Control of Myeloid Cell Trafficking in Resolution

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Key Words

Apoptosis · Efferocytosis · Annexin A1 · Resolvin · G-protein-coupled receptor

Abstract

Following tissue injury or microbial invasion, neutrophils are robustly recruited to inflammatory loci, which is a hallmark of the host inflammatory response. This event initiates a series of processes required to activate resolution, including recruitment of monocytes, clearance of microbes, cellular debris and apoptotic neutrophils, the egress of phagocytes and, ultimately, regain of tissue homeostasis. Substantial evidence now signifies that resolution of inflammation is a highly coordinated, active process dictated by the spatial-temporal generation of proresolving mediators that act on specific receptors to modulate cell and tissue reactivity. This review will focus on the mediators, targets and pathways initiated to orchestrate resolution. Importantly, disruption of the key processes involved in inflammatory resolution could result in delayed restoration of tissue homeostasis, leading to fibrosis and/or persistent inflammation.

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Active Termination of Inflammation: Mediators and Their Actions on Leukocytes

It is now widely recognized within the scientific community that the host immune response to inflammation does not simply dissipate, but is actively switched off by

engagement of proresolving pathways [1]. In recent years, a repertoire of mediators has been uncovered that fall under this umbrella of effectors of resolution. These proresolving mediators include canonical mediators such as cortisol and adenosine (which will be little discussed here) as well as more recently identified lipid, protein and peptide agonists such as the lipoxins (LXs), resolvins (Rvs), annexin A1 (AnxA1) and melanocortins (MCs), amongst several others. It is important to define that a proresolving mediator stimulates and activates endogenous pathways to regulate cellular trafficking, modulate myeloid cell lifespan and phenotype, and enhance tissue restoration to accelerate resolution; as such, a proresolving mediator is not equivalent to an anti-inflammatory mediator, which acts as a blocker of specific pathways or enzymes involved in the initiation of an inflammatory response. Table 1 summarizes the proresolving effects activated by effectors of resolution on myeloid cells. Thus, in contrast to current anti-inflammatories that typically target the 'heating-up' period of the immune response, new therapeutics based on proresolution mediators would accelerate the way our body naturally regulates an inflammatory reaction, prompting the regain of physiological tissue function.

LXs were the first anti-inflammatory and proresolving lipid mediators recognized that signal the resolution of acute inflammation [2]. During the time course of inflammation, mediator generation switches from arachidonate-derived eicosanoids, such as prostaglandins and leukotrienes, to anti-inflammatory LXs [3] and omega-3 eicosapentaenoic acid and docosahexaenoic acid-derived

Table 1. Summary of the major proresolving effects activated by effectors of resolution on myeloid cells

Proresolving property	Resolution agonists	References
↓ Neutrophil migration	adenosine, AnxA1 and N-terminal peptide Ac2-26, cortisol, DHEA metabolites, E ₂ , LXA ₄ , MaR1, α-MSH, γ-MSH PD1, RvD series, RvE series	3, 8, 68–74
↑ Neutrophil apoptosis	AnxA1, LXA ₄ , RvE1	75–77
↑ Microorganism phagocytosis	AnxA1, Ac2-26, chemerin-derived peptides, LXA ₄ , MaR1, α-MSH, RvD series, RvE series	9, 30, 71, 78
↑ Monocyte recruitment	LXA ₄ , LXB ₄ , Ac2-26 and shorter peptides	17, 18
→ Macrophage skewing	adenosine, RvD1	36, 37
↑ Efferocytosis	α-MSH, adenosine, AnxA1 and N-terminal peptide Ac2-26, chemerin-derived peptides, cortisol, LysoPS, LXA ₄ , MaR1, PD1, RvD series, RvE series	7, 9, 30, 34, 78–82
↑ Phagocyte exit to lymphatics	LXA ₄ , RvE1, PD1	30

DHEA metabolites = Docosahexaenoyl ethanolamide derivatives, e.g., 10,17-diHDHEA and 15-HEDPEA; E₂ = estradiol; MaR1 = maresin-1 (omega-3 derivative, docosahexaenoic acid derived); MSH = melanocyte-stimulating hormone; PD1 = protectin D1 (omega-3 derivative, docosahexaenoic acid derived); Rv = resolvin (omega-3 derivative, E series, eicosapentaenoic acid derived, D series, docosahexaenoic acid derived); LysoPS = lysophosphatidylserine.

Rvs, protectins and maresins [4]. These local chemical mediators (autacoids) are biosynthesized via distinct cellular or transcellular enzymatic pathways yielding unique stereospecific molecules [for a review, see ref. 4].

AnxA1 is a glucocorticoid-regulated protein that exerts a variety of proresolving properties, spanning from inhibition of myeloid cell extravasation to modulation of white blood cell lifespan in the inflamed tissue, from phagocytosis and efferocytosis (containment and clearance) to instruction of the adaptive immune system [5]. Work in the last decade has not only allowed the definition of these biological properties but also defined AnxA1 biology distinctly from the physiopharmacological control exerted by glucocorticoids. As an example, migrated neutrophils upregulate their AnxA1 content and release [6] to promote specific proresolving actions (table 1). Macrophages can also release AnxA1 to attain the same biological functions [7]: these events can occur both in a glucocorticoid-independent and -dependent fashion.

MCs are another group of less en-vogue endogenous proresolving mediators; these include α- and γ-melanocyte-stimulating hormone and adrenocorticotrophin. These polypeptides block neutrophilic infiltration [8, 9] and the production of proinflammatory cytokines from target cells [for a review, see ref. 10], yet also activate proresolving processes including the induction of heme oxygenase 1 [11] via signaling through specific MC receptors as further discussed *vide infra*.

As elegantly depicted in a recent review by Soehnlein and Lindbom [12], a self-limited inflammatory reaction can be categorized into 4 distinct phases: (1) resident macrophages signal neutrophil extravasation; (2) neu-

trophil secretion products recruit monocytes; (3) phagocytes release mediators to block further neutrophil influx, and (4) reprogrammed macrophages restore homeostasis. Initiation of the inflammatory response (phase 1 above) will be extensively covered by other articles within this special issue and will not be discussed in detail herein.

Distinct mechanisms are in place to recruit classical inflammatory versus nonclassical patrolling/resident monocytes to an inflammatory site. These two subsets are distinguished by their expression levels of chemokine receptors, most notably, CCR2⁺CX₃CR1^{hi} versus CCR2⁺CX₃CR1^{low} in mice [13] – or CD14⁺CD16⁺ versus CD14^{hi}CD16⁺ in humans [14] – for patrolling versus inflammatory cells, respectively. Patrolling monocytes crawl along the vascular endothelium and rapidly exit the bloodstream upon sensing signs of inflammation or infection, whereupon they produce cytokines that contribute, together with a plethora of other proinflammatory and danger signal mediators, to the initiation of an inflammatory response. Comparatively, classical inflammatory monocytes typically enter the tissue after neutrophils and are the predominant leukocyte subtype at 48–72 h in experimental peritonitis or pleurisy reactions, and differentiate locally into inflammatory macrophages or dendritic cells [14].

Neutrophils are equipped with several types of intracellular granules, which contain receptors, cell adhesion molecules and proteases that can be rapidly mobilized upon activation, allowing prompt and efficient innate effector functions [15]. During diapedesis, neutrophils liberate proteinases such as cathepsin G and proteinase 3, which not only facilitate neutrophil transmigration, but

also activate the endothelium. These mediators, along with azurocidin and the cathelicidin peptide LL-37 play important roles in recruiting classical inflammatory monocytes [16]. Interestingly, some of these cellular responses are mediated via a family of formyl peptide receptors (FPRs) that are also targeted by proresolving mediators and will be discussed *vide infra*. LXA₄ and LXB₄ are potent stimuli for human monocyte chemotaxis and adhesion [17]. Although this study predates the distinction of monocyte subtypes, these autacoids recruit monocytes in a nonphlogistic manner without causing release of proinflammatory mediators or reactive oxygen species production.

It is interesting how the same biological property of promoting nonphlogistic monocyte recruitment is emerging also for AnxA1 (unpublished) and AnxA1-derived peptides [18], the latter feasibly produced at the site of inflammation by the activity of serine proteases such as proteinase 3 or elastase [19] that are released as part of the trafficking process. Of interest, the central role played by AnxA1 in this context is also evident from its release by necrotic neutrophils, with the protein acting as a fail-safe signal [18] and possibly also able to promote monocyte recruitment to aid in debris removal and clearance. Additional properties of AnxA1-derived peptide Ac2-26 include the promotion of neutrophil chemokinesis [20], apoptosis [21] and efferocytosis [7] as well as monocyte recruitment [18]. In the latter case, peptide Ac2-26 is more efficacious than shorter peptides generated from the N-terminal domain of AnxA1 by the activity of metalloproteases, indicating perhaps the existence of a hierarchy with the sequential engagement of specific proteolytic activities. The actions of highlighted proresolving mediators are reviewed in table 1 and figure 1.

A common feature of these endogenous proresolving mediators is that they act essentially as brake signals to circumvent neutrophil-mediated tissue injury, by limiting neutrophil adhesion, diapedesis and chemotaxis [1]. Whilst the molecular mechanisms governing neutrophil recruitment and directional movement have been extensively investigated over the last few decades (see other reviews in this issue), the signals curtailing neutrophil migration are less well characterized. Signaling analysis has indicated that a dynamic balance between two canonical mitogen-activated protein kinases, extracellular signal-related kinase (ERK) and p38, controls neutrophil arrest and locomotor activity in response to the end-target chemoattractant formyl Met-Leu-Phe [22]. Detailed analyses showed G-protein-coupled receptor kinase 2

(GRK2) mediates the stop signal for chemotaxis via phosphorylation, internalization and desensitization of FPR1. The migrational response to formyl Met-Leu-Phe can be further dissected using specific mitogen-activated protein kinase inhibitors. Inhibition of p38 enhanced the stop signal, whereas inhibition of ERK (or activation of p38) overcame the arrest signal and warranted chemotaxis. Liu et al. [22] discovered that p38 acts as a GRK that phosphorylates the carboxy terminal of FPR1 at Ser³⁴², which is a distinct site to GRK2, yet prevents FPR1 from interacting with GRK2 and thus blocking receptor internalization to permit sustained migration. The signaling pathways initiated downstream of FPR2/ALX that block neutrophil chemotaxis yet stimulate monocyte recruitment in response to the proresolving agonists AnxA1 and LXA₄ are likely to be divergent. For example, LXA₄ blocks actin polymerization and downregulates CD11b expression on polymorphonuclear leukocytes (PMN), inhibiting chemotaxis and reducing adhesion without inducing Ca²⁺ mobilization, whereas in monocytes, LXA₄ triggers intracellular Ca²⁺ release and promotes adhesion [23, 24]. Thus, it is likely that cell-specific, pertussis toxin-sensitive G-protein subtypes couple to the intracellular domain of ALX/FPR2, allowing divergent downstream signaling, and hence, different cellular responses [25]. It is now appreciated that β -arrestins are not only involved in receptor desensitization and internalization but may also act as multifunctional adaptor proteins that modulate a number of signaling networks [26]. Therefore, it is plausible that in different myeloid cells, proresolving mediators might exhibit biased agonism towards G-protein- or β -arrestin-mediated signaling. LXA₄ and RvD1 recruit β -arrestin in a concentration-dependent manner in cells stably expressing GPR32 or ALX/FPR2, as demonstrated using a G-protein-coupled receptor (GPCR)- β -arrestin-coupled system to monitor receptor-ligand interaction [27]; whether there is β -arrestin-biased agonism in different myeloid cells remains to be determined.

An interesting twist has recently been reported for a specific myeloid cell, the eosinophil. Eosinophils are typically associated with allergic diseases or parasitic infections, yet Yamada et al. [28] reported that depletion of eosinophils led to augmented neutrophil influx and reduced phagocyte efflux to the draining lymphatics in a model of peritonitis. Of relevance, this lack of resolution in acute peritonitis was associated with diminished 12/15-lipoxygenase-derived lipid mediators including protectin D1. Recent studies indicate that eosinophils also produce another mediator of resolution, RvE3, to aid

Fig. 1. Key protective actions of proresolving mediators on myeloid cells stem downstream agonism at specific GPCRs. During inflammation, a number of endogenous anti-inflammatory and proresolving mediators are generated locally and act via specific GPCRs to initiate resolution. Proresolving mediators such as AnxA1, RvD1 and LXA₄ (that signal via FPR2/ALX), and adenosine (via A_{2A}) potentially block further neutrophil migration (1). LXA₄ as well as AnxA1 peptides, including peptide Ac2-26 and shorter peptides, increase monocyte recruitment to aid in microbial/debris removal

(2). A common feature of these protective mediators is containment and clearance of microorganisms (3); the chemerin peptide C15 signals via ChemR23 to mediate this response. Some of these mediators are also known to enhance neutrophil apoptosis (4), as well as their subsequent uptake via macrophages (5). Macrophage efferocytosis is known to skew macrophages towards a healing phenotype to assist with the restoration of tissue homeostasis; this process can also be induced by adenosine and Rvs (6). LXA₄ and RvE1 also augment phagocyte egress to draining lymph nodes (7).

resolution [29]; whether this effect is indirect and mediated by an intermediate resident cell type such as the macrophage remains to be determined.

During the final stages of an inflammatory response, exudate tissue leukocytes either die locally (preferably by apoptosis) and are cleared by phagocytes, or leave the tissue by egress to the draining lymphatics or systemic recirculation. RvE1, LXA₄ and protectin D1 are able to enhance the number of phagocytes carrying ingested zymo-

san to the peripheral lymph nodes or spleen [30], indicating that these proresolving mediators are able to regulate postexudate myeloid cell trafficking.

Macrophages are fundamental to tissue restoration; they shift from a high microbicidal, yet poor efferocytic capacity to an enhanced efferocytic activity and a reparative phenotype during later resolution phases. Initial in vitro characterization led to two distinct subtypes, classically (M1) or alternatively activated (M2) macrophages;

Table 2. A selection of proresolving GPCRs active on myeloid cells

GPCR acronym (G-protein subtype)	Endogenous proresolving agonists	Myeloid cell expression	References
FPR2/ALX (Gi)	LXA ₄ , epi-LXA ₄ , AnxA1, RvD1	PMN, Mo, MØ, eosinophil, microglia	23, 24, 53
ChemR23 (Gi)	RvE1, chemerin and its peptides	PMN, MØ	83, 84
A _{2A} , A _{2B} (Gs)	adenosine	PMN, Mo, MØ	37, 74
CB2 (Gi)	anandamide, DHEA	MØ, microglia	72
SST2 (Gi)	somatostatin	Mo, MØ	85, 86
DRV1/GPR32 (Gi)	RvD1, RvD5, LXA ₄	PMN, Mo, MØ	53
GPR30 (Gs)	estrogen	MØ	87
MC1 (Gs)	ACTH, α -MSH	PMN, MØ, eosinophil, basophil	10, 88
MC3 (Gs)	ACTH, α -MSH, γ -MSH	MØ, exudated PMN	10, 89
G2A (Gs)	Lyso-PS	PMN, MØ	90
GPR120 (Gq/11)	DHA and EPA	PMN, Mo, MØ	91

epi-LXA₄ = Aspirin-induced epimer of LXA₄; Mo = monocyte; MØ = macrophage; CB = cannabinoid receptor; DHEA = docosa-hexaenoyl ethanolamide; SST = somatostatin receptor; DRV = resolvin D series receptor; GPR = orphan receptor; ACTH = adrenocorticotrophin; MSH = melanocyte-stimulating hormone; Lyso-PS = lysophosphatidylserine; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid.

however, we now know that this is a far too simplistic view. Recently, an extensive transcriptome analysis of murine macrophages during the resolution phase of zymosan peritonitis revealed many overlapping aspects of both subtypes, suggesting that macrophages exhibit substantial plasticity during inflammation [31]. Efficient efferocytosis is essential for resolution; this process is a dynamic and rapid phenomenon to avoid secondary necrosis of the apoptotic neutrophil and perpetuation of tissue damage [32], as well as to promote reprogramming macrophages to a 'healing' phenotype. Effectors of resolution (such as LXA₄) are able to induce neutrophil apoptosis to accelerate resolution [33], promote efferocytosis [34, 35] as well as skew macrophages towards an M2 phenotype and reduce proinflammatory cytokine generation (as with RvD1) [36]. Adenosine also induces macrophage reprogramming, augmenting arginase 1 and tissue inhibitor of matrix metalloproteinase 1 expression [37]. Recent data indicate that low CD11b expression correlates with the propensity of macrophages to emigrate to the lymphatics [38], and matrix metalloproteinase-mediated CD11b shedding promotes their efflux [39]. Mice treated with proresolving lipid mediators RvD1/RvE1, or with dexamethasone, during experimental peritonitis have significantly more CD11b^{low} macrophages, implicating another means by which mediators of resolution may control postexudate phagocyte trafficking and differentiation [38].

Active Termination of Inflammation: Proresolving Receptors

From a pharmacological perspective, appreciation of the resolution of inflammation as an investigative area for drug discovery programs requires a detailed characterization of the receptor targets that are engaged by proresolving mediators. Here, we will focus on a subset of anti-inflammatory GPCRs as effectors on resolution. In most cases, the G protein coupled to the inhibitory receptor is an inhibitory G protein or a G protein associated with adenylate cyclase (table 2).

AnxA1 and peptides derived from its N-terminal domain bind to FPR2/ALX, a member of the FPR family of receptors, in transfected cells as well as in human PMN and compete with other known ligands [40]. Subsequent research demonstrated that the N-terminal peptide Ac2-26 signals via both FPR1 and FPR2/ALX [41, 42] and retains the ability to promote leukocyte detachment in Fpr1 null mice corroborating in vitro data that mouse ortholog(s) of human FPR2/ALX might be the candidate proresolving receptor [43]. In addition to a range of protein/peptide agonists, FPR2/ALX conveys the biological functions of high-affinity endogenous lipid ligands, including the already discussed LXA₄ and RvD1, as well as their aspirin-triggered epimers. Using tritiated LXA₄, specific binding sites were determined on human PMN, with a notable K_d = 0.5 nM [44]. Chimeric receptors were

generated to determine binding sites for LXA₄, using receptors with opposing functions, namely ALX/FPR2 and BLT₁ (a leukotriene B₄ receptor), revealing that the seventh transmembrane segment and third extracellular loop are required for high-affinity binding [45]. Using this receptor-cloning strategy, distinct binding sites for peptide versus non-peptide ligands were identified within ALX/FPR2 likely explaining the diversity of ligands that recognize and signal via this single GPCR. A similar approach using FPR1 and FPR2/ALX chimeras was recently utilized to identify the binding sites for AnxA1 [46]: these results indicated that the N-terminal domain of FPR2/ALX was sufficient for rapid downstream Ca²⁺ mobilization and ERK phosphorylation, whereas the second extracellular loop was necessary for gene activation and sustained AnxA1 responses. Therefore, the small lipid, the large protein and the shorter peptides that are generated from the N-terminal region of AnxA1 interact with distinct receptor domains to generate distinct downstream signals to modulate cell phenotype. It should not go unnoticed that FPR2/ALX is also the receptor for protein ligands that activate myeloid cells (for instance in terms of chemotaxis or chemokinesis) [24], a prime example being LL-37 we briefly mentioned above that engages FPR2/ALX to promote recruitment of inflammatory monocytes [16], nurturing the obvious question of how this receptor operates on the myeloid cell surface and whether it can engage distinct partners in a ligand-biased fashion. Future work will surely clarify this important aspect of the biology of this intriguing receptor.

In the same vein, there is need for further investigations regarding the mouse ortholog of human FPR2/ALX. Thus, Dufton et al. [47] have described the first animal colony lacking mouse Fpr2 (along with Fpr3, which together are thought to represent human FPR2/ALX), reporting loss of anti-inflammatory effects by AnxA1 and LXA₄. In addition, in some models (but not all), these mice displayed augmented inflammatory responses indicative of 'an anti-inflammatory nature' of this receptor [47]. A mouse colony generated with a different strategy was subsequently described by Chen et al. [48], with a phenotype suggestive of 'a pro-inflammatory nature' for Fpr2 since, in models of allergic airway inflammation, these mice were unable to mount a proper inflammatory reaction. It is plausible that the distinct strategies to nullify the receptor may underlie these apparent discrepancies [47, 48] and that, in rodents, human FPR2/ALX is genuinely represented by both mouse Fpr2 and Fpr3. On the other hand, a subsequent report by Chen et al. [49]

suggests that the phenotype of the null mice could also be disease/organ specific. Using a model of dextran sulfate sodium-induced colitis, Fpr2 null mice exhibit exacerbated inflammation, colon thickening and shortening and significantly reduced survival rates, thus emphasizing the anti-inflammatory role for Fpr2. In any case, we also note that pharmacological delivery of FPR2/ALX agonists elicits tissue protection in antigen plus endotoxin-induced allergy by inhibiting dendritic cell infiltration, migration and maturation as well as blocking Th1 and Th17 polarization and protects from endotoxin-induced lung neutrophilia [50], pointing towards a true inhibitory function for the receptor also during lung inflammatory reactions. Of interest, FPR2/ALX agonists are also protective in other models of complex disease such as those mimicking sepsis [51].

Recently, two GPCRs for RvD1 were identified using a GPCR β-arrestin-coupled system, namely FPR2/ALX and orphan receptor GPR32 (newly termed 'DRV1') [52] on human leukocytes [53]. Compared to LXA₄, RvD1 specifically binds with high affinity to human PMN with a K_d = 0.2 nM. Competition binding experiments revealed that RvD1-specific binding was partially displaced by LXA₄ (approximately 60%), whilst no competition was observed with the AnxA1 peptide Ac2-12, supporting the notion of independent peptide/lipid-binding sites [53], a finding corroborated by the chimeric experiments of Bena et al. [46]. We recently investigated which of these RvD1 receptors signals to limit human neutrophil recruitment. Low RvD1 concentrations (nM) appeared to be DRV1/GPR32 specific, and increased concentrations elicited FPR2/ALX-dependent responses [54]. An interesting dichotomy emerged when neutrophil surface expression of these receptors was monitored: FPR2/ALX, but not DRV1/GPR32, membrane levels increased following cell activation with pro-inflammatory stimuli, a process secondary to secretory vesicle mobilization. We proposed that in human neutrophils, DRV1/GPR32 may play a homeostatic role, transmitting signals for low concentrations of RvD1, whereas FPR2/ALX is rapidly increased upon leukocyte activation to counter-regulate overzealous neutrophil recruitment. Accordingly, FPR2/ALX surface expression is elevated on human blister exudate PMN [55], further supporting the notion that this receptor can be rapidly mobilized on the cell surface as part of a counter-regulatory process. Thus, accumulating evidence implicates FPR2/ALX as a stereoselective yet multi-recognition receptor that modulates the host immune response.

Modulation of Proresolving Receptor Expression

An important yet understudied aspect in resolution is the ‘flexible and rapid’ adaptation of the host response towards the termination of inflammation and regain of tissue function. Whilst this can be achieved by the synthesis and release of proresolving mediators, another possible checkpoint may be at the level of receptor expression. Above, we have discussed the distinct nongenomic regulation of FPR2/ALX on the neutrophil cell surface, but it should also be noted that FPR2/ALX and DRV1/GPR32 are upregulated on monocytes (24- to 48-hour time scale) following exposure to zymosan particles or granulocyte macrophage colony-stimulating factor [53]. Thus, proresolving receptors are also upregulated through a process of promoter activation and gene induction. Along this vein, recent studies have examined FPR2/ALX promoter activity and found that LXA₄ enhances the transcription of this receptor, in a forward feedback circuit [56]. In the context of MC receptors (GPCRs that convey the anti-inflammatory and proresolving activities of adrenocorticotrophin and α -melanocyte-stimulating hormone) [57], we have observed high and relatively stable expression of MC receptor type 1 (MC1) in macrophages, synovial tissue and subendothelial tissue in the mesenteric vasculature, with little expression of the MC3 receptor. However, upon application of an insult such as an ischemia-reperfusion procedure [8] or induction of joint arthritis [58], a marked upregulation of MC3 gene product occurred, with MC1 remaining stable. We postulate that in these settings, MC3 mRNA is overexpressed in the affected tissue not only in view of the marked influx of myeloid cells, but also through genuine gene promoter activation, and that this is part of a homeostatic process aiming at directing the host response through resolution pathways. Similar observations have been obtained for mouse Fpr1 and Fpr2/3 in two distinct organs/tissues, such as the arthritic joint [59] and the emphysema lung [60]. In humans, smokers and individuals with emphysema have elevated levels of FPR2/ALX on peripheral blood neutrophils [61, 62], and elevated levels of FPR2/ALX are detected in synovial tissue biopsies of rheumatoid arthritis compared with osteoarthritis patients [63].

We propose that future research will investigate the modulation of the promoter activity of specific proresolving receptors and that this could represent an innovative strategy to rebalance uncontrolled inflammatory pathologies. Similarly, it will not be inconceivable that specific pathologies, from vascular diseases to those of the gut or the joint, could be associated with single nucleotide poly-

morphisms of coding regions yielding functional changes in the biology of proresolving receptors. Although in their infancy, a few reports have recently emerged, indicating an association between alterations in proresolving receptor expression levels on myeloid cells and human pathologies. Simiele et al. [56] have observed a single nucleotide polymorphism in the FPR2/ALX promoter region in a patient with cardiovascular disease, which was associated with a 3-fold reduction in receptor levels on neutrophils compared with individuals with the unaffected allele. There is also a reported association between a FPR2/ALX polymorphism and reduced risk of aspirin hypersensitivity in asthmatics. Patients homozygous for -4209T>G exhibit a significantly higher expression of FPR2/ALX on monocytes; these individuals are protected from aspirin-induced decline in forced expiratory volume [64]. A polymorphism in human MC3R was recently identified in patients suffering from tuberculosis [65], though it remains to be determined if it entails loss of MC receptor function (hence potential absence of proresolving activities, e.g., safe disposal of bacteria by phagocytosis).

Perspective

The process termed the ‘resolution of inflammation’ has recently gained momentum; this entails appreciation of its pathophysiological importance, with (1) the description of mediators (effectors of resolution), (2) the definition of their actions on myeloid cells, and (3) the identification of their receptor targets.

There are now over 20 mediators of resolution, covering families of autacoids, bioactive lipids as well as proteolytically generated peptides and proteins. Over recent years, the definition of an endogenous proresolving mediator has evolved. Inhibition of leukocyte egress from the vasculature into the tissue was originally the main criteria for classification (then referred to as endogenous inhibitors of inflammation) [66]. It is now known that proresolving mediators also regulate antimicrobial defense, apoptosis and efferocytosis as well as monocyte recruitment and myeloid cell egress from the tissue into the lymphatic system. These fundamental processes define not only areas that require further investigation, but also the true dynamic nature of the process of resolution. Furthering our knowledge in this area will produce clear advancements in the pathophysiology of experimental and clinical disease, and together with elucidating the biological behavior of proresolving receptors, will impact on innovative approaches to drug discovery.

We have reasoned in this review article on the complex nature and biology of FPR2/ALX, chosen as exemplar of proresolving receptors, yet it is important to note that this has not deterred drug development both in terms of LX analogs [67] or the development of selective small molecule agonists by Actelion (e.g., see patent No. WO2010/143158).

We are confident this is still a dawn era in resolution and that the scientific interest in the processes operative in our body to switch off inflammation, and their mediator/receptor pairs, will incite wholehearted involvement of Pharma and Biotechs. It is only with appropriate investment that we can deliver the anti-inflammatory drugs

of the next decade, capitalizing on the concept and biology of resolution, and propose the exploitation – in terms of new molecules – of a totally novel approach to the way we consider homeostasis and intervene when this is no longer guaranteed.

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