Review

Resolvins, Specialized Proresolving Lipid Mediators, and Their Potential Roles in Metabolic Diseases

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Inflammation is associated with the development of diseases characterized by altered nutrient metabolism. Although an acute inflammatory response is host-protective and normally self-limited, chronic low-grade inflammation associated with metabolic diseases is sustained and detrimental. The resolution of inflammation involves the termination of neutrophil recruitment, counterregulation of proinflammatory mediators, stimulation of macrophage-mediated clearance, and tissue remodeling. Specialized proresolving lipid mediators (SPMs)–resolvins, protectins, and maresins–are novel autacoids that resolve inflammation, protect organs, and stimulate tissue regeneration. Here, we review evidence that the failure of resolution programs contributes to metabolic diseases and that SPMs may play pivotal roles in their resolution.

Initiation and Resolution of Acute Inflammation: Identification of the Proresolving Mediators

Acute inflammatory responses are protective for the host, yet, when uncontrolled or inappropriately activated, acute inflammation can lead to persistent chronic inflammation that is unresolved and can promote organ fibrosis and dysfunction (Figure 1A) (Majno and Joris, 2004). Although chronic inflammation is classically associated with arthritis and periodontitis (Gilroy, 2010; Karp, 2010; Ward, 2010), there is increasing evidence that uncontrolled inflammation is also associated with many other chronic diseases, such as asthma and neurological degenerative disorders, as well as metabolic diseases, including diabetes, obesity, and cardiovascular disease (reviewed in Nathan and Ding, 2010; Tabas and Glass, 2013). An acute inflammatory response is, by definition, divided into an initiation phase and a resolution phase. The initiation phase is accompanied by the cardinal signs of inflammation known to ancient civilizations as heat, swelling, and pain (Majno and Joris, 2004) with eventual losses of function that are controlled, for the most part, by local chemical autacoids (Houck, 1979). A majority of these chemical messengers are in the form of peptides (cytokines and chemokines), proteins, and lipid-derived mediators (prostaglandins and leukotrienes) that form chemical gradients that regulate leukocyte trafficking via chemotaxis and diapedesis from the blood stream into the injured tissue. The phagocytes contain invading microbes and clear tissue debris or remove environmental toxins that appear in tissues as a result of barrier disruption. The repertoire of edema, polymorphonuclear neutrophil (PMN) infiltration, and monocyte-macrophage accumulation ensues as a characteristic sequence of events during the initiation of the acute inflammatory response (Figure 1A) (Cassatella, 2003; Gilroy, 2010; Karp, 2010; Ward, 2010). Without an appropriate termination and clearance of phagocytes, the continued presence of activated leukocytes within tissues is associated with collateral tissue damage, amplification, and the persistence

of tissue inflammation. Hence, controlling PMN infiltration, cessation, and removal from tissues as well as macrophage accumulation, activation, and removal could attenuate nonresolving chronic tissue inflammation.

Many initiation-phase proinflammatory mediators are well known (i.e., prostaglandins and proinflammatory cytokines), and popular anti-inflammatory treatments are directed toward either blocking or antagonizing these mediators in a quest to control unwanted excessive inflammation (Samuelsson et al., 1987; Flower, 2006; Dinarello et al., 2012). What controls or limits the number of leukocytes that congregate within inflammatory exudates? How are signaling events organized toward resolving the acute inflammatory response (i.e., the removal of apoptotic PMNs and cellular debris), promoting the return to homeostasis, which is the ideal outcome of an inflammatory challenge (Serhan et al., 2000; Levy et al., 2001; Serhan, 2004)? The resolution of inflammation and return to homeostasis was widely believed to occur via the dissipation of initiating chemotactic signals in the acute inflammatory response (Majno and Joris, 2004). This dissipation can arise partly because of extensive negative feedback regulation of Toll-like receptor (TLR) signaling via the induction of $IkB\text{-}\alpha$ and A20 as well as transcriptional repressors, including activating transcription factor 3, for example (Olefsky and Glass, 2010). In addition, anti-inflammatory cytokines such as IL-10 also blunt inflammatory gene transcription. However, in addition to a decrescendo of proinflammatory mediators, ample evidence now shows that the resolution of contained inflammatory exudates is an actively programmed biochemical process regulated by the temporal biosynthesis of novel chemical mediators during the resolution phase. As described below, these proresolving mediators not only counterregulate inflammatory gene transcription but also directly block and limit excessive PMN migration and stimulate distinct cellular processes, such as macrophage uptake of apoptotic PMNs, microbes, and cellular debris that are required for tissue homeostasis to
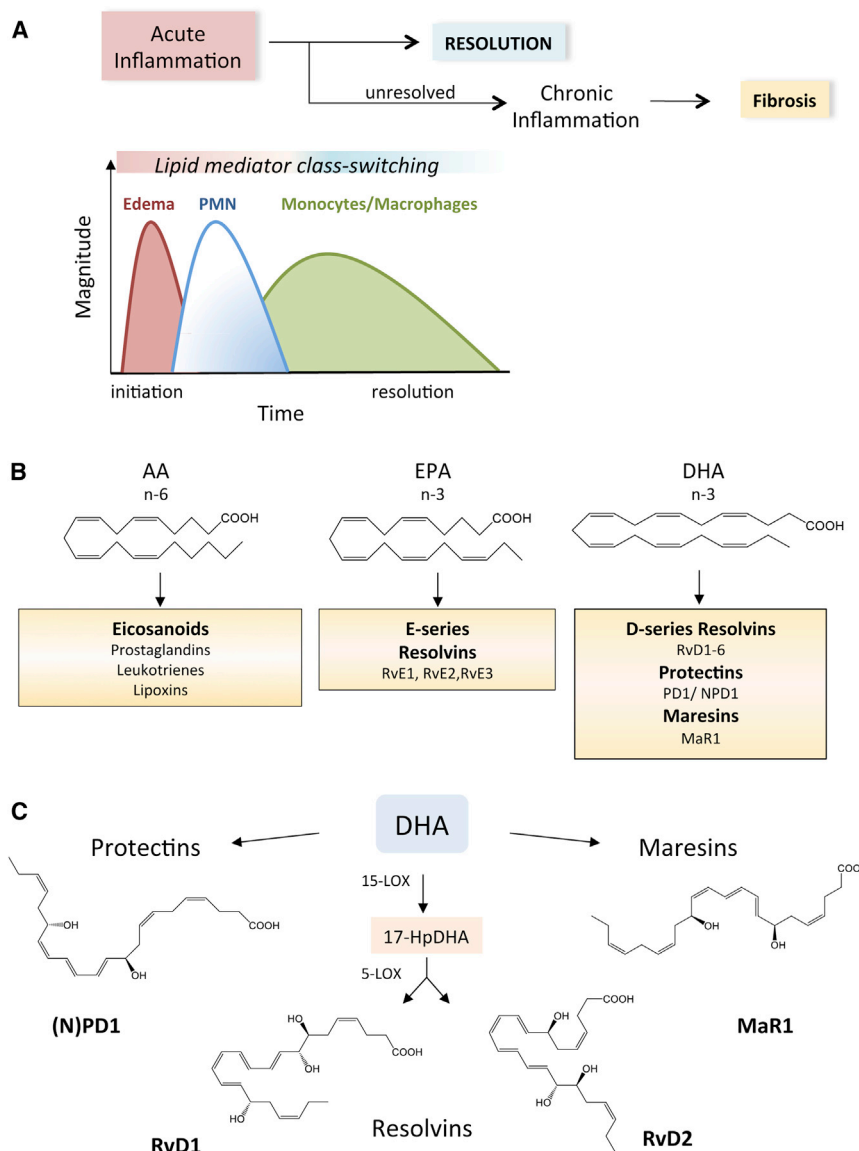
Figure 1. Specialized Proresolving Lipid Mediator Biosynthesis during Resolution of Inflammation

(A) Complete resolution is the ideal outcome of inflammation, although, if not properly regulated, it can lead to chronic inflammation, fibrosis, and loss of function. Inflammation and its resolution involves a temporal series of leukocyte trafficking events coupled with lipid mediator class switching, in which proinflammatory lipid mediators signal the generation of proresolving lipid mediators.

(B) A depiction of classic and novel lipid mediator families generated from essential omega-6 (n-6) and omega-3 (n-3) fatty acids, arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA).

(C) Abbreviated biosynthetic pathways and structures of resolvins, protectins, and maresins generated enzymatically from DHA. Their complete stereochemical structures are established; see Lipid Mediator Class Switching during Inflammation and Its Resolution: Alpha Signals Omega for details. LOX, lipoxygenase; RvD1, resolvin D1; PD1, protectin D1; MaR1, maresin 1.

identified a novel genus of bioactive mediators that comprise four families of distinct structures, namely lipoxins, resolvins, protectins, and maresins, all of which are biosynthesized within the resolution phase of acute inflammation (Levy et al., 2001; Serhan et al., 2000, 2002; Hong et al., 2003). These new local mediators activate previously unappreciated proresolving mechanisms, and their identification demonstrated that the resolution phase of acute inflammation is a biosynthetically active process (Serhan and Savill, 2005).

Lipid Mediator Class Switching during Inflammation and Its Resolution: Alpha Signals Omega

The initiation of acute inflammation is controlled by a number of autacoids, including lipid mediators such as the eicosanoids, prostaglandins (PGs), and leukotrienes, which are formed from arachidonic acid (AA; omega 6) and play key roles in regulating blood flow, endothelial permeability, and PMN diapedesis (Samuelsson et al., 1987). Transendothelial migration and chemotaxis of PMNs toward injured tissue and/or pathogens is governed in part by leukotriene B_4 (LTB_4) and chemokines (Figures 1A and 1B). Unexpectedly, we found that there is a temporal switch in lipid mediators from the initiation phase to resolution; that is, different lipid mediators are generated at different times during the evolution of the inflammatory response, and these mediators coincide with distinct cellular traffic and events. Although maximal levels of LTB_4 occur as PMN infiltrate tissues, other eicosanoids, including the proinflammatory cyclooxygenase products PGE_2 and PGD_2, initiate a lipid mediator class switch—a mediator circuit in exudates that activates leukocyte translational regulation of the enzymes required for producing

be re-established (Serhan et al., 2000; Levy et al., 2001; Serhan et al., 2002; Serhan and Savill, 2005). These findings swiftly raised the possibility that the failed resolution of an inflammatory challenge could potentially lead to recurring bouts of persistent tissue inflammation and diseases associated with chronic inflammation as well as the notion that the resolution phase is exciting new terrain for targeted innovative therapeutics (Serhan et al., 2007; Buckley et al., 2013).

Resolution is defined at the tissue level with the cessation of leukocyte infiltration in response to chemotactic signals, timely apoptosis of PMNs, and the accompanied active clearance of apoptotic cells and debris by macrophages (Serhan, 2004; Ward, 2010). Using a systems approach with liquid chromatography tandem mass spectrometry (LC-MS/MS)-based analysis of self-limited inflammatory exudates (ones that resolve to homeostasis on their own) formed in vivo in animal models as well as in isolated human cells, the Serhan laboratory, over several years,

proresolving lipid mediators (Levy et al., 2001). That is, PGE_2 and PGD_2 stimulate the biosynthesis of lipoxin A_4 (LXA_4), which appears in exudates at the time point at which PMN levels decline. Indeed, LXA_4 serves as an endogenous "stop" signal that decreases PMN infiltration. Thus, signals that mediate the resolution of a contained acute inflammatory response are tightly linked to mediators of the initiation phase; i.e., the beginning programs the end of inflammation (Serhan and Savill, 2005). Specialized proresolving lipid mediators (SPMs) can be generated via transcellular biosynthesis, and their appearance increases when macrophages are actively clearing apoptotic PMNs (Dalli and Serhan, 2012). Indeed, macrophage phagocytosis of apoptotic cells also leads to the biosynthesis of proresolving lipid mediators, which act in an autocrine manner to facilitate phagocytosis. This mechanism of biosynthesis, coupled with the potent regulation of inflammatory gene transcription, is similar to that of other "find me" and "eat me" signals (e.g., adenosine, ATP, and CX_3CL1) generated by phagocytes that play a key role in the immunologically silent process of apoptotic cell clearance (Han and Ravichandran, 2011; Köröskényi et al., 2011).

To emphasize the specificity of their actions and specialized roles in inflammation, we coined the term "specialized proresolving lipid mediators." This genus includes several families of chemically and functionally distinct mediators, namely the lipoxins, resolvins, protectins, and maresins, because they blunt PMN infiltration, decrease proinflammatory mediator production (both lipid mediators and cytokines), and stimulate macrophage-dependent uptake of apoptotic PMNs as well as bacterial clearance (Serhan and Savill, 2005; Chiang et al., 2012). Notably, SPMs also regulate PMN apoptosis (see below) and stimulate chemokine scavenging (e.g., CCL3 and CCL5) during resolution via the upregulation of CCR5 expression on apoptotic PMNs and T cells (Ariel et al., 2006). This mechanism facilitates the clearance of these chemokines from sites of inflammation, given that the apoptotic cells and bound chemokines are cleared by macrophages and that CCR5-dependent signaling is lost in apoptotic cells (Ariel et al., 2006). Systematic identification of these endogenous mediators indicated that they are novel structures and that the precursors for resolvins, protectins, and maresins are the omega-3 essential fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), whereas the lipoxins are generated from the omega-6 fatty acid AA (Figure 1B). It should be noted that, in addition to the SPMs described here, other resolution agonists, including glucocorticoid-induced annexin A1, are also important players (Perretti and D'Acquisto, 2009).

E-series resolvins (e.g., RvE1, RvE2, and RvE3) are produced from EPA, and DHA is a precursor of three novel families of distinct SPMs that include the D-series resolvins, protectins (including PD1 or neuroprotectin [N]PD1 when formed in neural tissues), and maresins (Serhan, 2007; Isobe et al., 2012) (Figure 1C). There are also temporal relationships between different SPM families and individual mediators produced in response to pathogens (e.g., viral versus bacterial) that trigger the production of select host SPMs (Chiang et al., 2012; Koltsida et al., 2013; Morita et al., 2013). This suggests that, even within the SPM genus, there are distinct roles for individual SPMs and that the complexities of their biosynthesis are just beginning to be appreciated.

It is extensively documented that, in certain clinical settings, omega-3 essential fatty acids (EPA and DHA) regulate both innate and acquired immune responses (reviewed in De Caterina, 2011; Calder, 2013). SPMs are generated in humans taking omega-3 dietary supplements, and SPM levels are increased above those produced normally in transgenic mice expressing an omega-3 fatty acid desaturase (*fat-1*) (Hudert et al., 2006; Mas et al., 2012). The identification of the SPM structures, mapping of their biosynthetic pathways, and stereochemical assignments for each of the SPMs, coupled with the elucidation of their potent actions with human cells and in numerous animal models of inflammation, collectively suggests that SPM formation may underlie some of the beneficial effects attributed to their precursors EPA and DHA (Serhan and Chiang, 2013). Like other small molecules, SPMs evoke stereospecific bioactions mediated in the nanomolar range by their binding to specific G protein-coupled receptors (GPCRs; Figure 2). Systematic receptor screening approaches along with radioligand-specific binding and results from competition studies identified GPCRs activated by SPMs, namely, ChemR23/ERV for RvE1 and RvE2 and ALX/FPR2 and GPR32/DRV for LXA_4 and RvD1 (Serhan and Chiang, 2013). Interestingly, GPR32 is also activated by RvD5 and RvD3 (Dalli et al., 2013), whereas other proresolving mediators, including annexin A1, activate ALX/FPR2 (Norling and Perretti, 2013). NPD1/PD1 displays specific binding to PMNs and human epithelial cells where neither RvE1 nor LXA_4 competes for PD1-specific binding, indicating that PD1 actions are most likely mediated by separate receptors. Binding studies using specific receptor expression constructs corroborate their potent actions on isolated cell types and in vivo, K_d values being in the picomolar-nanomolar range (Serhan and Chiang, 2013).

RvE1 and RvE2 are also endogenous receptor antagonists for the LTB_4 receptor BLT-1, which most likely explains their ability to potently regulate PMN trafficking to sites of inflammation. The endogenous role of these specific GPCRs in transmitting SPM signals has now been elucidated in vivo in mice with transgenic overexpression of ChemR23 and ALX/FPR2 as well as mice deficient in Fpr2 (the murine isoform of ALX/FPR2), and this has provided insights into the biological role of proresolving receptors (Serhan and Chiang, 2013). It should be noted that the substrate precursors of SPM, such as EPA and DHA, do not activate these receptors (Figure 2) (Arita et al., 2005; Chiang et al., 2012; Dalli et al., 2013) but, rather, other receptors (i.e., GPR120), although these sensors are activated by these essential fatty acids at high micromolar concentrations. Other fatty acids, including monounsaturated fatty acids (e.g., palmitoleic acid and oleic acid) also appear to activate GPR120 (Oh et al., 2010), and, thus, receptor-mediated actions of fatty acids remain to be elucidated.

During in vivo studies evaluating SPM bioactions, it was found that, in addition to halting PMN recruitment and promoting macrophage phagocytosis, SPMs also enhance phagocyte efflux from inflamed tissues to draining lymph nodes in order to aid in host defense. When leukocytes exit the inflamed site or exudate, they traverse perinodal adipose tissue en route to local lymph nodes (Schwab et al., 2007). Excessive and persistent inflammation during this lipopassage (or failure of leukocytes to reach the lymphatics [Schwab et al., 2007] that, hence, get stuck within adipose) can lead to adipose inflammation, which may contribute to metabolic syndrome (see below). Because
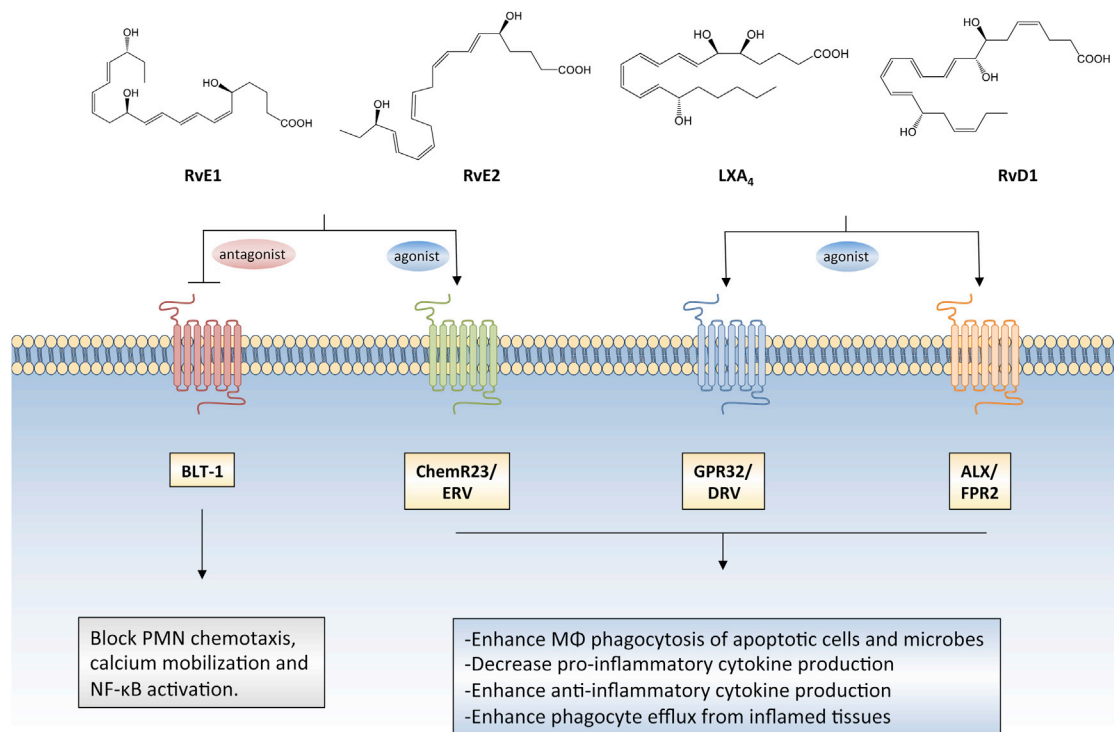
Figure 2. Proresolving Receptors and Biological Actions of SPMs
E series resolvins (RvE1 and RvE2) are agonists for ChemR23/ERV and antagonists for the leukotriene B_4 receptor, BLT-1. Lipoxin A_4 (LXA_4) and D series resolvins, RvD1, RvD3, and RvD5 (RvD1 structure shown) are agonists for GPR32/DRV1 and ALX/FPR2. Acting via these specific receptors, SPMs elicit their biological actions on leukocytes, which include the attenuation of PMN trafficking, activation, and inflammatory gene transcription as well as the stimulation of macrophage phagocytosis of both apoptotic cells and bacteria.

dysregulated inflammation is associated with many etiologically diverse diseases, it is likely that SPMs may play a critical role in preventing chronic inflammatory diseases and, potentially, organ fibrosis. We refer readers to detailed recent reviews covering SPM biosynthesis (Bannenberg et al., 2005), stereoselective receptor-dependent actions, and total organic synthesis (Serhan, 2007; Serhan and Petasis, 2011; Serhan and Chiang, 2013), given that a comprehensive analysis of these topics is outside of the scope of this review. Here, we evaluate recent evidence indicating that a failure of resolution and SPM biosynthesis contribute to chronic inflammation in the context of nutrient excess and that resolution agonists such as SPMs improve several clinically relevant outcomes in metabolic syndrome. This work posits that resolution agonists may represent a novel pharmacologic genus that is distinct from traditional anti-inflammatory therapies that impair host defense. Importantly, it also suggests that SPMs may be more effective from a therapeutic standpoint than parent omega-3 fatty acids, which are subject to multiple downstream metabolic checkpoints for eliciting their biological effects.

Defective Resolution of Inflammation in Metabolic Syndrome

Effector cells of the innate immune system, including PMNs and macrophages, are sensitive to changes in nutrient tone, and dysregulated systemic metabolism leads to chronic activation and inflammatory signaling in these cells (Lumeng and Saltiel,

2011). Likewise, robust inflammatory signaling occurs in nonimmune cells such as endothelial cells and adipocytes during states of nutrient stress (Hotamisligil, 2006). As such, chronic inflammation is associated with several diseases characterized by systemic alterations in metabolism, such as obesity, diabetes, and atherosclerosis (Rocha and Libby, 2009; Bornfeldt and Tabas, 2011). Although persistent metabolic dysfunction drives inflammation, it is clear that inflammation in turn could disrupt metabolic homeostasis. Thus, it is plausible that a deficiency in the processes that normally resolves inflammation after the induction of an inflammatory response could underlie the vicious feedforward cycle observed in chronic states of overnutrition.

Hypercholesterolemia

Hypercholesterolemia and subendothelial retention of cholesterol-rich oxidized lipoproteins in arteries lead to the recruitment and activation of macrophages, forming atherosclerotic lesions. Macrophage uptake of modified lipoproteins, coupled with defective cholesterol efflux through ATP binding cassette transporters (i.e., ABCA1 and G1), leads to the formation of cholesterol-loaded macrophage foam cells that persist in the vessel wall, initiate robust proinflammatory signaling and eventually undergo postapoptotic secondary necrosis due to defective egress or clearance by healthy phagocytes (Rocha and Libby, 2009; Bornfeldt and Tabas, 2011). It is currently held that deficient efferocytosis (apoptotic cell clearance) is related to the evolution of benign early fatty streaks (Merched et al., 2011) to more

advanced lesions containing necrotic cores, suggesting that a failure to promote efferocytosis may be a key determinant of lesion progression (Bornfeldt and Tabas, 2011). Although multiple mechanisms could underlie these defects, including a deficiency of receptors and bridging molecules involved in apoptotic cell uptake (i.e., Mertk and Mfge8), a lack of stimulatory mediators of efferocytosis could also play an important role (see below) (Bornfeldt and Tabas, 2011; Han and Ravichandran, 2011). In addition to macrophages, recent evidence indicates that PMNs also contribute to plaque progression and destabilization (Weber et al., 2008). In humans and animal models, atherosclerosis is associated with increased circulating PMN numbers, which contributes to plaque progression by increasing the generation of reactive intermediates from high levels of myeloperoxidase and promoting monocyte recruitment and dendritic cell (DC) activation (Della Bona et al., 2013). Moreover, the accumulation of apoptotic PMNs in plaques could result from deficient macrophage-mediated clearance. Thus, persistent recruitment of leukocytes coupled with a failure of phagocyte egress suggests a lack of endogenous proresolving mediators that are normally operative during acute inflammation.

Plasma levels of proresolving lipid mediator LXA_4 (aspirin-triggered form; ATL) inversely correlate with the development of both peripheral and coronary atherosclerosis in humans (Ho et al., 2010). This inverse relationship between ATL and peripheral atherosclerosis remains significant even after adjusting for age, gender, and high-sensitivity C-reactive protein. In mouse atherosclerosis models, macrophage-specific overexpression of a biosynthetic enzyme important in SPM biosynthesis (i.e., 12/15-LOX) decreases lesion formation in apolipoprotein E-deficient mice, and macrophages isolated from these transgenic mice biosynthesize more LXA_4 than WT (Merched et al., 2008). In macrophages, LXA_4 and other SPMs, including RvD1 and PD1 blunt production of cytokines such as CCL2, IFNγ, KC (murine isoform of IL-8), and IL-1β. In contrast, the phagocytosis of apoptotic cells is markedly enhanced by all three SPMs. In addition to macrophage-targeted actions, SPMs also reduce adhesion receptor expression (i.e., VCAM-1 and P-selectin) and chemokine production in isolated endothelial cells stimulated with TNFα. These atheroprotective actions were extended in more recent studies demonstrating that D-series resolvins are generated during vascular injury in vivo and that therapeutic administration of resolvins decreases intimal hyperplasia and leukocyte trafficking to injured arteries (Miyahara et al., 2013). In vitro studies demonstrated that SPMs block proliferation, migration, monocyte adhesion, and inflammatory signaling in human primary vascular smooth muscle cells in a receptor-dependent manner. Collectively, these findings demonstrate that the perpetuating inflammatory events that lead to advanced atherosclerosis may be related to the defective biosynthesis of mediators that resolve local inflammation and promote efferocytosis. Importantly, they highlight that resolution agonists (e.g., resolvins) may be novel immunomodulators that could "resolve" chronic inflammation induced by hypercholesterolemia.

Nonesterified Free Fatty Acids and Triglycerides

Similar to hypercholesterolemia, high circulating levels of nonesterified free fatty acids (FFAs) associated with obesity and type 2 diabetes also lead to the profound activation of inflammatory signaling in both immune and nonimmune cells. The liberation

of FFAs via lipolysis of triglyceride stores in insulin-resistant adipocytes drives macrophage recruitment to hypertrophied adipose tissue and promotes classical activation, whereas even an acute elevation of FFAs has been shown to increase macrophage accumulation in the heart (Ko et al., 2009; Gregor and Hotamisligil, 2011). Ectopic triglyceride accumulation in other tissues, such as the skeletal muscle and liver, is also associated with increased inflammatory signaling and leukocyte accumulation. Results from several studies demonstrate that pattern recognition receptors important in sensing exogenous pathogens are activated by FFAs in micromolar concentrations. For instance, high levels of saturated FFAs activate TLR2 and TLR4, which leads to immune cell activation, production of inflammatory cytokines, and defective insulin signaling (Nguyen et al., 2007). Indeed, TLR4-null mice are protected against insulin resistance and adipose inflammation in response to acute FFA challenge (Shi et al., 2006). More recently, nod-like receptors (NLRs), which are intracellular receptors and key components of inflammasome, are implicated as sensors of FFAs. Activation of the NLRP3-ASC inflammasome by FFAs in hematopoietic cells leads to the production of IL1-β and IL-18, and mice deficient in inflammasome components, namely *Pycard* and *Nlrp3*, are protected from systemic insulin resistance and hyperglycemia in the context of obesity (Wen et al., 2011). Hence, nutrient sensing by pattern recognition receptors sustains inflammation, which contributes to the development of insulin resistance. However, it should be noted that several other mechanisms have been proposed to mediate the diverse signaling roles of FFAs, such as alterations in membrane fluidity, nuclear receptor activation, and lipid raft formation (Hotamisligil, 2006; Li et al., 2009). Moreover, because circulating FFAs encompass a diverse array of both saturated and unsaturated fatty acids, their overall inflammatory potential is likely to be regulated by the relative distribution of FFA species in vivo.

Consistent with increased inflammatory signaling via elevated FFAs in leukocytes, we recently documented that saturated FFAs promote PMN survival during acute inflammation (Hellmann et al., 2013). We utilized an acute model of sterile peritonitis because we established a set of resolution indices (Bannenberg et al., 2005) that serve as useful criteria for establishing how specific components of resolution are modulated in different scenarios (e.g., experimental inflammation-modifying drugs, etc.). These indices include Ψ_{max}, which represents the magnitude of maximal PMN infiltration; T_{max} (the time point at which PMNs reach their maximum levels), T_{50} (the time point at which PMNs decline to half of their maximum value), and R_i (resolution interval; the period of time during which PMNs decrease to half of their maximum value) (Bannenberg et al., 2005). Using this system, we determined that, despite similar infiltration of PMNs during acute inflammation in obese diabetic mice, PMNs accumulate during the phase at which they normally undergo apoptosis and are cleared from the inflamed site (Figures 3A and 3B). The incubation of human PMNs with saturated FFAs promotes signaling via the extracellular signal-related kinase pathway and decreases caspase-3 cleavage—effects that are similar to that of lipopolysaccharide (LPS)-mediated activation of TLRs. Given that increased PMN survival is associated with the delayed resolution of inflammation, these results suggest that chronically elevated FFAs may modulate the resolution of
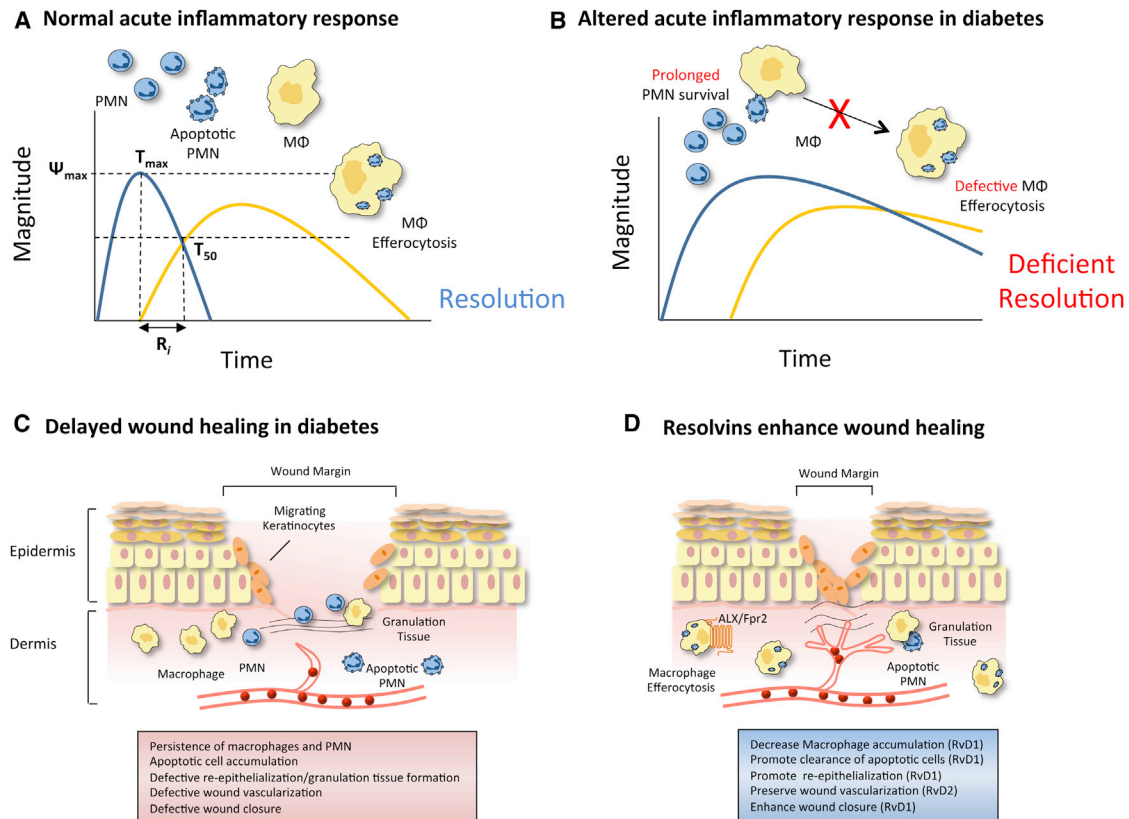
Figure 3. Altered Resolution of Inflammation in Diabetic Wounds

(A) Cellular events during a normal acute inflammatory response depicting the rapid macrophage-dependent clearance of apoptotic neutrophils (PMN). Established resolution indices are shown, with Ψ_{max} representing maximum PMN infiltration, T_{max} representing the time point at which PMN reach their maximum levels, T_{50} representing the time point at which PMN decline to half of their maximum value, and R_i representing the resolution interval, the period of time during which PMNs decrease to half of their maximum value; see Bannenberg et al. (2005) for details.

(B) Altered resolution of acute inflammation in the context of obesity and diabetes in which PMN apoptosis is delayed and macrophage efferocytosis is defective.

(C) Depiction of defects in the wound-healing program in diabetes, including persistent leukocyte and apoptotic cell accumulation and defective wound closure.

(D) Enhancement of wound healing in diabetes by SPMs by promoting macrophage-mediated apoptotic cell clearance and re-epithelialization.

acute inflammation (Serhan et al., 2007). Interestingly, other studies have recently documented that proresolving lipid mediators, such as RvE1, override survival signaling induced by inflammatory mediators (i.e., serum amyloid A) and, thus, enhance resolution (El Kebir et al., 2012). Hence, in contrast to rapid apoptosis and clearance of PMNs during acute inflammation in health, the altered metabolic environment in obesity and type 2 diabetes leads to chronic leukocyte accumulation in part from prolonged survival at sites of inflammation; e.g., within the adipose tissue. This continued presence of activated PMNs can precipitate unintentional tissue injury.

Along with promoting low-grade inflammation, nutrient excess also paradoxically impairs host defense and promotes leukocyte dysfunction. Indeed, results from several studies show that both obesity and diabetes are associated with increased susceptibility to respiratory, skin, odontogenic, and postsurgical nosocomial infections (Falagas and Kompoti, 2006). As noted, innate immune cells, such as macrophages and PMNs, play critical roles in bacterial containment via phagocytosis and lysosomal-dependent bacterial killing. Defects in macrophage phagocytosis have been established in animal models of obesity and diabetes, and the exposure of macrophages to saturated FFAs

induces defects in both FcR-mediated phagocytosis and apoptotic cell uptake (O'Brien et al., 2002, 2006; Li et al., 2009; Khanna et al., 2010; Hodgson et al., 2011). Recently, we demonstrated that macrophage-mediated clearance of apoptotic thymocytes, as well as IgG-opsonized zymosan, is defective in obese diabetic mice (Hellmann et al., 2013; Tang et al., 2013). In these studies, we also observed a persistence of apoptotic PMNs and zymosan in the peritoneum during acute sterile inflammation. Given that the macrophage-mediated clearance of apoptotic cells is required to resolve inflammation, defects in this specific process in obesity and diabetes suggests that endogenous mediators involved in active stimulation of immunologically silent efferocytosis are diminished (Figures 3A and 3B). These results in the setting of acute sterile inflammation largely recapitulate findings in models of chronic inflammation, such as atherosclerosis (see above), suggesting that this and related acute models may be useful in identifying molecular events in inflammation that are dysregulated by nutrient excess.

Although the precise molecular mechanisms underlying defects in macrophage phagocytosis induced by saturated FFAs are not complete, recent results indicate that autocrine production of prostanoids, such as PGE_2 and PGD_2 may play a role.

These mediators have roles in blunting macrophage phagocytosis by receptor-mediated activation of cAMP pathway, effects that are in part due to the downstream activation of phosphatase and tensin homolog on chromosome 10 and subsequent inactivation of phosphatidyl inositol 3-kinase (PI3K) (Canetti et al., 2007). Our recent results demonstrate that FFAs drive inflammatory signaling, which leads to the upregulation of COX-2 and subsequent production of PGE_2 and PGD_2 in macrophages, and that autocrine actions of these prostanoids are causally related to defective phagocytosis induced by FFAs (Hellmann et al., 2013). Li et al. (2009) found that the ratio of omega-6 to omega-3 polyunsaturated fatty acids in macrophages may be an important determinant of defective phagocytosis induced by FFAs. Although altered uptake of apoptotic cells was evident in macrophages incubated with FFAs, these defects were restored by the addition of EPA and DHA. Although alterations in membrane fluidity could play a role in the regulation of phagocytosis at higher local concentrations by these diverse fatty acids (Calder, 2013), it is also possible that substrate diversion to anti-inflammatory and proresolving lipid mediators may partly underlie the protective effects of omega-3 polyunsaturated fatty acid in this context, given that SPMs are biosynthesized during macrophage phagocytosis (Chiang et al., 2012; Serhan and Chiang, 2013). In support of this view, defects in diabetic macrophage phagocytosis are acutely reversed by RvD1 in a receptor-dependent manner (Tang et al., 2013). The activation of ALX/FPR2 by RvD1 blocks cAMP accumulation via coupling ALX/FPR2 to $G_{\alpha i}$ and the subsequent inhibition of adenylate cyclase (Krishnamoorthy et al., 2010; Hellmann et al., 2013; Tang et al., 2013). Given that cAMP-PKA signaling impairs phagosome formation at the level of PI3K, it is likely that the chronic activation of this pathway may underlie an inability of macrophages to undergo phagocytosis in the context of nutrient excess. Indeed, FFAs alter tyrosine phosphorylation of the regulatory p85 subunit of PI3K in macrophages, which is related to defective efferocytosis (Li et al., 2009). This dysfunction and its correction by SPMs is potentially of high significance, given that defects in phagocytosis lead to apoptotic cell accumulation and bacterial proliferation and are thus associated with atherosclerosis, autoimmunity and increased susceptibility to infections. These results also highlight that temporal imbalances between proinflammatory versus proresolving lipid mediators sustains inflammation.

Hyperglycemia

The defining metabolic feature of both type 1 and 2 diabetes is hyperglycemia, which is due to the deficient insulin production or systemic insulin resistance, respectively. Because of the highly integrated nature of glucose and lipid metabolism, it is not surprising that the changes in lipid metabolism in states of obesity and nutrient excess lead ultimately to systemic hyperglycemia. The common downstream effector in these conditions of nutrient stress is uncontrolled inflammation, which most likely explains the strong association of diabetes with increased risk of inflammatory diseases, such as cardiovascular disease and cancer. With respect to cardiovascular disease, recent studies suggest that hyperglycemia regulates myelopoiesis and sustained monocyte recruitment to atherosclerotic lesions (Nagareddy et al., 2013). Interestingly, this enhanced myelopoiesis results from the increased production of inflammatory mediators (S100A8) released by PMNs. Other recent results demonstrate that mac-

rophages isolated from type 1 diabetic mice exhibit an inflammatory phenotype associated with the increased expression of long-chain acyl-CoA synthetase 1 (ACSL1) and that myeloid deletion of *Acsl1* decreases atherosclerosis in the context of type 1 diabetes (Kanter et al., 2012). Mechanistically, hyperglycemia induces ACSL1 in macrophages, which results in increased AA-CoA esters and the production of inflammatory lipid mediators, such as PGE_2. The production of PGE_2 enhances inflammatory signaling in macrophages and is markedly reduced in *Acsl1*-deficient macrophages. These results are in alignment to those obtained with macrophages incubated with the FFAs reviewed above, indicating that sustained production of proinflammatory lipid mediators may be a common downstream pathway in macrophages activated by nutrient stress.

Similar to elevated levels of FFAs, hyperglycemia also promotes leukocyte dysfunction. Hyperglycemia impairs PMN chemotaxis, yet it increases the production of superoxide and inflammatory cytokines. In Akita mice, which are a model of human type 1 diabetes, increased PMN adherence to the microvasculature and associated tissue damage were observed in a model of ligature-induced bone loss (Gyurko et al., 2006). In macrophages, hyperglycemia impairs the phagocytosis of both apoptotic cells and bacteria, and nonobese diabetic mice have a deficiency in the clearance of apoptotic cells (Abrass and Hori, 1984; O'Brien et al., 2002; O'Brien et al., 2006). Given that both hyperglycemia and elevated FFA levels initiate inflammatory signaling, which gives rise to PGE_2 production in macrophages (see above), these results suggest that autocrine actions of proinflammatory lipid mediators may underlie phagocyte defects in the context of nutrient excess. Thus, leukocyte dysfunction leading to persistent inflammation and altered resolution are common features of nutrient excess and play a causal role in promoting chronic metabolic disease.

Delayed Resolution of Inflammation in Diabetic Wounds

The complete resolution of tissue injury involves a temporally coordinated program that is evolutionarily conserved. After tissue damage from blunt trauma, surgical incision, burn, or ischemia, a prompt response is mounted that has the ultimate goal of returning tissue(s) to their previous state. Although, in some tissues and organisms, the regeneration process is complete, in most cases, a fibrotic scar is left at the site of injury after the host-protective inflammatory response has resolved (Figure 1A). After primary hemostasis, there is an early infiltration of PMNs, which carry an armament for destroying foreign invaders. As noted above, endogenous counterregulatory mediators such as SPMs regulate the magnitude of the PMN infiltrate appropriate for the level of injury and/or pathogen load. Subsequent infiltration of macrophages leads to the clearance of apoptotic cells and tissue debris, and macrophages also assist with pathogen eradication and participate in notifying the adaptive immune system of a potential threat through differentiation into antigen-presenting cells (i.e., monocyte-derived DCs). Macrophages persist in wounds for an extended period of time and exist in multiple phenotypic states in order to carry out specific tasks in the wound-healing program, such as tissue remodeling, revascularization, and fibrosis (Mosser and Edwards, 2008; Brancato and Albina, 2011). It has recently been shown that so-called "inflammatory" monocytes, which in mice are defined

as Ly6C^{hi}CCR2^{hi}CX3CR1^{low}, have considerable plasticity and differentiate into reparative monocytes and macrophages during the resolution of fibrosis and tissue regeneration (Ramachandran et al., 2012; Godwin et al., 2013; Nahrendorf and Swirski, 2013; Wynn et al., 2013). Ultimately, blood supply is restored to the injured tissue, and carefully orchestrated tissue fibrosis ensues. In the case of cutaneous wounds, re-epithelialization and the reinstatement of barrier function proceeds with the extent of scar tissue formation related to the depth of the wound.

Several steps in the intricately orchestrated wound-healing program are disrupted in the context of metabolic disease, and defective wound healing (accompanied by tissue necrosis and infection) is one of the most prominent clinical manifestations of diabetes (Jeffcoate and Harding, 2003). Because of peripheral neuropathies and both macrovascular and microvascular disease, diabetics often sustain wounds in the extremities. Insufficient blood supply increases susceptibility to wounds and also impairs wound healing, and, thus, clinical management of tissue vascularization through vascular and endovascular surgical procedures is often necessary in order to prevent chronic ischemia. Obesity and diabetes are both associated with increased risk of infection in open wounds, which can be related to defects in leukocyte-mediated pathogen killing and containment (Figure 3C). In fact, diabetic wounds are usually characterized by excessive leukocyte accumulation, indicating that leukocyte dysfunction and failure to promote phagocyte egress may be important determinants of delayed wound healing in diabetes (Figure 3C) (Wetzler et al., 2000; Khanna et al., 2010).

Recently, we reported that SPM biosynthetic pathways are perturbed in the wounds of obese diabetic mice. In a murine model of cutaneous excisional wound healing, obese diabetic mice had significant defects in wound closure, and this was associated with decreased conversion of DHA to intermediates and pathway biomarkers involved in the biosynthesis of maresins, resolvins, and protectins; e.g., 14-HDHA and 17-HDHA (Tang et al., 2013). Leukocytes and apoptotic cells accumulate in the wounds of diabetic mice, and, because the clearance of apoptotic cells is a primary feature of active resolution of inflammation, we asked whether SPMs would enhance apoptotic cell clearance and wound closure. Indeed, local application of RvD1 significantly decreases apoptotic cells and macrophages in diabetic wounds, and this translates into enhanced wound closure and granulation tissue formation (Figure 3D). RvD1 rescues the defective clearance of apoptotic cells in the thymus of diabetic mice, indicating that correcting phagocyte defects is a primary protective action of SPMs in diabetes. In addition to the clearance of apoptotic cells, RvD1 rescues defective FcR-mediated phagocytosis, suggesting that SPMs can also serve as effective adjunctive therapeutics to antibiotics in the context of diabetic wound infection. Along these lines, cotreatment of wild-type mice with ciproflaxacin and RvD5 significantly enhances survival in the context of *Escherichia coli* infection in comparison to antibiotic treatment alone, whereas RvD1, RvD5, and PD1 enhance vancomycin-mediated clearance of *Staphylococcus aureus* during skin infection (Chiang et al., 2012). Altogether, these findings suggest that defective SPM biosynthesis in diabetes may be related to increased susceptibility to wound infection and prolonged inflammation.

In addition to macrophage phagocytosis, SPMs regulate other distinct cellular events during the wound-healing program and may have actions on nonimmune cells as well. For instance, RvD1 enhances human keratinocyte migration in vitro, which is a critical step in wound closure (Norling et al., 2011). Lipoxygenase-mediated biosynthesis of MaR1, which is the newest member of the SPM genus, was recently uncovered during tissue regeneration in brown planaria (*Dugesia tigrina*) subject to surgical injury (Serhan et al., 2012). Adding synthetic MaR1 back significantly enhances tissue regeneration, suggesting that SPMs play an evolutionarily conserved role in wound remodeling and tissue regeneration. As noted, resolvins, specifically RvD1, RvD2, and RvD5, potently regulate bacterial containment by phagocytes, and, in support of this notion, RvD2 was found to enhance survival in a rodent model of cutaneous burn injury (Bohr et al., 2013; Kurihara et al., 2013) and sepsis (Spite et al., 2009). Treatment of burn wounds with RvD2 largely prevents dermal necrosis and preserves the vasculature, whereas untreated mice show progressive tissue necrosis and thrombosis in the deep dermal vascular network. Mechanistically, RvD2 rescues defective directional migration of PMNs isolated from burn wounds and simultaneously decreases proinflammatory cytokine production by PMNs. These findings suggest that SPM display diverse roles in wound-healing programs and that they may be effective mediators in modulating innate cells (e.g., phagocytes) that could rescue defective wound healing in the context of metabolic disease and prevent tissue loss and susceptibility to infection.

Nonresolving Adipose Tissue Inflammation

Adiposity is a dominant risk factor for the metabolic syndrome and related comorbidities. In mammals, the two types of fat tissue are white adipose tissue (WAT) and brown adipose tissue (BAT). WAT is characterized by the presence of adipocytes containing large unilocular lipid droplets, whereas BAT is mainly composed by multiloculated adipocytes containing large numbers of mitochondria (Rosen and Spiegelman, 2006). WAT is widely distributed through the body, and its main function is to store excess energy as triglycerides. In contrast, BAT is located in discrete pockets and is specialized to generate heat by dissipating chemical energy and counteracting hypothermia (Rosen and Spiegelman, 2006). However, BAT is difficult to find in adult humans, given that brown fat pads existing within the posterior neck in neonatal humans to provide cold adaptive nonshivering thermogenesis for newborns are lost soon after birth (Cristancho and Lazar, 2011).

WAT is mainly recognized for its role as a major secretory organ responsible for lipolysis and release of fatty acids in the circulation. WAT is the major source of fatty acids that are used as energy substrates for generating ATP (Redinger, 2009). Until the last decade, energy storage and lipolysis were seen essentially as the unique roles of WAT. Nowadays, WAT is regarded as a highly active metabolic tissue and an important endocrine organ involved in the balance of body homeostasis beyond the paradigm of fuel storage. Indeed, since the cloning of the "*ob*" gene coding for leptin in 1994 (Zhang et al., 1994), an ever-increasing number of hormones, signaling peptides, and lipid mediators secreted from WAT, now numbering more than 50 different molecular entities, have been identified. These molecules, collectively known as adipokines, are widely

recognized as key regulators of inflammation and immune functions, blood pressure homeostasis and metabolic processes such as appetite and satiety as well as glucose and lipid metabolism.

Adiposity develops when food availability exceeds the metabolic demand ("nutrient excess"). In this setting, adipocytes expand nearly 1,000-fold in volume and 10-fold in diameter in order to store the excess of fuel as triglycerides (Redinger, 2009). In the setting of obesity, an imbalance between oxygen supply and demand in enlarged adipocytes inadvertently triggers tissue hypoxia, which initiates a cascade of events leading to chronic "low-grade" inflammation in the adipose tissue (Eltzschig and Carmeliet, 2011). This "low-grade" inflammation can be regarded as a long-term inflammatory response triggered by nutrients and metabolic surplus and, therefore, is also known as "metabolic-triggered inflammation" or "metainflammation" (Hotamisligil, 2006). Metabolic inflammation is usually not systemic, but it may affect disease processes in target organs such as the liver and pancreas and increase the incidence of classical inflammatory diseases such as rheumatoid arthritis (Jeppesen, 2011; Gukovsky et al., 2013). It involves molecules and pathways similar to those of classical inflammation, but, in this case, these signals play a dual role as inflammatory mediators as well as regulators of energy storage and metabolism. A rise in proinflammatory adipokines such TNFα, IL-6, IL-1β, MCP-1, leptin, and resistin, accompanied by a reduction in the anti-inflammatory and insulin-sensitizing adipokine adiponectin, has been reported to signal the onset of metabolic dysfunction (Ouchi et al., 2011). This unbalanced secretion of inflammatory adipokines results in the recruitment of circulating inflammatory cells, especially macrophages, into WAT, thus perpetuating an inflammatory vicious cycle.

The ability of adipose tissue to produce bioactive local mediators derived from enzymatic oxygenation of polyunsaturated fatty acids was first described in the late 1960s (Shaw and Ramwell, 1968). PGE_2 is an abundant COX product in adipose tissue, where it plays a role in adipogenesis and lipolysis, although its role in WAT inflammation remains unclear. Interestingly, 5-LOX products are identified to play a proinflammatory role in adipose tissue. Indeed, all enzymes necessary for the formation of 5-LOX products (5-LO, 5-LO-activating protein [FLAP], LTA_4 hydrolase, and LTC_4 synthase), as well as receptors involved in LT signaling (BLT-1, BLT-2, CysLT1, and CysLT2), are present in adipose tissue (Horrillo et al., 2010). FLAP overexpression and excessive generation of 5-LOX products are common findings in adipose tissue of obese patients and animals with insulin resistance (Bäck et al., 2007; Horrillo et al., 2010; Chakrabarti et al., 2011). In obese adipose tissue, a direct connection between LTB_4 and enhanced release of the adipokines, MCP-1 and IL-6 has been established, and mice deficient in LTB_4 receptor BLT-1 show reduced monocyte recruitment to hypertrophied adipose tissue (Horrillo et al., 2010; Spite et al., 2011). Consistently, the inhibition of the 5-LOX pathway with a selective FLAP inhibitor or genetic deletion of BLT-1 alleviates adipose tissue inflammation and insulin resistance in obesity (Horrillo et al., 2010; Spite et al., 2011). In addition to 5-LOX, inflammatory lipid mediators generated from AA via 12/15-LOX (i.e., 12-HETE) also play an important role in the development of adipose tissue inflammation in obesity (for review, see Cole et al., 2013). This

further emphasizes that lipid mediator pathways are skewed toward the continued production of inflammatory lipid mediators in the context of nutrient excess and that the "lipid mediator tone" (see below) may be an important determinant as to whether inflammation resolves or persists in obesity.

In addition to the heightened production of proinflammatory lipid- and peptide-derived chemical mediators (i.e., LTB_4, IL-6, TNFα, and MCP-1), unresolved inflammation in obese adipose tissue also appears to be associated with impaired biosynthesis of anti-inflammatory mediators. Indeed, obesity entails a consistent reduction in the circulating levels of adiponectin, one of the few adipokines that possesses anti-inflammatory and insulin-sensitizing properties (Trayhurn and Wood, 2005). Notably, a deficit in SPM levels is also present in adipose tissues from patients with metabolic syndrome and experimental models of obesity and insulin resistance. In particular, recent studies have uncovered the existence of a marked deficit in PD1 and its precursor 17-HDHA in subcutaneous fat from patients with peripheral vascular disease in whom the inflammatory status in adipose tissue is remarkably exacerbated in comparison to healthy subcutaneous fat (Clària et al., 2013). In this study, LC-MS/MS-based metabolo-lipidomic analyses of fat from selected human anatomic locations identified unique signature profiles in the content of bioactive lipid mediators. Importantly, these analyses demonstrated a heterogeneous capacity for SPM biosynthesis among different adipose tissue depots, a higher activation of resolution circuits occurring in perivascular fat in comparison to subcutaneous fat (Clària et al., 2013). This is relevant for vascular pathologies because, for its tissue mass and anatomic proximity surrounding systemic vessels, perivascular adipose tissue plays an emerging role in vascular biology homeostasis. A failure in the endogenous anti-inflammatory peptide annexin A1 to respond to increased systemic inflammation has also been demonstrated in human obesity (Kosicka et al., 2013). At the experimental level, a deficit in tissue SPM levels (RvD1, PD1, and 17-HDHA) has been characterized in inflamed visceral and subcutaneous fat compartments from *ob/ob* obese and *db/db* obese diabetic mice (González-Périz et al., 2009; Neuhofer et al., 2013). Given that SPM autacoids are made locally, act in their surrounding tissue milieu, and are metabolically inactivated, the loss of SPM in obese adipose tissue can be the consequence of omega-3 fatty acid deficiency in the tissue, which are substrates for SPM biosynthesis (Canetti et al., 2007). Along these lines, the transgenic restoration of omega-3 fatty acids reversed the inefficient resolution capacity in adipose tissue from obese mice (White et al., 2010). Alternatively, the loss of SPMs in obesity may reflect accelerated tissue SPM conversion and clearance to inactive further metabolites because 15-PG-dehydrogenase (also known as eicosanoid oxidoreductase), a key enzyme in SPM inactivation, is markedly upregulated in obese adipose tissue (Clària et al., 2012). In adipose tissue, both RvD1 and RvD2 are each further metabolically converted to oxo-resolvin products, some of which appear inactive (Clària et al., 2012). It is noteworthy that SPM deficiencies in obesity appear to be a generalized defect in all metabolic tissues, given that, in addition to adipose, deficiencies are noted in the liver, cutaneous wounds, and skeletal muscle (White et al., 2010; Tang et al., 2013). Collectively, these findings are consistent with the notion that unresolved chronic "low-grade"

inflammation in obese adipose tissue is the result of inappropriate resolution-capacity allowing the inflammatory response to proceed without controlled checkpoints.

Consistent with the notion that defective SPM biosynthesis promotes adipose tissue inflammation, the administration of exogenous SPM successfully rescues the impaired resolution capacity of obese adipose tissue. In this regard, the administration of nanogram doses of RvD1 to *db/db* obese diabetic mice improves glucose tolerance, decreases fasting blood glucose, and increases insulin-stimulated Akt phosphorylation in adipose tissue (Hellmann et al., 2011). This SPM also reduces the formation of crown-like structures rich in inflammatory macrophages in adipose tissue. Similarly, intraperitoneal (i.p.) injection (nanogram amounts) of RvE1 to obese *ob/ob* mice confers significant insulin-sensitizing effects by mechanisms related to the AMPK-adiponectin axis and the induction of GLUT-4 and IRS-1 expression (González-Périz et al., 2009). Also, 17-HDHA treatment (i.p. injection of nanogram doses) reduces adipose tissue expression of inflammatory cytokines (MCP-1, TNFα, IL-6, and osteopontin), increases adiponectin expression, and improves glucose tolerance in parallel to insulin sensitivity in *db/db* obese diabetic mice (Neuhofer et al., 2013). Ex vivo, in fat explants, RvD1 and RvD2 each rescue the impaired phenotype of obese adipose tissue by enhancing the expression and secretion of adiponectin in parallel with decreasing the secretion of proinflammatory adipokines and cytokines including leptin, TNFα, IL-6, and IL-1β (Clària et al., 2012). Using adipose tissue explants from aging female mice as a model of age-associated adipose inflammation, Börgeson et al. (2012) recently discovered that LXA$_4$ in nanomolar concentrations decreases IL-6 while restoring GLUT-4 and IRS-1 expression, indicating improved inflammation and insulin sensitivity. Additional investigations reveal that LXA$_4$ preserves Akt signaling and glucose uptake in cultured adipocytes. In human monocyte-adipocyte coincubations, both RvD1 and RvD2 reduce MCP-1- and LTB$_4$-stimulated monocyte adhesion to adipocytes as well as monocyte transadipose migration (Clària et al., 2012). These interactions between monocytes and adipocytes are likely events in the progression of inflamed adipose tissue (see below). Importantly, RvD1 stimulates macrophage phagocytosis and enhances the phagocytic activity of macrophages isolated from the adipose tissue stromal vascular cell fraction (Titos et al., 2011). A summary of the main actions of SPMs uncovered to date in adipocytes and macrophages from the stromal vascular cell fraction is illustrated in Figure 4. Altogether, these findings suggest that the lack of intrinsic capacity of adipose tissue to produce endogenous "stop signals" required in the resolution of inflammation is a critical factor(s) that can contribute to obesity-linked inflammation and insulin resistance.

An intriguing aspect of adipose tissue inflammation is that the inflammatory response in this organ and tissue appears to be a unique process driven in large part by tissue macrophages (Lumeng and Saltiel, 2011). Indeed, the presence of an increased number of adipose-tissue-infiltrating macrophages, which form the characteristic "crown-like structures" that surround necrotic adipocytes and scavenge adipocyte debris, is a hallmark of obesity (Cancello et al., 2005). Although enlarged adipocytes were initially thought to be the cellular source of proinflammatory mediators in obesity, it was later established that infiltrated mac-

rophages in obese fat are the major driver of exacerbated production of proinflammatory mediators (Odegaard and Chawla, 2013). The contribution of other inflammatory cell types, including T lymphocytes and eosinophils, to adipose tissue inflammation and homeostasis has also been acknowledged (Odegaard and Chawla, 2013). In addition to the augmented infiltration of macrophages into adipose tissue, obesity also induces a phenotypic switch in these cells toward the classically activated M1 phenotype (Lumeng and Saltiel, 2011). Depending on the disease stage and the signals they are exposed to, macrophages are broadly characterized by their activation (polarization) status according to the M1-M2 classification system (Mosser and Edwards, 2008). On the basis of this classification, the M1 designation is reserved for classically activated macrophages after stimulation with IFNγ and LPS, whereas the M2 designation is applied to the alternatively activated macrophages after in vitro stimulation with IL-4 and IL-13. M1 macrophages display enhanced proinflammatory biosynthetic capacity and increased superoxide anion (O$_2^-$) levels (Mosser and Edwards, 2008). Conversely, M2 macrophages produce less proinflammatory cytokines and are essential for tissue repair and resolution of inflammation. M1-M2 macrophage polarization can be easily monitored in rodents by assessing the expression of selected markers. M1-associated genes include inducible nitric oxide synthase (*iNos*) and classical proinflammatory mediators such as *Tnfα*, *Il-1β*, *Il-6*, and *Ccl2*. M2 macrophages display the upregulation of scavenger, mannose (CD206) and galactose (*Mgl-1*) receptors, arginase 1 (*Arg-1*), which antagonizes iNOS activity, and *Il-10* as well as the upregulation of other genes such as chitinases *Ym1* and *Ym2* and resistin-like molecule (*Relm*) α (also known as FIZZ) (Mosser and Edwards, 2008). Notably, *Alox15* is an IL-4- and 1L-13-responsive gene, and recent transcriptomics analyses have demonstrated that *Alox15* is highly upregulated in macrophages isolated during the resolution of acute inflammation (Stables et al., 2011). Along these lines, Th2-skewed human peripheral blood monocytes express high levels of *Alox15* and produce PD1 from endogenous DHA (Ariel et al., 2005). Moreover, M2 macrophages produce elevated SPM levels, including MaR1, PD1, and RvD5 in comparison to M1 polarized macrophages in vitro, as assessed with metabolipidomic analysis (Dalli and Serhan, 2012).

In line with SPM protective actions against adipose tissue inflammation and in addition to their increased biosynthesis by M2 macrophages, SPMs skew adipose tissue macrophages toward an M2 phenotype. RvD1 upregulates a panel of M2 markers that includes *Il-10*, CD206, *Relm-α*, and *Ym1* in macrophages from obese adipose tissue (Figure 4) (Titos et al., 2011). RvD1 also remarkably stimulates *Arg-1* expression while promoting nonphlogistic macrophage phagocytosis and attenuating IFNγ- and LPS-induced Th1 cytokine secretion (Titos et al., 2011). Similar findings have been reported on the ability of RvD1 to improve insulin sensitivity by increasing the percentage of macrophages expressing the M2 marker Mgl-1 in adipose tissue from obese diabetic mice (Hellmann et al., 2011). The ability of SPMs to modify macrophage plasticity has also been demonstrated by Schif-Zuck et al. (2011). The administration of RvD1 or RvE1 to mice enhances appearance of CD11b^{low} macrophages during acute peritonitis by reducing the number of engulfment-related events required for macrophage
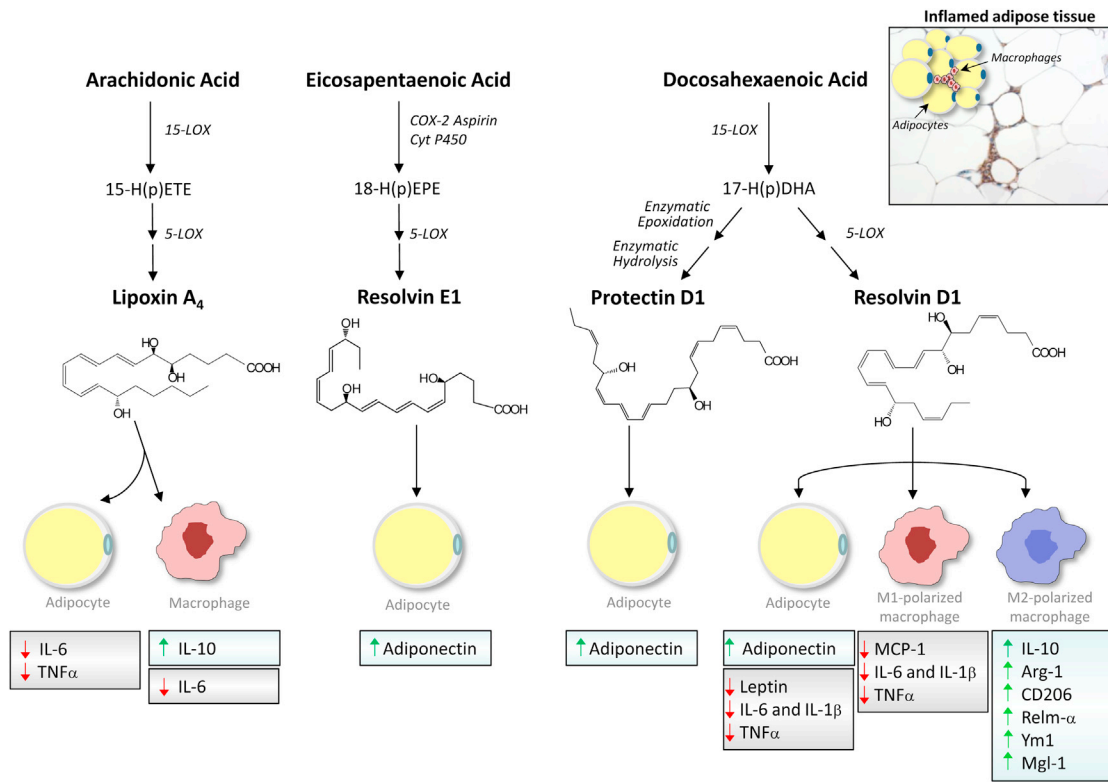
Figure 4. Biosynthesis and Biological Role of SPMs in Adipose Tissue
In the adipose tissue, the major long-chain polyunsaturated fatty acids, arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid are endogenously converted into potent anti-inflammatory and proresolving SPM lipoxin A_4, resolvin E1, protectin D1, and resolvin D1. In inflamed adipose tissue, these mediators evoke cell-specific regulatory actions on adipokine expression and/or secretion by adipocytes and macrophages. Resolvin D1 also promotes macrophage polarization toward the anti-inflammatory M2 phenotype. A section of adipose tissue immunostained with an F4/80 antibody, depicting a remarkable infiltration of macrophages forming "crown-like" structures within surrounding adipocytes, is shown in the upper right corner. COX-2, cyclooxygenase-2; Cyt P450, cytochrome P450; LOX, lipoxygenase; IL, interleukin; TNFα, tumor necrosis factor α; MCP-1, monocyte chemoattractant protein-1.

deactivation and by reducing the ability of peritoneal macrophages to produce proinflammatory cytokines upon LPS stimulation. Altogether, the ability of SPMs to modulate the plasticity of tissue macrophages offers new opportunities for facilitating the resolution of adipose tissue inflammation in metabolic diseases.

Steatohepatitis: Failed Resolution of Metabolic Liver Disease?

Nonalcoholic fatty liver disease (NAFLD) is a condition ranging from steatosis or simple accumulation of triglycerides in the cytoplasm of hepatocytes to steatosis combined with inflammation (steatohepatitis or NASH) in the absence of excessive alcohol consumption (Day, 2011). NAFLD is considered to be the hepatic manifestation of the metabolic syndrome, given their prevalence is coincidental in western society (Day, 2011). The strong association between NAFLD and obesity has been well documented in the European DIONYSOS study cohort (3,000 participants), in which NAFLD was present in 25% of participants with a normal weight (body mass index [BMI] 20.0–24.9 kg/m^2), 67% of overweight participants (BMI 25.0–29.9 kg/m^2), and 94% of participants with obesity (BMI ∼30 kg/m^2) (Bedogni et al., 2005). Although hepatic steatosis is generally an asymptomatic premorbid condition, it increases the vulnerability of the liver to

progress to more advanced and irreversible forms of liver disease (Day, 2011). Steatotic livers are indeed more susceptible to tissue-damaging effects of oxidative stress and inflammatory mediators, which pave the way for progressive liver damage into NASH, fibrosis, and, ultimately, cirrhosis (Day, 2011). In addition to increased FFA release and exacerbated production of proinflammatory adipokines by adipose tissue (Tilg and Moschen, 2008; Gregor and Hotamisligil, 2011), hepatic insulin resistance and hepatic steatosis in obesity are also driven by the activation of classical inflammatory lipid-mediator pathways. This view is consistent with earlier observations reporting that omega-6-derived eicosanoids, especially LTB$_4$, play a role in the progression of metabolic diseases. Indeed, leukotrienes would promote hyperlipidemia-dependent vascular complications and represent a risk factor in atherosclerosis (Zhao et al., 2004). Given that the sequence of molecular and cellular events underlying atherosclerosis (i.e., lipid accumulation, mounting inflammation, and progression to fibrous plaque in the arterial vessel wall) is fundamentally similar to that described for NAFLD, it was not surprising that leukotrienes also contribute to metabolic liver disease. Indeed, the 5-LOX pathway is markedly activated in patients and animals with NAFLD (Puri et al., 2009; Horrillo et al., 2010). Consistently, either pharmacological or genetic inhibition of the leukotriene pathway protects against HFD-induced

inflammatory liver injury, insulin resistance, and TNFα-induced hepatocyte cell death (Horrillo et al., 2010; Martínez-Clemente et al., 2010; Spite et al., 2011).

Omega-3 fatty acid precursors and SPMs exert opposite roles in hepatic steatosis to those described for omega-6-derived eicosanoids. In this regard, the administration of an omega-3-enriched diet for 5 weeks significantly alleviates hepatic steatosis in *ob/ob* mice, an experimental model of obesity-induced insulin resistance and fatty liver disease (González-Périz et al., 2009). This antisteatotic effect is associated with improved insulin tolerance and changes in the expression of specific adipocyte-derived factors (i.e., adipokines) that orchestrate the interaction between adipose tissue and the liver (González-Périz et al., 2009). In parallel with this antisteatotic effect, omega-3-enriched diets also ameliorate inflammatory liver injury in mice (González-Périz et al., 2006). The hepatoprotective actions of omega-3 diets are associated with the increased generation of SPMs in liver tissue (i.e., PD1 and 17S-HDHA). These SPMs are able to attenuate DNA damage and oxidative stress in hepatocytes and reduce TNFα release in macrophages (González-Périz et al., 2006). A proof of concept of the beneficial role of omega-3 fatty acids in metabolic liver disease has recently been gathered in mice with the transgenic expression of the *C. elegans fat-1* gene, which encodes an omega-3 desaturase capable of generating omega-3 fatty acids from the omega-6 type (see above) (López-Vicario et al., 2013). These *fat-1* mice have a more balanced omega-6/omega-3 ratio and are protected from obesity-induced hepatic insulin resistance, steatosis and inflammation. Interestingly, transgenic *fat-1* mice show increased formation of resolvins (Hudert et al., 2006). Consistently, we reproduce the protective actions of omega-3 fatty acids against inflammatory injury observed in *fat-1* hepatocytes by incubating wild-type hepatocytes with nanomolar concentrations of RvD1 (López-Vicario et al., 2013). Little information is available on the role of other SPMs (i.e., lipoxins and maresins) in metabolic liver disease, although the liver is a rich source of LXA$_4$ and 15-epi-LXA$_4$ (Titos et al., 1999), the formation of which attenuates IL-8 secretion by hepatocytes (Planagumà et al., 2002).

Concluding Remarks

Although partial gastrectomy and other types of bariatric surgery are successful in weight loss (Carlin et al., 2013), new approaches are needed. Recent efforts have uncovered extensive interactions between immunity and metabolism and have led to the identification of salient mechanisms underlying the initiation of inflammatory signaling in the context of metabolic diseases. Less is known about the resolution of inflammation or SPM biosynthesis in the context of metabolic diseases. As discussed in the present review, some of the features of active resolution appear to be deficient in metabolic disease, and treatment with specific SPMs improves metabolism and immunity. Nevertheless, several key questions remain. Is resolution impaired in states of nutrient excess or insulin resistance? How do the proresolving mediators impact intermediary metabolism, and how are these mediators, in turn, affected by insulin resistance? How do metabolic diseases perturb lipid mediator class switching? Is this merely a problem of substrate ratios in the diet and utilization (e.g., AA versus EPA and DHA), or are there unappreciated changes in the expression or regulation of the enzymes involved in SPM biosynthesis? How does nutrient metabolism regulate SPM biosynthesis at the molecular level within adipose tissues? Given that SPM circuits are active in adipose tissue and are decreased in obesity (Clària et al., 2013), what are the roles of specific SPMs in lean noninflamed adipose tissue homeostasis?

Mapping of lipid mediator networks during the resolution of inflammation has increased our understanding of the process of resolution itself and has informed new therapeutic strategies for treating inflammation. Although animal studies show that dietary intake of omega-3 fatty acids reduces inflammation while, at the same time, improving systemic metabolism and increasing SPM biosynthesis, human studies have, in some cases, failed to consistently demonstrate a beneficial effect of omega-3 fatty acids on systemic metabolism (De Caterina, 2011). This discrepancy highlights the need for a more thorough understanding of the impact of nutrient excess on local SPM biosynthesis and SPM levels. It also raises the possibility that the use of SPMs might represent a more targeted therapeutic approach than simply increasing omega-3 fatty acids in the diet (Tabas and Glass, 2013). These fatty acids are precursors of lipid mediators that are several orders of magnitude more potent and display stereospecific biological roles in regulating inflammation (Serhan and Petasis, 2011; Serhan and Chiang, 2013). Moreover, the anti-inflammatory effects of omega-3 fatty acids are clearly regulated at multiple levels (e.g., receptors and enzymatic activities), which might be independently affected by disease. Hence, direct treatment with lipid mediators, rather than the parent omega-3-fatty acids, may be more effective in targeting inflammatory responses. The notion that nonselective or off-target effects of omega-3 fatty acids might be problematic is supported by results suggesting that, although a high intake of omega-3 fatty acids is beneficial in some contexts, it can increase the appearance of auto-oxidation products and may also increase the risk of certain types of cancer (Brasky et al., 2013), or it is possible that these disease populations are at risk because they are unable to produce SPMs.

In resolution, all SPMs have two main functions: they stop and limit further PMN entry and stimulate macrophage intake and the clearance of apoptotic cells, debris, and bacteria; hence, additional research is needed to identify how different SPMs are biosynthesized in response to inflammatory challenges within adipose tissues and how individual SPMs differ in their individual actions. Therefore, it will be interesting to determine whether different SPMs regulate distinct events in chronic inflammation and to identify how individual SPMs differ in their ability to regulate discrete processes in tissue-specific resolution program(s) and tissue regeneration. In summation, it should be pointed out that certain therapeutic strategies impact resolution, either positively (e.g., carbon monoxide and cyclin-dependent kinase inhibitors) or negatively (e.g., selective COX-2 and LOX inhibitors) (Chiang et al., 2013), and, thus, determining how the resolution of inflammation in obesity is impacted may be an essential criterion in developing future therapeutic interventions aimed at combating inappropriate inflammation in metabolic disease.

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