the hair follicle.

sPLA$_2$ and reproduction

Various sPLA$_2$s are expressed in male reproductive organs at relatively high levels. In both mouse and human testes, sPLA$_2$-X and -IID are localized in the head and midpiece of elongating spermatids, respectively, and both sPLA$_2$-IIF and -V are distributed in interstitial Leydig cells (*128*). A high level of sPLA$_2$-IIA is detected in human seminal plasma (*129*), where the enzyme originates from the prostate epithelium and may act as an anti-microbial factor to protect this organ and spermatozoa against microbial invasion (*130*). sPLA$_2$-III is localized in testicular Sertoli cells

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

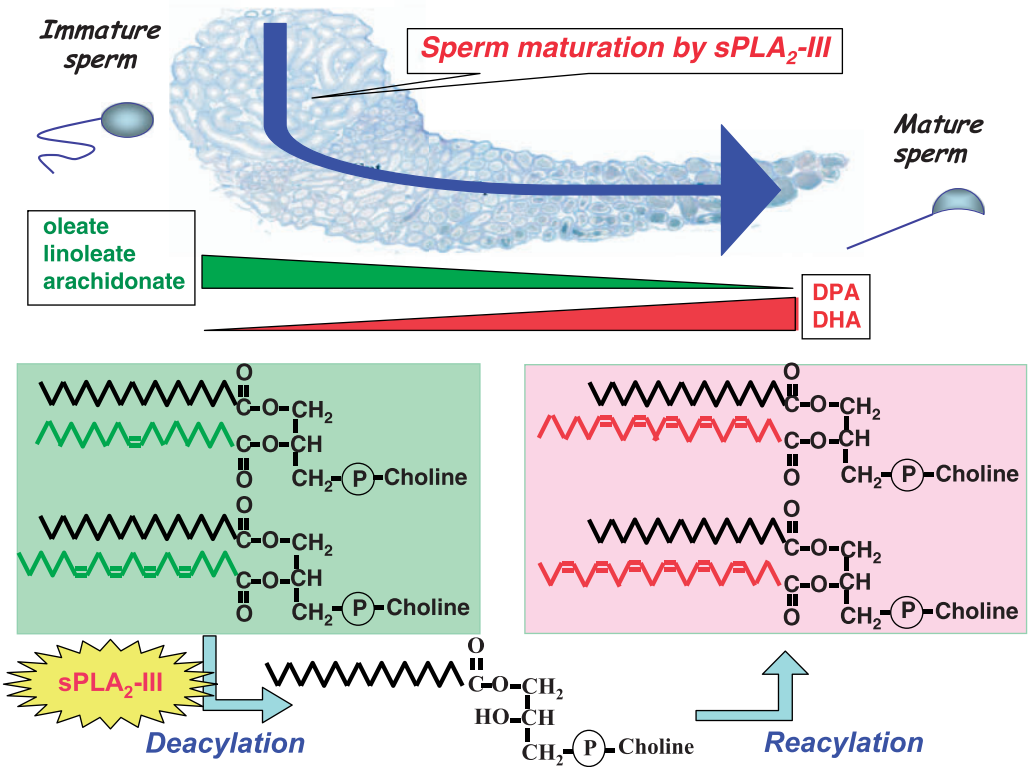

Fig. 6 The role of sPLA$_2$-III in epididymal sperm maturation. During their passage through the epididymis, immature sperm cells exiting from the testis undergo proper maturation towards mature motile spermatozoa. sPLA$_2$-III is expressed in the luminal epithelium in the caput (head) epididymidis and is secreted into the lumen, where it encounters immature sperm passing through the duct. During the sperm maturation process, PC in the wild-type sperm membrane undergoes a dramatic shift in its acyl groups from oleic acid, linoleic acid and AA to DPA and DHA, whereas this membrane lipid remodelling event is compromised in *Pla2g3*$^{-/-}$ sperm. Thus, sPLA$_2$-III may participate in the hydrolysis of PC with oleic acid, linoleic acid and AA in the sperm membrane during epididymal transit, and this event may be followed by reacylation of LPC with DHA and DPA, leading to an increase of PC with DPA/DHA in mature spermatozoa. In the *Pla2g3*$^{-/-}$ epididymis, impairment of the deacylation step may eventually perturb the subsequent reconstitution of DPA/DHA in the sperm membrane, culminating in the asthenozoospermia (sperm with low motility and abnormal morphology) phenotype.

and caput epididymal epithelial cells (*131*). These locations suggest that individual sPLA$_2$ isoforms play distinct roles in the gonadal microenvironments. Studies using sPLA$_2$ knockout mice have revealed that two particular sPLA$_2$s, sPLA$_2$-III and sPLA$_2$-X, play non-redundant roles in post-testicular sperm maturation and functions in the mouse male genital tract (*131*, *132*).

We have recently found that *Pla2g3*$^{-/-}$ mice displayed a striking defect in male, but not female, reproduction due to impaired sperm motility (*131*). This was because sperm maturation in the epididymis, but not spermatogenesis in the testis, was hampered in the null mice. During epididymal transit, PC in the sperm membrane undergoes a dramatic shift in its acyl groups from oleic acid, linoleic acid and AA to docosapentaenoic acid (DPA) and DHA, and this increased proportion of DHA consequently contributes to an increase of sperm membrane fluidity, and thereby motility (*133*–*137*). In *Pla2g3*$^{-/-}$ mice, this sperm membrane remodelling in the epididymis was severely compromised, and as a result, cauda epididymal spermatozoa from *Pla2g3*$^{-/-}$ mice had aberrant acrosomes and flagella with an abnormal axoneme configuration, and displayed hypomotility and reduced ability to fertilize intact eggs (*131*). This indicates that sPLA$_2$-III may facilitate the hydrolysis of PC with oleic acid, linoleic acid and AA in the sperm membrane during epididymal transit and that this event may be followed by reacylation of LPC with DHA and DPA, leading to an increase of PC with DPA/DHA in mature sperm (Fig. 6). In the *Pla2g3*$^{-/-}$ epididymis, preturbation of the deacylation step may alter the subsequent incorporation of DPA/DHA in the sperm membrane, resulting in the asthenozoospermia (spermatozoa with low motility and abnormal morphology) phenotype. Additionally, the gonads of *Pla2g3*$^{-/-}$ mice contained less 12/15-lipoxygenase metabolites than those of wild-type mice, implying preferential functional coupling of the gonadal sPLA$_2$-III with a specific arm (the 12/15-lipoxygenase pathway) of the PUFA-metabolic pathway (*131*).

It had been proposed that a certain germ cell-associated sPLA$_2$ might play a role in the sperm acrosome reaction by producing LPC (*138*–*140*). We found that sPLA$_2$-X was stored in acrosomes of mature spermatozoa and was released upon exocytosis (so-called 'acrosome reaction'), a key step essential for fertilization with oocytes (*132*). Although cauda epididymal spermatozoa from *Pla2g10*$^{-/-}$ mice showed normal motility, they exhibited a lower acrosome reaction that was associated with a decreased outcome of

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

in vitro fertilization, and accordingly, the litter size of male *Pla2g10*$^{-/-}$ mice was significantly smaller than that of wild-type mice (*132*). In agreement, the acrosome reaction of mouse spermatozoa was suppressed by addition of a pan-sPLA$_2$ inhibitor or an anti-sPLA$_2$-X antibody, an event that was reversed by addition of recombinant sPLA$_2$-X or its product LPC. Although a recent *in vitro* study showed that exogenous sPLA$_2$-X reduces sperm motility (*141*), the physiological relevance of this observation remains uncertain, since the motility of *Pla2g10*$^{-/-}$ sperm is identical to that of wild-type sperm (*104*).

Taken together, these two particular sPLA$_2$s (sPLA$_2$-III and -X), which are expressed in different locations within the male reproductive organs, exert non-redundant but interrelated functions in two major steps of male fertility; one during sperm maturation in the epididymis, in which sPLA$_2$-III plays a role in enrichment of DHA/DPA-rich mature sperm, and the other during capacitation and the acrosome reaction, likely after ejaculation into the uterus and oviduct, in which sPLA$_2$-X hydrolyses the DHA/DPA-rich sperm membrane to release LPC, which in turn promotes the acrosome reaction. As an additional note, sPLA$_2$-X is also localized in the endometrial epithelium in female reproductive organs (*104*). Considering that exogenous sPLA$_2$-X can powerfully boost the spontaneous acrosome reaction of spermatozoa (*132*), one may speculate that sPLA$_2$-X secreted from the endometrial epithelium into the lumen may assist, in a paracrine manner, the spontaneous acrosome reaction of sperm cells passing through the uterine duct. As such, sPLA$_2$-X boosts the premature acrosome reaction of a likely phospholipid-damaged sperm subpopulation to eliminate them from the fertilization race.

sPLA$_2$ and neurons

sPLA$_2$-X augments neuritogenesis through production of LPC in neuronal cell culture (*142*) suggesting its neuronal role. *In vivo*, sPLA$_2$-X is localized in ganglia or neuronal fibres in peripheral tissues of both mouse and human, and is found in both A-fibre and C-fibre neurons in mouse dorsal root ganglion (DRG) (*104*). *Ex vivo* neuritogenesis of DRG explants was slightly reduced in *Pla2g10*$^{-/-}$ mice and enhanced in *Pla2g10*-Tg mice relative to the respective controls. DRG isolated from E12.5 *Pla2g10*$^{-/-}$ foetus revealed reduced expression of several neuron-associated genes. Furthermore, the late phase of pain nociception, as assessed by the acetic acid writhing test, was significantly reduced in *Pla2g10*$^{-/-}$ mice and conversely augmented in *Pla2g10*-Tg mice, an event that appeared to be independent of the spinal levels of nociceptive PGs (*104*). Collectively, sPLA$_2$-X in certain neuronal microdomains may have a spatiotemporal effect on neuritogenesis and/or neuronal transmission leading to persistent pain nociception.

It is tempting to speculate that the neuronal location of sPLA$_2$-X in several peripheral tissues may underlie an alternative mechanism for the regulation of tissue-specific homoeostasis by this enzyme. For instance, localization of sPLA$_2$-X in neuronal fibres in male genital organs suggests that the enzyme might be involved in the autonomic nervous response for ejaculation. The expression of sPLA$_2$-X in ganglion cells of the myenteric plexus between smooth muscle cell fibres in the gastrointestinal tract (*142*, *143*) might be a reflection of its potential role in the peristaltic reflex controlled by the enteric nervous system, which could exert further influence on gastrointestinal lipid digestion and absorption. Finally, besides the reported negative regulatory role of sPLA$_2$-X in LXR-dependent metabolic processes in the adrenal glands and adipose tissues (*95*, *96*), the neuronal location of sPLA$_2$-X in adipose tissue might be related to the neuroendocrine regulation of adiposity.

sPLA$_2$-III is also expressed in neurons (*144*). In cultured neuronal cells, overexpression of sPLA$_2$-III facilitated neurite growth and survival, while knockdown of endogenous sPLA$_2$-III by siRNA suppressed these processes. The neuritogenic action of sPLA$_2$-III was correlated with the cellular level of LPC. Moreover, among the sPLA$_2$ members, sPLA$_2$-III showed the highest expression in mouse DRG, and *Pla2g3* siRNA-silenced DRG explants showed decreased axonal branching (*144*). The precise role of sPLA$_2$-III in neurons *in vivo* should be clarified using *Pla2g3*$^{-/-}$ mice.

More about sPLA$_2$s

The physiological roles of other group II subfamily sPLA$_2$ isoforms (IIC, IID, IIE and IIF) remain elusive, since mice with knockout, or those that are transgenic, for these enzymes have not yet been reported. sPLA$_2$-IIC is expressed in rodent testis, but is not expressed as a functional protein in humans (*145*). As mentioned above, sPLA$_2$-IID has been proposed to have an immunosuppressive function (*77*), but this awaits confirmation using *Pla2g2d*$^{-/-}$ mice. sPLA$_2$-IIE is expressed constitutively in several tissues at low levels, but its role is entirely unknown (*146*). sPLA$_2$-IIF has a unique C-terminal extension that contains an additional Cys residue, which might contribute to formation of a homodimer or a heterodimer with a second protein (*147*, *148*). This enzyme is expressed abundantly in the skin, suggesting its role in the epidermal barrier (unpublished results).

Group XII sPLA$_2$s (sPLA$_2$-XIIA and -XIIB) represent a unique collection of the sPLA$_2$ family. High expression of sPLA$_2$-XIIA, relative to other sPLA$_2$s, is found in many tissues (*149–151*). Interestingly, despite its weak enzymatic activity in a standard PLA$_2$ assay, sPLA$_2$-XIIA kills Gram-negative bacteria efficiently at least *in vitro* (*152*). A study using *Xenopus* suggests the role of this enzyme in early neuronal development (*153*). sPLA$_2$-XIIB is structurally related to sPLA$_2$-XIIA and is expressed in liver and intestine (*151*). A recent study has demonstrated that the expression of sPLA$_2$-XIIB was up-regulated by the transcription factor HNF-4α and its co-activator PGC-1α, and deletion of the *Pla2g12b* gene resulted in increased fat accumulation in the liver leading to steatohepatitis, a phenotype similar to that seen in *Hnf4a*$^{-/-}$ mice (*154*).

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

The aberrant fat accumulation in *Pla2g12b*$^{-/-}$ liver was due to impaired hepatic secretion of VLDL. However, since sPLA$_2$-XIIB lacks the catalytic activity due to replacement of the catalytic centre His with Leu (*151*), the molecular mechanism whereby this catalytically inactive sPLA$_2$ isoform regulates VLDL secretion remains unknown.

sPLA$_2$ receptor

Finally, we would like to offer some comments on the catalysis-independent functions of sPLA$_2$s. Over the last two decades, a number of membrane receptors and soluble binding proteins interacting with sPLA$_2$s have been described (*15*), although the non-enzymatic actions of sPLA$_2$s largely lack physiological evidence *in vivo*. Nevertheless, the presence of catalytically inactive or very weak sPLA$_2$s may indicate that some functions of sPLA$_2$s depend on their ligand-like action. Such examples include the expression of the catalytically weak or inactive group XII sPLA$_2$s at high levels in various tissues (see above), the activation of synovial cells by a catalytically inactive sPLA$_2$-IIA mutant (*155*, *156*), and the anti-inflammatory effect of a catalytically inactive sPLA$_2$-IID mutant when injected into mice (*77*). In addition, otoconin-90/95, another catalytically inactive sPLA$_2$-like protein that is most similar to sPLA$_2$-IB, acts as a structural component of otoconia (*157*). Knockout studies have shown that otoconin-90/95 is essential for the formation of the organic matrix of otoconia by specifically recruiting other matrix components, given that its deletion led to imbalance but normal hearing (*158*, *159*).

Clearly, the M-type sPLA$_2$ receptor (PLA2R1) is the best-known binding partner of various conventional sPLA$_2$s with different affinities (*160*, *161*). However, the biological roles of PLA2R1 are rather controversial and need more careful evaluation. In essence, PLA2R1 is a mammalian homologue of type α PLA$_2$ inhibitory protein (PLAI) in snakes. Both PLA2R1 and PLAI are made up of multiple C-type lectin domains, and their interaction with sPLA$_2$ leads to inhibition of enzymatic activity or biological effects (*162*, *163*). Furthermore, PLA2R1 internalizes the bound sPLA$_2$ into phagolysosomes, in which the enzyme is rapidly degraded (*164*, *165*). On the basis of these properties, it is likely that PLA2R1 plays a role in sequestration or clearance of sPLA$_2$s from biological fluids, and thereby termination of sPLA$_2$ actions. In contrast, PLA2R1 has also been proposed to participate in sPLA$_2$-evoked signalling including cell proliferation and/or pro-inflammatory effects (*166*). Such a pro-inflammatory role is supported by evidence that *Pla2r1*$^{-/-}$ mice are resistant to endotoxin shock (*167*). Alternatively, *Pla2r1*$^{-/-}$ mice might be protected from inflammation because of a defect in clearance of an immunosuppressive sPLA$_2$.

However, several features of PLA2R1 hold some questions regarding the signalling role of this protein. First, although various mouse sPLA$_2$s bind to mouse PLA2R1 with high to moderate affinity, this ligand specificity is not conserved in other animal species, including humans (*163*). This seems odd, since a ligand–receptor pair, as seen for a variety of cytokines, hormones, growth factors and neurotransmitters, is generally highly specific and well conserved among animal species. Exceptions include several classes of pattern recognition receptors, which broadly recognize microbial and host components (*168–170*). Second, a signalling receptor generally has a long cytoplasmic region bearing one or more signalling motifs, which are essential for interaction with downstream kinases or adaptor molecules. However, PLA2R1 possesses only a short stretch in the cytoplasmic tail, which does not contain any known signalling module except for a motif for endocytosis (*164*). With this structural property, it is difficult to envisage that PLA2R1 itself could act as a signalling receptor. The α chain of cytokine receptors with a short cytoplasmic region transmits a cytokine signal by forming a complex with β and/or γ receptor chains bearing a long cytoplasmic region containing signalling motifs (*171*). By analogy, the presence of the second, as yet unknown signal-transmitting subunit that could form a functional complex with PLA2R1 should be taken into consideration. Third, the most commonly observed signalling event associated with PLA2R1 is the activation of MAP kinases and NF-κB. However, the same signalling pathways are readily activated if the recombinant sPLA$_2$ preparations (mostly *Escherichia coli*-derived) are contaminated with even a tiny amount of bacterial components such as LPS or peptidoglycan, which activate Toll-like receptors (TLRs) (*168*, *169*). Hence, some previous studies might have erroneously reported the responses to contaminating LPS rather than to sPLA$_2$. Nonetheless, it is interesting to note that some C-type lectins, upon binding with their ligands, can act cooperatively with TLRs (*168–170*). For instance, mannose-binding lectin enhances TLR2/TLR6 signalling from the phagosome (*172*), dectin-1, another C-type lectin that recognizes the fungal wall, can collaborate with TLR2 to induce inflammatory responses (*173*), and CD36, another class of pattern recognition receptor that binds to polycationic ligands of both pathogen and self origin, promotes sterile inflammation through assembly of a TLR4/TLR6 heterodimer (*174*). By analogy, therefore, the sPLA$_2$–PLA2R1 interaction might be functionally linked to TLR signalling pathways in innate or adaptive immunity, leading to activation of MAP kinases and NF-κB and thereby to cell proliferation and inflammatory responses.

Recently, two novel roles of PLA$_2$R1 have been proposed (*175*, *176*). First, PLA2R1 has been found to control cellular senescence in human fibroblasts (*175*). PLA2R1 expression is increased during cellular senescence, and overexpression of the receptor promotes senescence while its knockdown prevents the onset of senescence. Interestingly, the expression of sPLA$_2$-IIA is also increased during senescence, and this enzyme appears to play a role in cellular senescence via PLA2R1. These findings suggest a novel role of the receptor as a tumour suppressor gene. Second, PLA$_2$R1 represents the main antigen target in idiopathic membranous nephropathy, an autoimmune human kidney disease (*176*). A majority of

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

Table II. Functions of $sPLA_2$s *in vivo* as revealed by gene-manipulated mice.

In vivo models	$sPLA_2$s	Localizations	Functions	Underlying mechanisms
Airway diseases	$sPLA_2$-V	Bronchial epithelium, DC/macrophages	Promotion	Antigen processing by DC, surfactant hydrolysis
	$sPLA_2$-X	Alveolar epithelium	Promotion	Production of cysLTs, surfactant hydrolysis?
Arthritis	$sPLA_2$-IIA	Synovial cells, chondrocytes	Promotion	Production of PGE_2?
	$sPLA_2$-V	Macrophages	Protection	Clearance of pathogenic immune complex
Myocardial ischemia	$sPLA_2$-V	Myocardiocytes	Promotion	Production of LTB_4 and TXA_2
	$sPLA_2$-X	Neutrophils	Promotion	Production of LTB_4, respiratory burst
Atherosclerosis	$sPLA_2$-V	Atherosclerotic lesion	Promotion	Production of atherogenic small-dense LDL
	$sPLA_2$-X	Atherosclerotic lesion	Promotion	Production of atherogenic small-dense LDL
	$sPLA_2$-IIA	Atherosclerotic lesion	Promotion	Production of atherogenic small-dense LDL
	$sPLA_2$-III	Atherosclerotic lesion	Promotion	Production of atherogenic small-dense LDL
Obesity	$sPLA_2$-X	Adipose tissue, adrenal gland	Protection	PUFA-dependent LXR repression
		Gastrointestinal tract	Promotion	Gastrointestinal phospholipid digestion
	$sPLA_2$-IB	Pancreas	Promotion	Gastrointestinal phospholipid digestion
Cancer	$sPLA_2$-IIA	Intestinal Paneth cells, gastric gland	Protection	Maintenance of intestinal microflora?
Microbial defence	$sPLA_2$-IIA	Various tissues after infection	Protection	Degradation of bacterial membranes
	$sPLA_2$-V	Macrophages	Protection	Phagocytotic killing of fungi
Alopecia	$sPLA_2$-X	Hair follicular ORS	Promotion	ORS cell growth
Reproduction	$sPLA_2$-III	Epididymal epithelium	Promotion	Sperm maturation by membrane remodeling
	$sPLA_2$-X	Sperm acrosome	Promotion	Sperm acrosome reaction by LPC
Pain	$sPLA_2$-X	DRG neurons	Promotion	Neuritogenesis by LPC
Steatohepatitis	$sPLA_2$-XIIB	Liver, intestine	Protection	VLDL secretion

DC, dendritic cells.

patients with idiopathic membranous nephropathy have serum antibodies against a conformation-dependent epitope in PLA_2R1. The receptor is present in glomerular podocytes in human adult kidney and is likely to be the target of human IgG_4 immunoglobulin that specifically recognizes the receptor. The binding of the autoantibodies to PLA_2R1 triggers an unknown cellular signalling cascade that eventually leads to podocyte dysfunction.

Concluding remarks

By the 1980s, research on $sPLA_2$ had become central to the field of lipid biochemistry. After the discovery of $cPLA_2\alpha$ in the early 1990s, however, the main interest of researchers shifted to the regulatory functions of this enzyme, as well as other newly identified intracellular PLA_2s, whereas $sPLA_2$s no longer represented the mainstream of PLA_2 research. Despite this, efforts by several groups including ours over the past decade have prompted reassessment of the biological functions of $sPLA_2$s and their target substrates using transgenic/knockout mice and specific inhibitors, in combination with the newly developed, highly sensitive, lipidomics technology. It has become apparent that individual $sPLA_2$s play important and diverse roles in various biological events, often acting through 'classical' mechanisms involving lipid mediators, and in other cases probably through 'alternative' mechanisms that are dependent upon their unique actions on extracellular phospholipid targets.

We are currently on the threshold of a new era of $sPLA_2$ biology. Although a growing body of evidence from transgenic or knockout studies has delineated unique roles of individual $sPLA_2$s in various pathophysiological events, in many cases the mechanistic insights are still speculative and incomplete. Continued efforts to analyse $sPLA_2$ gene-manipulated mice in the context of pathology, histology, molecular biology and biochemistry, in combination with lipidomics techniques and informatics, will shed further light on the true functional aspects of $sPLA_2$s including their target substrates and products associated with particular biological events. It is interesting that two or more $sPLA_2$s can function in a given pathophysiological event in a cooperative, complementary or counter-regulatory manner (Table II). Therefore, it seems logical that the control of particular $sPLA_2$s, alone or through a combination of multiple isoforms, would be advantageous for inhibition of selective lipid pathways in the treatment of various diseases. Currently, the pan-$sPLA_2$ inhibitor varespladib, which inhibits the conventional class of $sPLA_2$s, can markedly reduce the area of atherosclerotic lesions in experimental animals, and even in humans in early-phase clinical studies (*92*). These facts point to the $sPLA_2$ family as a potential therapeutic target for atherosclerosis, and probably other diseases in which one or more $sPLA_2$s are involved, such as asthma, arthritis and metabolic syndrome among others. Hopefully, given all these basic and clinical standpoints, the upcoming full elucidation of the $sPLA_2$ networks underlying each pathophysiology will ensure that $sPLA_2$s return to the centre stage of biomedical science in the next decade.

Funding

Grants-in aid for scientific research from the Ministry of Education, Science, Culture, Sports and Technology of Japan. Toray Foundation (to M.M), the Mitsubishi Science Foundation, the Terumo Life Science Foundation and the Uehara Memorial Foundation. In the interest of brevity, we have referenced other reviews whenever possible and apologize to the authors of the numerous primary papers that were not explicitly cited.

Conflict of interest

None declared.

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

References

1. Murakami, M. and Kudo, I. (2002) Phospholipase A_2. *J. Biochem.* **131**, 285–292
2. MacPhee, M., Chepenik, P.K., Liddel, A.R., Nelson, K.K., Siracusa, D.L., and Buchberg, M.A. (1995) The secretory phospholipase A_2 gene is a candidate for the *mom1* locus, a major modifier of Apc^{min}-induced intestinal neoplasia. *Cell* **81**, 957–966
3. Grass, D.S., Felkner, R.H., Chiang, M.Y., Wallace, R.E., Nevalainen, T.J., Bennett, C.F., and Swanson, M.E. (1996) Expression of human group II PLA_2 in transgenic mice results in epidermal hyperplasia in the absence of inflammatory infiltrate. *J. Clin. Invest.* **97**, 2233–2241
4. Weinrauch, Y., Elsbach, P., Madsen, L.M., Foreman, A., and Weiss, J. (1996) The potent anti-*Staphylococcus aureus* activity of a sterile rabbit inflammatory fluid is due to a 14-kD phospholipase A_2. *J. Clin. Invest.* **97**, 250–257
5. Laine, V.J., Grass, D.S., and Nevalainen, T.J. (1999) Protection by group II phospholipase A_2 against *Staphylococcus aureus*. *J. Immunol.* **162**, 7402–7408
6. Ivandic, B., Castellani, L.W., Wang, X.P., Qiao, J.H., Mehrabian, M., Navab, M., Fogelman, A.M., Grass, D.S., Swanson, M.E., de Beer, M.C., de Beer, F., and Lusis, A.J. (1999) Role of group II secretory phospholipase A_2 in atherosclerosis: 1. Increased atherogenesis and altered lipoproteins in transgenic mice expressing group IIa phospholipase A_2. *Arterioscler. Thromb. Vasc. Biol.* **19**, 1284–1290
7. Koduri, R.S., Gronroos, J.O., Laine, V.J., Le Calvez, C., Lambeau, G., Nevalainen, T.J., and Gelb, M.H. (2002) Bactericidal properties of human and murine groups I, II, V, X, and XII secreted phospholipases A_2. *J. Biol. Chem.* **277**, 5849–5857
8. Uozumi, N., Kume, K., Nagase, T., Nakatani, N., Ishii, S., Tashiro, F., Komagata, Y., Maki, K., Ikuta, K., Ouchi, Y., Miyazaki, J., and Shimizu, T. (1997) Role of cytosolic phospholipase A_2 in allergic response and parturition. *Nature* **390**, 618–622
9. Bonventre, J.V., Huang, Z., Taheri, M.R., O'Leary, E., Li, E., Moskowitz, M.A., and Sapirstein, A. (1997) Reduced fertility and postischaemic brain injury in mice deficient in cytosolic phospholipase A_2. *Nature* **390**, 622–625
10. Takaku, K., Sonoshita, M., Sasaki, N., Uozumi, N., Doi, Y., Shimizu, T., and Taketo, M.M. (2000) Suppression of intestinal polyposis in $Apc^{Delta716}$ knockout mice by an additional mutation in the cytosolic phospholipase A_2 gene. *J. Biol. Chem.* **275**, 34013–34016
11. Clark, J.D., Lin, L.L., Kriz, R.W., Ramesha, C.S., Sultzman, L.A., Lin, A.Y., Milona, N., and Knopf, J.L. (1991) A novel arachidonic acid-selective cytosolic PLA_2 contains a Ca^{2+}-dependent translocation domain with homology to PKC and GAP. *Cell* **65**, 1043–1051
12. Lin, L.L., Wartmann, M., Lin, A.Y., Knopf, J.L., Seth, A., and Davis, R.J. (1993) $cPLA_2$ is phosphorylated and activated by MAP kinase. *Cell* **72**, 269–278
13. Murakami, M., Taketomi, Y., Miki, Y., Sato, H., Hirabayashi, T., and Yamamoto, K. (2011) Recent progress in phospholipase A_2 research: from cells to animals to humans. *Prog. Lipid Res.* **50**, 152–192
14. Murakami, M., Taketomi, Y., Girard, G., Yamamoto, K., and Lambeau, G. (2010) Emerging roles of secreted phospholipase A_2 enzymes: lessons from transgenic and knockout mice. *Biochimie* **92**, 561–582
15. Lambeau, G. and Gelb, M.H. (2008) Biochemistry and physiology of mammalian secreted phospholipases A_2. *Annu. Rev. Biochem.* **77**, 495–520
16. Das, S., Rafter, J.D., Kim, K.P., Gygi, S.P., and Cho, W. (2003) Mechanism of group IVA cytosolic phospholipase A_2 activation by phosphorylation. *J. Biol. Chem.* **278**, 41431–41442
17. Lamour, N.F., Subramanian, P., Wijesinghe, D.S., Stahelin, R.V., Bonventre, J.V., and Chalfant, C.E. (2009) Ceramide 1-phosphate is required for the translocation of group IVA cytosolic phospholipase A_2 and prostaglandin synthesis. *J. Biol. Chem.* **284**, 26897–26907
18. Tucker, D.E., Ghosh, M., Ghomashchi, F., Loper, R., Suram, S., John, B.S., Girotti, M., Bollinger, J.G., Gelb, M.H., and Leslie, C.C. (2009) Role of phosphorylation and basic residues in the catalytic domain of cytosolic phospholipase $A_2\alpha$ in regulating interfacial kinetics and binding and cellular function. *J. Biol. Chem.* **284**, 9596–9611
19. San Pietro, E., Capestrano, M., Polishchuk, E.V., DiPentima, A., Trucco, A., Zizza, P., Mariggiò, S., Pulvirenti, T., Sallese, M., Tete, S., Mironov, A.A., Leslie, C.C., Corda, D., Luini, A., and Polishchuk, R.S. (2009) Group IV phospholipase $A_2\alpha$ controls the formation of inter-cisternal continuities involved in intra-Golgi transport. *PLoS Biol.* **7**, e1000194
20. Bao, S., Miller, D.J., Ma, Z., Wohltmann, M., Eng, G., Ramanadham, S., Moley, K., and Turk, J. (2004) Male mice that do not express group VIA phospholipase A_2 produce spermatozoa with impaired motility and have greatly reduced fertility. *J. Biol. Chem.* **279**, 38194–38200
21. Bao, S., Jacobson, D.A., Wohltmann, M., Bohrer, A., Jin, W., Philipson, L.H., and Turk, J. (2008) Glucose homeostasis, insulin secretion, and islet phospholipids in mice that overexpress $iPLA_2\beta$ in pancreatic β-cells and in $iPLA_2\beta$-null mice. *Am. J. Physiol. Endocrinol. Metab.* **294**, E217–E229
22. Morgan, N.V., Westaway, S.K., Morton, J.E., Gregory, A., Gissen, P., Sonek, S., Cangul, H., Coryell, J., Canham, N., Nardocci, N., Zorzi, G., Pasha, S., Rodriguez, D., Desguerre, I., Mubaidin, A., Bertini, E., Trembath, R.C., Simonati, A., Schanen, C., Johnson, C.A., Levinson, B., Woods, C.G., Wilmot, B., Kramer, P., Gitschier, J., Maher, E.R., and Hayflick, S.J. (2006) *PLA2G6*, encoding a phospholipase A_2, is mutated in neurodegenerative disorders with high brain iron. *Nature Genet.* **38**, 752–754
23. Mancuso, D.J., Sims, H.F., Han, X., Jenkins, C.M., Guan, S.P., Yang, K., Moon, S.H., Pietka, T., Abumrad, N.A., Schlesinger, P.H., and Gross, R.W. (2007) Genetic ablation of calcium-independent phospholipase $A_2\gamma$ leads to alterations in mitochondrial lipid metabolism and function resulting in a deficient mitochondrial bioenergetic phenotype. *J. Biol. Chem.* **282**, 34611–34622
24. Mancuso, D.J., Kotzbauer, P.T., Wozniak, D.F., Sims, H.F., Jenkins, C.M., Guan, S., Han, X., Yang, K., Sun, G., Malik, I., Conyers, S., Green, K.G., Schmidt, R.E., and Gross, R.W. (2009) Genetic ablation of calcium-independent phospholipase $A_2\gamma$ leads to alterations in hippocampal cardiolipin content and molecular species distribution, mitochondrial degeneration, autophagy and cognitive dysfunction. *J. Biol. Chem.* **284**, 35632–35644
25. Haemmerle, G., Lass, A., Zimmermann, R., Gorkiewicz, G., Meyer, C., Rozman, J., Heldmaier, G., Maier, R., Theussl, C., Eder, S., Kratky, D., Wagner, E.F.,

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

Klingenspor, M., Hoefler, G., and Zechner, R. (2006) Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. *Science* **312**, 734–737

26. Romeo, S., Kozlitina, J., Xing, C., Pertsemlidis, A., Cox, D., Pennacchio, L.A., Boerwinkle, E., Cohen, J.C., and Hobbs, H.H. (2008) Genetic variation in *PNPLA3* confers susceptibility to nonalcoholic fatty liver disease. *Nat. Genet.* **40**, 1461–1465
27. Akassoglou, K., Malester, B., Xu, J., Tessarollo, L., Rosenbluth, J., and Chao, M.V. (2004) Brain-specific deletion of neuropathy target esterase/swisscheese results in neurodegeneration. *Proc. Natl Acad. Sci. USA* **101**, 5075–5080
28. Ghosh, M., Tucker, D.E., Burchett, S.A., and Leslie, C.C. (2006) Properties of the Group IV phospholipase A_2 family. *Prog. Lipid Res.* **45**, 487–510
29. Kienesberger, P.C., Oberer, M., Lass, A., and Zechner, R. (2009) Mammalian patatin domain containing proteins: a family with diverse lipolytic activities involved in multiple biological functions. *J. Lipid Res.* **50**, S63–68
30. Mallat, Z., Lambeau, G., and Tedgui, A. (2010) Lipoprotein-associated and secreted phospholipases A_2 in cardiovascular disease: roles as biological effectors and biomarkers. *Circulation* **122**, 2183–2200
31. Bezzine, S., Koduri, R.S., Valentin, E., Murakami, M., Kudo, I., Ghomashchi, F., Sadilek, M., Lambeau, G., and Gelb, M.H. (2000) Exogenously added human group X secreted phospholipase A_2 but not the group IB, IIA, and V enzymes efficiently release arachidonic acid from adherent mammalian cells. *J. Biol. Chem.* **275**, 3179–3191
32. Murakami, M., Shimbara, S., Kambe, T., Kuwata, H., Winstead, M.V., Tischfield, J.A., and Kudo, I. (1998) The functions of five distinct mammalian phospholipase A_2s in regulating arachidonic acid release. Type IIA and type V secretory phospholipases A_2 are functionally redundant and act in concert with cytosolic phospholipase A_2. *J. Biol. Chem.* **273**, 14411–14423
33. Murakami, M., Kambe, T., Shimbara, S., Higashino, K., Hanasaki, K., Arita, H., Horiguchi, M., Arita, M., Arai, H., Inoue, K., and Kudo, I. (1999) Different functional aspects of the group II subfamily (Types IIA and V) and type X secretory phospholipase A_2s in regulating arachidonic acid release and prostaglandin generation. Implications of cyclooxygenase-2 induction and phospholipid scramblase-mediated cellular membrane perturbation. *J. Biol. Chem.* **274**, 31435–31444
34. Murakami, M., Koduri, R.S., Enomoto, A., Shimbara, S., Seki, M., Yoshihara, K., Singer, A., Valentin, E., Ghomashchi, F., Lambeau, G., Gelb, M.H., and Kudo, I. (2001) Distinct arachidonate-releasing functions of mammalian secreted phospholipases A_2 in fibroblastic and mastocytoma cells through heparan sulfate shuttling and external plasma membrane mechanisms. *J. Biol. Chem.* **276**, 10083–10096
35. Murakami, M., Masuda, S., Shimbara, S., Bezzine, S., Lazdunski, M., Lambeau, G., Gelb, M.H., Matsukura, S., Kokubu, F., Adachi, M., and Kudo, I. (2003) Cellular arachidonate-releasing function of novel classes of secretory phospholipase A_2s (groups III and XII). *J. Biol. Chem.* **278**, 10657–10667
36. Murakami, M., Kambe, T., Shimbara, S., Yamamoto, S., Kuwata, H., and Kudo, I. (1999) Functional association of type IIA secretory phospholipase A_2 with the glycosylphosphatidylinositol-anchored heparan sulfate proteoglycan in the cyclooxygenase-2-mediated delayed prostanoid-biosynthetic pathway. *J. Biol. Chem.* **274**, 29927–29936
37. Sato, H., Taketomi, Y., Isogai, Y., Masuda, S., Kobayashi, T., Yamamoto, K., and Murakami, M. (2009) Group III secreted phospholipase A_2 transgenic mice spontaneously develop inflammation. *Biochem. J.* **421**, 17–27
38. Yamamoto, K., Taketomi, T., Isogai, Y., Miki, Y., Sato, H., Masuda, S., Nishito, Y., Morioka, K., Ishimoto, Y., Suzuki, N., Yokoya, Y., Hanasaki, K., Ishikawa, Y., Ishii, T., Kobayashi, T., Fukami, K., Ikeda, K., Nakanishi, H., Taguchi, R., and Murakami, M. (2011) Hair follicular expression and function of group X secreted phospholipase A_2 in mouse skin. *J. Biol. Chem.* **286**, 11616–11631
39. Satake, Y., Diaz, B.L., Balestrieri, B., Lam, B.K., Kanaoka, Y., Grusby, M.J., and Arm, J.P. (2004) Role of group V phospholipase A_2 in zymosan-induced eicosanoid generation and vascular permeability revealed by targeted gene disruption. *J. Biol. Chem.* **279**, 16488–16494
40. Diaz, B.L., Satake, Y., Kikawada, E., Balestrieri, B., and Arm, J.P. (2006) Group V secretory phospholipase A_2 amplifies the induction of cyclooxygenase 2 and delayed prostaglandin D_2 generation in mouse bone marrow culture-derived mast cells in a strain-dependent manner, *Biochim. Biophys. Acta* **1761**, 1489–1497
41. Fujioka, D., Saito, Y., Kobayashi, T., Yano, T., Tezuka, H., Ishimoto, Y., Suzuki, N., Yokota, Y., Nakamura, T., Obata, J.E., Kanazawa, M., Kawabata, K., Hanasaki, K., and Kugiyama, K. (2008) Reduction in myocardial ischemia/reperfusion injury in group X secretory phospholipase A_2-deficient mice. *Circulation* **117**, 2977–2985
42. Kim, Y.J., Kim, K.P., Han, S.K., Munoz, N.M., Zhu, X., Sano, H., Leff, A.R., and Cho, W. (2002) Group V phospholipase A_2 induces leukotriene biosynthesis in human neutrophils through the activation of group IVA phospholipase A_2. *J. Biol Chem.* **277**, 36479–36488
43. Lai, Y., Oslund, R.C., Bollinger, J.G., Henderson, W.R. Jr., Santana, L.F., Altemeier, W.A., Gelb, M.H., and Hallstrand, T.S. (2010) Eosinophil cysteinyl leukotriene synthesis mediated by exogenous secreted phospholipase A_2 group X. *J. Biol Chem.* **285**, 41491–41500
44. Kikawada, E., Bonventre, J.V., and Arm, J.P. (2007) Group V secretory PLA_2 regulates TLR2-dependent eicosanoid generation in mouse mast cells through amplification of ERK and $cPLA_2\alpha$ activation. *Blood* **110**, 561–567
45. Ohtsuki, M., Taketomi, Y., Arata, S., Masuda, S., Ishikawa, Y., Ishii, T., Takanezawa, Y., Aoki, J., Arai, H., Yamamoto, K., Kudo, I., and Murakami, M. (2006) Transgenic expression of group V, but not group X, secreted phospholipase A_2 in mice leads to neonatal lethality because of lung dysfunction. *J. Biol. Chem.* **281**, 36420–36433
46. Curfs, D.M., Ghesquiere, S.A., Vergouwe, M.N., van der Made, I., Gijbels, M.J., Greaves, D.R., Verbeek, J.S., Hofker, M.H., and de Winther, M.P. (2008) Macrophage secretory phospholipase A_2 group X enhances anti-inflammatory responses, promotes lipid accumulation, and contributes to aberrant lung pathology. *J. Biol. Chem.* **283**, 21640–21648
47. Touqui, L. and Alaoui-El-Azher, M. (2001) Mammalian secreted phospholipases A_2 and their pathophysiological

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

significance in inflammatory diseases. *Curr. Mol. Med.* **1**, 739–754

48. Furue, S., Mikawa, K., Nishina, K., Shiga, M., Ueno, M., Tomita, Y., Kuwabara, K., Teshirogi, I., Ono, T., Hori, Y., Matsukawa, A., Yoshinaga, M., and Obara, H. (2001) Therapeutic time-window of a group IIA phospholipase A_2 inhibitor in rabbit acute lung injury: correlation with lung surfactant protection. *Crit. Care Med.* **2**, 9719–9727
49. Munoz, N.M., Meliton, A.Y., Arm, J.P., Bonventre, J.V., Cho, W., and Leff, A.R. (2007) Deletion of secretory group V phospholipase A_2 attenuates cell migration and airway hyperresponsiveness in immunosensitized mice. *J. Immunol.* **179**, 4800–4807
50. Masuda, S., Murakami, M., Mitsuishi, M., Komiyama, K., Ishikawa, Y., Ishii, T., and Kudo, I. (2005) Expression of secretory phospholipase A_2 enzymes in lungs of humans with pneumonia and their potential prostaglandin-synthetic function in human lung-derived cells. *Biochem. J.* **387**, 27–38
51. Hallstrand, T.S., Chi, E.Y., Singer, A.G., Gelb, M.H., and Henderson, W.R. Jr. (2007) Secreted phospholipase A_2 group X overexpression in asthma and bronchial hyperresponsiveness. *Am. J. Respir. Crit. Care Med.* **176**, 1072–1078
52. Seeds, M.C., Jones, K.A., Duncan Hite, R., Willingham, M.C., Borgerink, H.M., Woodruff, R.D., Bowton, D.L., and Bass, D.A. (2000) Cell-specific expression of group X and group V secretory phospholipases A_2 in human lung airway epithelial cells. *Am. J. Respir. Cell. Mol. Biol.* **23**, 37–44
53. Henderson, W.R. Jr., Chi, E.Y., Bollinger, J.G., Tien, Y.T., Ye, X., Castelli, L., Rubtsov, Y.P., Singer, A.G., Chiang, G.K., Nevalainen, T., Rudensky, A.Y., and Gelb, M.H. (2007) Importance of group X-secreted phospholipase A_2 in allergen-induced airway inflammation and remodeling in a mouse asthma model. *J. Exp. Med.* **204**, 865–877
54. Munoz, N.M., Meliton, A.Y., Meliton, L.N., Dudek, S.M., and Leff, A.R. (2009) Secretory group V phospholipase A_2 regulates acute lung injury and neutrophilic inflammation caused by LPS in mice. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **296**, L879–L887
55. Giannattasio, G., Fujioka, D., Xing, W., Katz, H.R., Boyce, J.A., and Balestrieri, B. (2010) Group V secretory phospholipase A_2 reveals its role in house dust mite-induced allergic pulmonary inflammation by regulation of dendritic cell function. *J. Immunol.* **185**, 4430–4438
56. Seilhamer, J.J., Pruzanski, W., Vadas, P., Plant, S., Miller, J.A., Kloss, J., and Johnson, L.K. (1989) Cloning and recombinant expression of phospholipase A_2 present in rheumatoid arthritic synovial fluid. *J. Biol. Chem.* **264**, 5335–5338
57. Kramer, R.M., Hession, C., Johansen, B., Hayes, G., McGray, P., Chow, E.P., Tizard, R., and Pepinsky, R.B. (1989) Structure and properties of a human non-pancreatic phospholipase A_2. *J. Biol. Chem.* **264**, 5768–5775
58. Pruzanski, W. and Vadas, P. (1991) Phospholipase A_2 - a mediator between proximal and distal effectors of inflammation. *Immunol. Today* **12**, 143–146
59. Boilard, E., Lai, Y., Larabee, K., Balestrieri, B., Ghomashchi, F., Fujioka, D., Gobezie, R., Coblyn, J.S., Weinblatt, M.E., Massarotti, E.M., Thornhill, T.S., Divangahi, M., Remold, H., Lambeau, G., Gelb, M.H., Arm, J.P., and Lee, D.M. (2010) A novel anti-inflammatory role for secretory phospholipase A_2 in immune complex-mediated arthritis. *EMBO Mol. Med.* **2**, 172–187
60. Hegen, M., Sun, L., Uozumi, N., Kume, K., Goad, M.E., Nickerson-Nutter, C.L., Shimizu, T., and Clark, J.D. (2003) Cytosolic phospholipase $A_2\alpha$-deficient mice are resistant to collagen-induced arthritis. *J. Exp. Med.* **197**, 1297–1302
61. Honda, T., Segi-Nishida, E., Miyachi, Y., and Narumiya, S. (2006) Prostacyclin-IP signaling and prostaglandin E_2-EP2/EP4 signaling both mediate joint inflammation in mouse collagen-induced arthritis. *J. Exp. Med.* **203**, 325–335
62. McCoy, J.M., Wicks, J.R., and Audoly, L.P. (2002) The role of prostaglandin E_2 receptors in the pathogenesis of rheumatoid arthritis. *J. Clin. Invest.* **110**, 651–658
63. Kim, N.D., Chou, R.C., Seung, E., Tager, A.M., and Luster, A.D. (2006) A unique requirement for the leukotriene B_4 receptor BLT1 for neutrophil recruitment in inflammatory arthritis. *J. Exp. Med.* **203**, 829–835
64. Mathis, S.P., Jala, V.R., Lee, D.M., and Haribabu, B. (2010) Nonredundant roles for leukotriene B_4 receptors BLT1 and BLT2 in inflammatory arthritis. *J. Immunol.* **185**, 3049–3056
65. Bradley, J.D., Dmitrienko, A.A., Kivitz, A.J., Gluck, O.S., Weaver, A.L., Wiesenhutter, C., Myers, S.L., and Sides, G.D. (2005) A randomized, double-blinded, placebo-controlled clinical trial of LY333013, a selective inhibitor of group II secretory phospholipase A_2, in the treatment of rheumatoid arthritis. *J. Rheumatol.* **32**, 417–423
66. Lapointe, S., Brkovic, A., Cloutier, I., Tanguay, J.F., Arm, J.P., and Sirois, M.G. (2010) Group V secreted phospholipase A_2 contributes to LPS-induced leukocyte recruitment. *J. Cell Physiol.* **224**, 127–134
67. Degousee, N., Ghomashchi, F., Stefanski, E., Singer, A., Smart, B.P., Borregaard, N., Reithmeier, R., Lindsay, T.F., Lichtenberger, C., Reinisch, W., Lambeau, G., Arm, J., Tischfield, J., Gelb, M.H., and Rubin, B.B. (2002) Groups IV, V, and X phospholipases A_2s in human neutrophils: role in eicosanoid production and Gram-negative bacterial phospholipid hydrolysis. *J. Biol. Chem.* **277**, 5061–5073
68. Chen, J., Engle, S.J., Seilhamer, J.J., and Tischfield, J.A. (1994) Cloning and recombinant expression of a novel human low molecular weight Ca^{2+}-dependent phospholipase A_2. *J. Biol. Chem.* **269**, 2365–2368
69. Masuda, S., Murakami, M., Ishikawa, Y., Ishii, T., and Kudo, I. (2005) Diverse cellular localizations of secretory phospholipase A_2 enzymes in several human tissues. *Biochim. Biophys. Acta* **1736**, 200–210
70. Yano, T., Fujioka, D., Saito, Y., Kobayashi, T., Nakamura, T., Obata, J.E., Kawabata, K., Watanabe, K., Watanabe, Y., Mishina, H., Tamaru, S., and Kugiyama, K. (2011) Group V secretory phospholipase A_2 plays a pathogenic role in myocardial ischaemia-reperfusion injury. *Cardiovasc. Res.* **90**, 335–343
71. Xiao, C.Y., Hara, A., Yuhki, K., Fujino, T., Ma, H., Okada, Y., Takahata, O., Yamada, T., Murata, T., Narumiya, S., and Ushikubi, F. (2001) Roles of prostaglandin I_2 and thromboxane A_2 in cardiac ischemia-reperfusion injury: a study using mice lacking their respective receptors. *Circulation* **104**, 2210–2215
72. Odegaard, J.I., Ricardo-Gonzalez, R.R., Red Eagle, A., Vats, D., Morel, C.R., Goforth, M.H., Subramanian, V., Mukundan, L., Ferrante, A.W., and Chawla, A. (2008) Alternative M2 activation of Kupffer cells by PPARδ

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

ameliorates obesity-induced insulin resistance. *Cell Metab*. **7**, 496–507

73. Kang, K., Reilly, S.M., Karabacak, V., Gangl, M.R., Fitzgerald, K., Hatano, B., and Lee, C.H. (2008) Adipocyte-derived Th2 cytokines and myeloid PPARδ regulate macrophage polarization and insulin sensitivity. *Cell Metab*. **7**, 485–495
74. Namgaladze, D., Morbitzer, D., von Knethen, A., and Brune, B. (2010) Phospholipase A_2-modified low-density lipoprotein activates macrophage peroxisome proliferator-activated receptors. *Arterioscler. Thromb. Vasc. Biol*. **30**, 313–320
75. Locksley, R.M. (2010) Asthma and allergic inflammation. *Cell* **140**, 777–783
76. Ishizaki, J., Suzuki, N., Higashino, K., Yokota, Y., Ono, T., Kawamoto, K., Fujii, N., Arita, H., and Hanasaki, K. (1999) Cloning and characterization of novel mouse and human secretory phospholipase A_2s. *J. Biol. Chem*. **274**, 24973–24979
77. von Allmen, C.E., Schmitz, N., Bauer, M., Hinton, H.J., Kurrer, M.O., Buser, R.B., Gwerder, M., Muntwiler, S., Sparwasser, T., Beerli, R.R., and Bachmann, M.F. (2009) Secretory phospholipase A_2-IID is an effector molecule of $CD4^+CD25^+$ regulatory T cells. *Proc. Natl Acad. Sci. USA* **106**, 11673–11678
78. Takabatake, N., Sata, M., Inoue, S., Shibata, Y., Abe, S., Wada, T., Machiya, J., Ji, G., Matsuura, T., Takeishi, Y., Muramatsu, M., and Kubota, I. (2005) A novel polymorphism in secretory phospholipase A_2-IID is associated with body weight loss in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med*. **172**, 1097–1104
79. Sato, H., Kato, R., Isogai, Y., Saka, G., Ohtsuki, M., Taketomi, Y., Yamamoto, K., Tsutsumi, K., Yamada, J., Masuda, S., Ishikawa, Y., Ishii, T., Kobayashi, T., Ikeda, K., Taguchi, R., Hatakeyama, S., Hara, S., Kudo, I., Itabe, H., and Murakami, M. (2008) Analyses of group III secreted phospholipase A_2 transgenic mice reveals potential participation of this enzyme in plasma lipoprotein modification, macrophage foam cell formation, and atherosclerosis. *J. Biol. Chem*. **283**, 33483–33497
80. Pruzanski, W., Stefanski, E., de Beer, F.C., de Beer, M.C., Vadas, P., Ravandi, A., and Kuksis, A. (1998) Lipoproteins are substrates for human secretory group IIA phospholipase A_2: preferential hydrolysis of acute phase HDL. *J. Lipid Res*. **39**, 2150–2160
81. Kugiyama, K., Ota, Y., Takazoe, K., Moriyama, Y., Kawano, H., Miyao, Y., Sakamoto, T., Soejima, H., Ogawa, H., Doi, H., Sugiyama, S., and Yasue, H. (1999) Circulating levels of secretory type II phospholipase A_2 predict coronary events in patients with coronary artery disease. *Circulation* **100**, 1280–1284
82. Mallat, Z., Steg, P.G., Benessiano, J., Tanguy, M.L., Fox, K.A., Collet, J.P., Dabbous, O.H., Henry, P., Carruthers, K.F., Dauphin, A., Arguelles, C.S., Masliah, J., Hugel, B., Montalescot, G., Freyssinet, J.M., Asselain, B., and Tedgui, A. (2005) Circulating secretory phospholipase A_2 activity predicts recurrent events in patients with severe acute coronary syndromes. *J. Am. Coll. Cardiol*. **46**, 1249–1257
83. Webb, N.R. (2005) Secretory phospholipase A_2 enzymes in atherogenesis. *Curr. Opin. Lipidol*. **16**, 341–344
84. Murakami, M. and Kudo, I. (2003) New phospholipase A_2 isozymes with a potential role in atherosclerosis. *Curr. Opin. Lipidol*. **14**, 431–436
85. Kimura-Matsumoto, M., Ishikawa, Y., Komiyama, K., Tsuruta, T., Murakami, M., Masuda, S., Akasaka, Y., Ito, K., Ishiguro, S., Morita, H., Sato, S., and Ishii, T. (2008) Expression of secretory phospholipase A_2s in human atherosclerosis development. *Atherosclerosis* **196**, 81–91
86. Webb, N.R., Bostrom, M.A., Szilvassy, S.J., van der Westhuyzen, D.R., Daugherty, A., and de Beer, F.C. (2003) Macrophage-expressed group IIA secretory phospholipase A_2 increases atherosclerotic lesion formation in LDL receptor-deficient mice. *Arterioscler. Thromb. Vasc. Biol*. **23**, 263–268
87. Yamamoto, K., Isogai, Y., Sato, H., Taketomi, Y., and Murakami, M. (2011) Secreted phospholipase A_2, lipoprotein hydrolysis, and atherosclerosis: integration with lipidomics. *Anal. Bioanal. Chem* **400**, 1829–1842
88. Bostrom, M.A., Boyanovsky, B.B., Jordan, C.T., Wadsworth, M.P., Taatjes, D.J., de Beer, F.C., and Webb, N.R. (2007) Group V secretory phospholipase A_2 promotes atherosclerosis: evidence from genetically altered mice. *Arterioscler. Thromb. Vasc. Biol*. **27**, 600–606
89. Zack, M., Boyanovsky, B.B., Shridas, P., Bailey, W., Forrest, K., Howatt, D.A., Gelb, M.H., de Beer, F.C., Daugherty, A., and Webb, N.R. (2011) Group X secretory phospholipase A_2 augments angiotensin II-induced inflammatory responses and abdominal aortic aneurysm formation in *apoE*-deficient mice. *Atherosclerosis* **214**, 58–64
90. Shridas, P., Bailey, W.M., Gizard, F., Oslund, R.C., Gelb, M.H., Bruemmer, D., and Webb, N.R. (2010) Group X secretory phospholipase A_2 negatively regulates ABCA1 and ABCG1 expression and cholesterol efflux in macrophages. *Arterioscler. Thromb. Vasc. Biol*. **30**, 2014–2021
91. Shaposhnik, Z., Wang, X., Trias, J., Fraser, H., and Lusis, A.J. (2009) The synergistic inhibition of atherogenesis in $apoE^{-/-}$ mice between pravastatin and the $sPLA_2$ inhibitor varespladib (A-002). *J. Lipid Res*. **50**, 623–629
92. Rosenson, R.S., Hislop, C., McConnell, D., Elliott, M., Stasiv, Y., Wang, N., and Waters, D.D. (2009) Effects of 1-H-indole-3-glyoxamide (A-002) on concentration of secretory phospholipase A_2 (PLASMA study): a phase II double-blind, randomised, placebo-controlled trial. *Lancet* **373**, 649–658
93. Wootton, P.T., Arora, N.L., Drenos, F., Thompson, S.R., Cooper, J.A., Stephens, J.W., Hurel, S.J., Hurt-Camejo, E., Wiklund, O., Humphries, S.E., and Talmud, P.J. (2007) Tagging SNP haplotype analysis of the secretory PLA_2-V gene, *PLA2G5*, shows strong association with LDL and oxLDL levels, suggesting functional distinction from $sPLA_2$-IIA: results from the UDACS study. *Hum. Mol. Genet*. **16**, 1437–1444
94. Pettersson, C., Fogelstrand, L., Rosengren, B., Stahlman, S., Hurt-Camejo, E., Fagerberg, B., and Wiklund, O. (2008) Increased lipolysis by secretory phospholipase A_2 group V of lipoproteins in diabetic dyslipidaemia. *J. Intern. Med*. **264**, 155–165
95. Shridas, P., Bailey, W.M., Boyanovsky, B.B., Oslund, R.C., Gelb, M.H., and Webb, N.R. (2010) Group X secretory phospholipase A_2 regulates the expression of steroidogenic acute regulatory protein (StAR) in mouse adrenal glands. *J. Biol. Chem*. **285**, 20031–20039
96. Li, X., Shridas, P., Forrest, K., Bailey, W., and Webb, N.R. (2010) Group X secretory phospholipase A_2

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

negatively regulates adipogenesis in murine models. *FASEB J.* **24**, 4313–4324

97. Carey, M.C., Small, D.M., and Bliss, C.M. (1983) Lipid digestion and absorption. *Annu. Rev. Physiol.* **45**, 651–677
98. Young, S.C. and Hui, D.Y. (1999) Pancreatic lipase/colipase-mediated triacylglycerol hydrolysis is required for cholesterol transport from lipid emulsions to intestinal cells. *Biochem. J.* **339**, 615–620
99. Gilham, D., Labonte, E.D., Rojas, J.C., Jandacek, R.J., Howles, P.N., and Hui, D.Y. (2007) Carboxyl ester lipase deficiency exacerbates dietary lipid absorption abnormalities and resistance to diet-induced obesity in pancreatic triglyceride lipase knockout mice. *J. Biol. Chem.* **282**, 24642–24649
100. Huggins, K.W., Boileau, A.C., and Hui, D.Y. (2002) Protection against diet-induced obesity and obesity-related insulin resistance in Group 1B PLA_2-deficient mice. *Am. J. Physiol. Endocrinol. Metab.* **283**, E994–E1001
101. Labonte, E.D., Kirby, R.J., Schildmeyer, N.M., Cannon, A.M., Huggins, K.W., and Hui, D.Y. (2006) Group 1B phospholipase A_2-mediated lysophospholipid absorption directly contributes to postprandial hyperglycemia. *Diabetes* **55**, 935–941
102. Wilson, S.G., Adam, G., Langdown, M., Reneland, R., Braun, A., Andrew, T., Surdulescu, G.L., Norberg, M., Dudbridge, F., Reed, P.W., Sambrook, P.N., Kleyn, P.W., and Spector, T.D. (2006) Linkage and potential association of obesity-related phenotypes with two genes on chromosome 12q24 in a female dizygous twin cohort. *Eur. J. Hum. Genet.* **14**, 340–348
103. Richmond, B.L., Boileau, A.C., Zheng, S., Huggins, K.W., Granholm, N.A., Tso, P., and Hui, D.Y. (2001) Compensatory phospholipid digestion is required for cholesterol absorption in pancreatic phospholipase A_2-deficient mice. *Gastroenterology* **120**, 1193–1202
104. Sato, H., Isogai, Y., Masuda, S., Taketomi, Y., Miki, Y., Kamei, D., Hara, S., Kobayashi, T., Ishikawa, Y., Ishii, T., Ikeda, K., Taguchi, R., Ishimoto, Y., Suzuki, N., Yokota, Y., Hanasaki, K., Suzuki-Yamamoto, T., Yamamoto, K., and Murakami, M. (2011) Physiological roles of group X secreted phospholipase A_2 in reproduction, gastrointestinal phospholipid digestion, and neuronal function. *J. Biol. Chem.* **286**, 11632–11648
105. Hui, D.Y., Cope, M.J., Labonte, E.D., Chang, H.T., Shao, J., Goka, E., Abousalham, A., Charmot, D., and Buysse, J. (2009) The phospholipase A_2 inhibitor methyl indoxam suppresses diet-induced obesity and glucose intolerance in mice. *Br. J. Pharmacol.* **157**, 1263–1269
106. Nevalainen, T.J., Graham, G.G., and Scott, K.F. (2008) Antibacterial actions of secreted phospholipases A_2. *Biochim. Biophys. Acta* **1781**, 1–9
107. Singer, A.G., Ghomashchi, F., Le Calvez, C., Bollinger, J., Bezzine, S., Rouault, M., Sadilek, M., Nguyen, E., Lazdunski, M., Lambeau, G., and Gelb, M.H. (2002) Interfacial kinetic and binding properties of the complete set of human and mouse groups I, II, V, X, and XII secreted phospholipases A_2. *J. Biol. Chem.* **277**, 48535–48549
108. Piris-Gimenez, A., Paya, M., Lambeau, G., Chignard, M., Mock, M., Touqui, L., and Goossens, P.L. (2005) *In vivo* protective role of human group IIa phospholipase A_2 against experimental anthrax. *J. Immunol.* **175**, 6786–6791
109. Laine, V.J., Grass, D.S., and Nevalainen, T.J. (2000) Resistance of transgenic mice expressing human group II phospholipase A_2 to *Escherichia coli* infection. *Infect. Immun.* **68**, 87–92
110. Balestrieri, B., Hsu, V.W., Gilbert, H., Leslie, C.C., Han, W.K., Bonventre, J.V., and Arm, J.P. (2006) Group V secretory phospholipase A_2 translocates to the phagosome after zymosan stimulation of mouse peritoneal macrophages and regulates phagocytosis. *J. Biol. Chem.* **281**, 6691–6698
111. Balestrieri, B., Maekawa, A., Xing, W., Gelb, M.H., Katz, H.R., and Arm, J.P. (2009) Group V secretory phospholipase A_2 modulates phagosome maturation and regulates the innate immune response against *Candida albicans*. *J. Immunol.* **182**, 4891–4898
112. Mitsuishi, M., Masuda, S., Kudo, I., and Murakami, M. (2006) Group V and X secretory phospholipase A_2s prevent adenovirus infection into mammalian cells. *Biochem. J.* **393**, 97–106
113. Mitsuishi, M., Masuda, S., Kudo, I., and Murakami, M. (2007) Human group III Phospholipase A_2 suppresses adenovirus infection into host cells: evidence that group III, V and X phospholipase A_2s act on distinct cellular phospholipids molecular species. *Biochim. Biophys. Acta* **1771**, 1389–1396
114. Limou, S., Coulonges, C., Foglio, M., Heath, S., Diop, G., Leclerc, S., Hirtzig, T., Spadoni, J.L., Therwath, A., Lambeau, G., Gut, I., and Zagury, J.F. (2008) Exploration of associations between phospholipase A_2 gene family polymorphisms and AIDS progression using the SNPlex method. *Biomed. Pharmacother.* **62**, 31–40
115. Guillaume, C., Deregnaucourt, C., Clavey, V., and Schrevel, J. (2004) Anti-Plasmodium properties of group IA, IB, IIA and III secreted phospholipases A_2 are serum-dependent. *Toxicon* **43**, 311–318
116. Cormier, R.T., Hong, K.H., Halberg, R.B., Hawkins, T.L., Richardson, P., Mulherkar, R., Dove, W.F., and Lander, E.S. (1997) Secretory phospholipase *Pla2g2a* confers resistance to intestinal tumorigenesis. *Nat. Genet.* **17**, 88–91
117. Leung, S.Y., Chen, X., Chu, K.M., Yuen, S.T., Mathy, J., Ji, J., Chan, A.S., Li, R., Law, S., Troyanskaya, O.G, Tu, I.P., Wong, J., So, S., Botstein, D., and Brown, P.O. (2000) Phospholipase A_2 group IIA expression in gastric adenocarcinoma is associated with prolonged survival and less frequent metastasis. *Proc. Natl Acad. Sci. USA* **99**, 16203–16208
118. Aggarwal, A., Guo, D.L., Hoshida, Y., Yuen, S.T., Chu, K.M., So, S., Boussioutas, A., Chen, X., Bowtell, D., Aburatani, H., Leung, S.Y., and Tan, P. (2006) Topological and functional discovery in a gene coexpression meta-network of gastric cancer. *Cancer Res.* **66**, 232–241
119. Dong, Q., Patel, M., Scott, K.F., Graham, G.G., Russell, P.J., and Sved, P. (2006) Oncogenic action of phospholipase A_2 in prostate cancer. *Cancer Lett.* **240**, 9–16
120. Mirtti, T., Laine, V.J., Hiekkanen, H., Hurme, S., Rowe, O., Nevalainen, T.J., Kallajoki, M., and Alanen, K. (2009) Group IIA phospholipase A as a prognostic marker in prostate cancer: relevance to clinicopathological variables and disease-specific mortality. *APMIS* **117**, 151–161

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

121. Mulherkar, R., Kirtane, B.M., Ramchandani, A., Mansukhani, N.P., Kannan, S., and Naresh, K.N. (2003) Expression of enhancing factor/phospholipase A_2 in skin results in abnormal epidermis and increased sensitivity to chemical carcinogenesis. *Oncogene* **22**, 1936–1944
122. Murakami, M., Masuda, S., Shimbara, S., Ishikawa, Y., Ishii, T., and Kudo, I. (2005) Cellular distribution, post-translational modification, and tumorigenic potential of human group III secreted phospholipase A_2. *J. Biol. Chem.* **280**, 24987–24998
123. Mounier, C.M., Wendum, D., Greenspan, E., Flejou, J.F., Rosenberg, D.W., and Lambeau, G. (2008) Distinct expression pattern of the full set of secreted phospholipases A_2 in human colorectal adenocarcinomas: sPLA$_2$-III as a biomarker candidate. *Br. J. Cancer* **98**, 587–595
124. Hoeft, B., Linseisen, J., Beckmann, L., Müller-Decker, K., Canzian, F., Hüsing, A., Kaaks, R., Vogel, U., Jakobsen, M.U., Overvad, K., Hansen, R.D., Knüppel, S., Boeing, H., Trichopoulou, A., Koumantaki, Y., Trichopoulos, D., Berrino, F., Palli, D., Panico, S., Tumino, R., Bueno-de-Mesquita, H.B., van Duijnhoven, F.J., van Gils, C.H., Peeters, P.H., Dumeaux, V., Lund, E., Huerta Castaño, J.M., Muñoz, X., Rodriguez, L., Barricarte, A., Manjer, J., Jirström, K., Van Guelpen, B., Hallmans, G., Spencer, E.A., Crowe, F.L., Khaw, K.T., Wareham, N., Morois, S., Boutron-Ruault, M.C., Clavel-Chapelon, F., Chajes, V., Jenab, M., Boffetta, P., Vineis, P., Mouw, T., Norat, T., Riboli, E., and Nieters, A. Polymorphisms in fatty-acid-metabolism-related genes are associated with colorectal cancer risk. *Carcinogenesis* **31**, 466–472
125. Alonso, L. and Fuchs, E. (2006) The hair cycle. *J. Cell Sci.* **119**, 391–393
126. Yamamoto, K., Taketomi, T., Isogai, Y., Miki, Y., Sato, H., Masuda, S., Nishito, Y., Morioka, K., Ishimoto, Y., Suzuki, N., Yokoya, Y., Hanasaki, K., Ishikawa, Y., Ishii, T., Kobayashi, T., Fukami, K., Ikeda, K., Nakanishi, H., Taguchi, R., and Murakami, M. (2011) Hair follicular expression and function of group X secreted phospholipase A_2 in mouse skin. *J. Biol. Chem.* **286**, 11616–11631
127. Müller-Decker, K., Neufang, G., Berger, I., Neumann, M., Marks, F., and Fürstenberger, G. (2002) Transgenic cyclooxygenase-2 overexpression sensitizes mouse skin for carcinogenesis. *Proc. Natl Acad. Sci. USA* **99**, 12483–12488
128. Masuda, S., Murakami, M., Matsumoto, S., Eguchi, N., Urade, Y., Lambeau, G., Gelb, M.H., Ishikawa, Y., Ishii, T., and Kudo, I. (2004) Localization of various secretory phospholipase A_2 enzymes in male reproductive organs. *Biochim. Biophys. Acta* **1686**, 61–76
129. Takayama, K., Hara, S., Kudo, I., and Inoue, K. (1991) Detection of 14-kDa group II phospholipase A_2 in human seminal plasma. *Biochem. Biophys. Res. Commun.* **178**, 1505–1511
130. Kallajoki, M., Alanen, K.A., Nevalainen, M., and Nevalainen, T.J. (1998) Grouup II phospholipase A_2 in human male reproductive organs and genital tumors. *Prostate* **35**, 263–72
131. Sato, H., Taketomi, T., Isogai, Y., Miki, Y., Yamamoto, K., Masuda, S., Hosono, T., Arata, S., Ishikawa, Y., Ishii, T., Kobayashi, T., Nakanishi, H., Ikeda, K., Taguchi, R., Hara, S., Kudo, I., and Murakami, M. (2010) Group III secreted phospholipase A_2 regulates epididymal sperm maturation and fertility in mice. *J. Clin. Invest.* **120**, 1400–1414
132. Escoffier, J., Jemel, I., Tanemoto, A., Taketomi, Y., Payre, C., Coatrieux, C., Sato, H., Yamamoto, K., Masuda, S., Pernet-Gallay, K., Pierre, V., Hara, S., Murakami, M., De Waard, M., Lambeau, G., and Arnoult, C. (2010) Group X phospholipase A_2 is released during sperm acrosome reaction and controls fertility outcome in mice. *J. Clin. Invest.* **120**, 1415–1428
133. Rejraji, H., Sion, B., Prensier, G., Carreras, M., Motta, C., Frenoux, J.M., Vericel, E., Grizard, G., Vernet, P., and Drevet, J.R. (2006) Lipid remodeling of murine epididymosomes and spermatozoa during epididymal maturation. *Biol. Reprod.* **74**, 1104–1113
134. Lenzi, A., Picardo, M., Gandini, L., and Dondero, F. (1996) Lipids of the sperm plasma membrane: from polyunsaturated fatty acids considered as markers of sperm function to possible scavenger therapy. *Hum. Reprod. Update* **2**, 246–256
135. Lenzi, A., Gandini, L., Maresca, V., Rago, R., Sgrò, P., Dondero, F., and Picardo, M. (2000) Fatty acid composition of spermatozoa and immature germ cells. *Mol. Hum. Reprod.* **6**, 226–231
136. Furimsky, A., Vuong, N., Xu, H., Kumarathasan, P., Xu, M., Weerachatyanukul, W., Bou Khalil, M., Kates, M., and Tanphaichitr, N. (2005) Percoll gradient-centrifuged capacitated mouse sperm have increased fertilizing ability and higher contents of sulfogalactosylglycerolipid and docosahexaenoic acid-containing phosphatidylcholine compared to washed capacitated mouse sperm. *Biol. Reprod.* **72**, 574–583
137. Aksoy, Y., Aksoy, H., Altinkaynak, K., Aydin, H.R., and Ozkan, A. (2006) Sperm fatty acid composition in subfertile men. *Prostaglandins Leukot. Essent. Fatty Acids* **75**, 75–79
138. Fleming, A.D. and Yanagimachi, R. (1984) Evidence suggesting the importance of fatty acids and the fatty acid moieties of sperm membrane phospholipids in the acrosome reaction of guinea pig spermatozoa. *J. Exp. Zool.* **229**, 485–489
139. Llanos, M.N., Morales, P., and Riffo, M.S. (1993) Studies of lysophospholipids related to the hamster sperm acrosome reaction *in vitro*. *J. Exp. Zool.* **267**, 209–216
140. Riffo, M and Nieto, A. (1999) Lysophosphatidylcholine induces changes in physicochemical, morphological, and functional properties of mouse zona pellucida: a possible role of phospholipase A_2 in sperm-zona pellucida interaction. *Mol. Reprod. Dev.* **53**, 68–76
141. Escoffier, J., Pierre, V.J., Jemel, I., Munch, L., Boudhraa, Z., Ray, P.F., De Waard, M., Lambeau, G., and Arnoult, C. (2011) Group X secreted phospholipase A_2 specifically decreases sperm motility in mice. *J. Cell Physiol.* in press
142. Masuda, S., Murakami, M., Takanezawa, Y., Aoki, J., Arai, H., Ishikawa, Y., Ishii, T., Arioka, M., and Kudo, I. (2005) Neuronal expression and neuritogenic action of group X secreted phospholipase A_2. *J. Biol. Chem.* **280**, 23203–23214
143. Surrel, F., Jemel, I., Boilard, E., Bollinger, J.G., Payré, C., Mounier, C.M., Talvinen, K.A., Laine, V.J., Nevalainen, T.J., Gelb, M.H., and Lambeau, G. (2009) Group X phospholipase A_2 stimulates the proliferation of colon cancer cells by producing various lipid mediators. *Mol. Pharmacol.* **76**, 778–790

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

144. Masuda, S., Yamamoto, K., Hirabayashi, T., Ishikawa, Y., Ishii, T., Kudo, I., and Murakami, M. (2008) Human group III secreted phospholipase A_2 promotes neuronal outgrowth and survival. *Biochem. J.* **409**, 429–438
145. Chen, J., Engle, S.J., Seilhamer, J.J., and Tischfield, J.A. (1994) Cloning and characterization of novel rat and mouse low molecular weight Ca^{2+}-dependent phospholipases A_2 containing 16 cysteines. *J. Biol. Chem.* **269**, 23018–23024
146. Suzuki, N., Ishizaki, J., Yokota, Y., Higashino, K., Ono, T., Ikeda, M., Fujii, N., Kawamoto, K., and Hanasaki, K. (2000) Structures, enzymatic properties, and expression of novel human and mouse secretory phospholipases A_2. *J. Biol. Chem.* **275**, 5785–5793
147. Valentin, E., Ghomashchi, F., Gelb, M.H., Lazdunski, M., and Lambeau, G. (1999) On the diversity of secreted phospholipases A_2. Cloning, tissue distribution, and functional expression of two novel mouse group II enzymes. *J. Biol. Chem.* **274**, 31195–31202
148. Murakami, M., Yoshihara, K., Shimbara, S., Lambeau, G., Gelb, M.H., Singer, A.G., Sawada, M., Inagaki, N., Nagai, H., Ishihara, M., Ishikawa, Y., Ishii, T., and Kudo, I. (2002) Cellular arachidonate-releasing function and inflammation-associated expression of group IIF secretory phospholipase A_2. *J. Biol. Chem.* **277**, 19145–19155
149. Gelb, M.H., Valentin, E., Ghomashchi, F., Lazdunski, M., and Lambeau, G. (2000) Cloning and recombinant expression of a structurally novel human secreted phospholipase A_2. *J. Biol. Chem.* **275**, 39823–39826
150. Ho, I.C., Arm, J.P., Bingham, C.O., Choi, A., Austen, K.F., and Glimcher, L.H. (2001) A novel group of phospholipase A_2 preferentially expressed in Th2 cells. *J. Biol. Chem.* **276**, 18321–18326
151. Rouault, M., Bollinger, J.G., Lazdunski, M., Gelb, M.H., and Lambeau, G. (2003) Novel mammalian group XII secreted phospholipase A_2 lacking enzymatic activity. *Biochemistry* **42**, 11494–11503
152. Huhtinen, H.T., Gronroos, J.O., Gronroos, J.M., Uksila, J., Gelb, M.H., Nevalainen, T.J., and Laine, V.J. (2006) Antibacterial effects of human group IIA and group XIIA phospholipase A_2 against *Helicobacter pylori in vitro*. *APMIS* **114**, 127–130
153. Munoz-Sanjuan, I. and Brivanlou, A.H. (2005) Induction of ectopic olfactory structures and bone morphogenetic protein inhibition by *Rossy*, a group XII secreted phospholipase A_2. *Mol. Cell. Biol.* **25**, 3608–3619
154. Guan, M., Qu, L., Tan, W., Chen, L., and Wong, C.W. (2011) Hepatocyte nuclear factor-4α regulates liver triglyceride metabolism in part through secreted phospholipase A_2 GXIIB. *Hepatology* **53**, 458–466
155. Bryant, K.J., Bidgood, M.J., Lei, P.W., Taberner, M., Salom, C., Kumar, V., Lee, L., Church, W.B., Courtenay, B., Smart, B.P., Gelb, M.H., Cahill, M.A., Graham, G.G., McNeil, H.P., and Scott, K.F. (2011) A bifunctional role for group IIA secreted phospholipase A_2 in human rheumatoid fibroblast-like synoviocyte arachidonic acid metabolism. *J. Biol. Chem.* **286**, 2492–2503
156. Tada, K., Murakami, M., Kambe, T., and Kudo, I. (1998) Induction of cyclooxygenase-2 by secretory phospholipase A_2s in nerve growth factor-stimulated rat serosal mast cells is facilitated by interaction with fibroblasts and mediated by a mechanism independent of their enzymatic functions. *J. Immunol.* **161**, 5008–5015
157. Wang, Y., Kowalski, P.E., Thalmann, I., Ornitz, D.M., Mager, D.L., and Thalmann, R. (1998) Otoconin-90, the mammalian otoconial matrix protein, contains two domains of homology to secretory phospholipase A_2. *Proc. Natl Acad. Sci. USA* **95**, 15345–15350
158. Zhao, X., Yang, H., Yamoah, E.N., and Lundberg, Y.W. (2007) Gene targeting reveals the role of Oc90 as the essential organizer of the otoconial organic matrix. *Dev. Biol.* **304**, 508–524
159. Zhao, X., Jones, S.M., Yamoah, E.N., and Lundberg, Y.W. (2008) Otoconin-90 deletion leads to imbalance but normal hearing: a comparison with other otoconia mutants. *Neuroscience* **153**, 289–299
160. Ishizaki, J., Hanasaki, K., Higashino, K., Kishino, J., Kikuchi, N., Ohara, O., and Arita, H. (1994) Molecular cloning of pancreatic group I phospholipase A_2 receptor. *J. Biol. Chem.* **269**, 5897–5904
161. Lambeau, G., Ancian, P., Barhanin, J., and Lazdunski, M. (1994) Cloning and expression of a membrane receptor for secretory phospholipases A_2. *J. Biol. Chem.* **269**, 1575–1578
162. Higashino, K., Yokota, Y., Ono, T., Kamitani, S., Arita, H., and Hanasaki, K. (2002) Identification of a soluble form phospholipase A_2 receptor as a circulating endogenous inhibitor for secretory phospholipase A_2. *J. Biol. Chem.* **277**, 13583–13588
163. Rouault, M., Le Calvez, C., Boilard, E., Surrel, F., Singer, A., Ghomashchi, F., Bezzine, S., Scarzello, S., Bollinger, J., Gelb, M.H., and Lambeau, G. (2007) Recombinant production and properties of binding of the full set of mouse secreted phospholipases A_2 to the mouse M-type receptor. *Biochemistry* **46**, 1647–1662
164. Zvaritch, E., Lambeau, G., and Lazdunski, M. (1996) Endocytic properties of the M-type 180-kDa receptor for secretory phospholipases A_2. *J. Biol. Chem.* **271**, 250–257
165. Yokota, Y., Notoya, M., Higashino, K., Ishimoto, Y., Nakano, K., Arita, H., and Hanasaki, K. (2001) Clearance of group X secretory phospholipase A_2 via mouse phospholipase A_2 receptor. *FEBS LETT.* **509**, 250–254
166. Hanasaki, K. and Arita, H. (1999) Biological and pathological functions of phospholipase A_2 receptor. *Arch. Biochem. Biophys.* **372**, 215–223
167. Hanasaki, K., Yokota, Y., Ishizaki, J., Itoh, T., and Arita, H. (1997) Resistance to endotoxic shock in phospholipase A_2 receptor-deficient mice. *J. Biol. Chem.* **272**, 32792–32797
168. Kawai, T. and Akira, S. (2010) The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat. Immunol.* **11**, 373–384
169. Takeuchi, O. and Akira, S. (2010) Pattern recognition receptors and inflammation. *Cell* **140**, 805–820
170. Geijtenbeek, T.B. and Gringhuis, S.I. (2009) Signalling through C-type lectin receptors: shaping immune responses. *Nat. Rev. Immunol.* **9**, 465–479
171. Miyajima, A., Kitamura, T., Harada, N., Yokota, T., and Arai, K. (1992) Cytokine receptors and signal transduction. *Annu. Rev. Immunol.* **10**, 295–331
172. Ip, W.K., Takahashi, K., Ezekowitz, R.A., and Stuart, L.M. (2009) Mannose-binding lectin and innate immunity. *Immunol. Rev.* **230**, 9–21

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011

173. Gantner, B.N., Simmons, R.M., Canavera, S.J., Akira, S., and Underhill, D.M. (2003) Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *J. Exp. Med.* **197**, 1107–1117
174. Stewart, C.R., Stuart, L.M., Wilkinson, K., van Gils, J.M., Deng, J., Halle, A., Rayner, K.J., Boyer, L., Zhong, R., Frazier, W.A., Lacy-Hulbert, A., El Khoury, J., Golenbock, D.T., and Moore, K.J. (2010) CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. *Nat. Immunol.* **11**, 155–161
175. Augert, A., Payre, C., de Launoit, Y., Gil, J., Lambeau, G., and Bernard, D. (2009) The M-type receptor PLA2R regulates senescence through the p53 pathway. *EMBO Rep.* **10**, 271–277
176. Beck, L.H. J, Bonegio, R.G. r, Lambeau, G., Beck, D.M., Powell, D.W., Cummins, T.D., Klein, J.B., and Salant, D.J. (2009) M-type phospholipase A_2 receptor as target antigen in idiopathic membranous nephropathy. *N. Engl. J. Med.* **36**, 111–121

Downloaded from jb.oxfordjournals.org by guest on August 27, 2011