Biochemical and Biophysical Research Communications xxx (2018) 1–9

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Metabololipidomic profiling of functional immunoresolvent clusters and eicosanoids in mammalian tissues

Paul C. Norris, Charles N. Serhan*

Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital and Harvard Medical School, 60 Fenwood Road, BTM 3016, Boston, MA, 02115, USA

ARTICLE INFO

Article history:
Received 1 March 2018
Accepted 6 March 2018
Available online xxx

Keywords:
Resolvin
Protectin
Leukotriene
Hemostasis
Coagulation
Platelet

ABSTRACT

Metabolomics enables a systems approach to interrogate the bioactive mediators, their pathways and further metabolites involved in the physiology and pathophysiology of human and animal tissues. New metabololipidomic approaches with mass spectrometry presented in this brief review can now be utilized for the identification and profiling of lipid mediator networks that control inflammation-resolution in human blood and healthy and diseased solid tissues. Coagulation of blood is a protective response that prevents excessive bleeding on injury of blood vessels. Here, we review novel approaches to understand the relationship(s) between coagulation and resolution of inflammation and infection. To determine whether coagulation is involved in host-protective actions by lipid mediators, we used a metabololipidomic-based profiling approach with human whole blood (WB) during coagulation. We identified recently temporal clusters of endogenously produced pro-thrombotic and proinflammatory lipid mediators (eicosanoids), as well as specialized proresolving mediators (SPMs) in this vital process. In addition to the classic eicosanoids (prostaglandins, thromboxanes and leukotrienes), a specific SPM cluster was identified that consists of resolvin E1 (RvE1), RvD1, RvD5, lipoxin B_4, and maresin 1, each of which present at bioactive concentrations (0.1–1 nM). The removal of adenosine from coagulating blood samples significantly enhances SPM amounts and unleashes the biosynthesis of RvD3, RvD4, and RvD6 evident following rapid snap freezing with centrifugation before extraction and LC-MS-MS. The classic cyclooxygenase inhibitors, celecoxib and indomethacin, that block thromboxanes and prostanoids do not block production of the clot-driven SPM cluster. Unbiased mass cytometry analysis demonstrated that the SPM cluster produced in human blood targets leukocytes at the single-cell level, directly activating extracellular signaling in human neutrophils and monocytes. Human whole blood treated with the components of this SPM cluster enhanced both phagocytosis and killing of *Escherichia coli* by leukocytes. Thus, we identified a pro-resolving lipid mediator circuit and specific SPM cluster that promotes host defense. This new lipid mediator (LM)-SPM metabololipidomic approach now provides accessible metabolomic profiles in healthy and diseased human tissues, including cancer, for precision and personalized medicine.

1. Introduction

In mammals, blood coagulation supports host defense through compartmentalization of pathogens within microvessels during systemic infection. Neutralization and clearance of the pathogens requires ongoing coordination between the cellular components of blood and surrounding tissues. Coagulation is linked to the inflammatory response via lipid mediators [1–3]. Initiation of coagulation via intrinsic and extrinsic pathways leads to inflammation through the activation of platelets, neutrophils, and monocytes within hemostatic plugs [4]. Arachidonic acid–derived eicosanoids play integral roles in hemostasis and inflammation; for example, thromboxane A_2 (TxA_2) is a potent prothrombotic mediator, and prostaglandins and leukotrienes, collectively known as eicosanoids [5,6], increase vascular permeability, recruit neutrophils (PMN) to injury sites, and position neutrophils for lipid mediator (LM) class switching from leukotriene biosynthesis to SPM production [2,3]. This enables the local biosynthesis of specialized pro-resolving

* Corresponding author.
E-mail address: cserhan@bwh.harvard.edu (C.N. Serhan).

https://doi.org/10.1016/j.bbrc.2018.03.037
0006-291X/

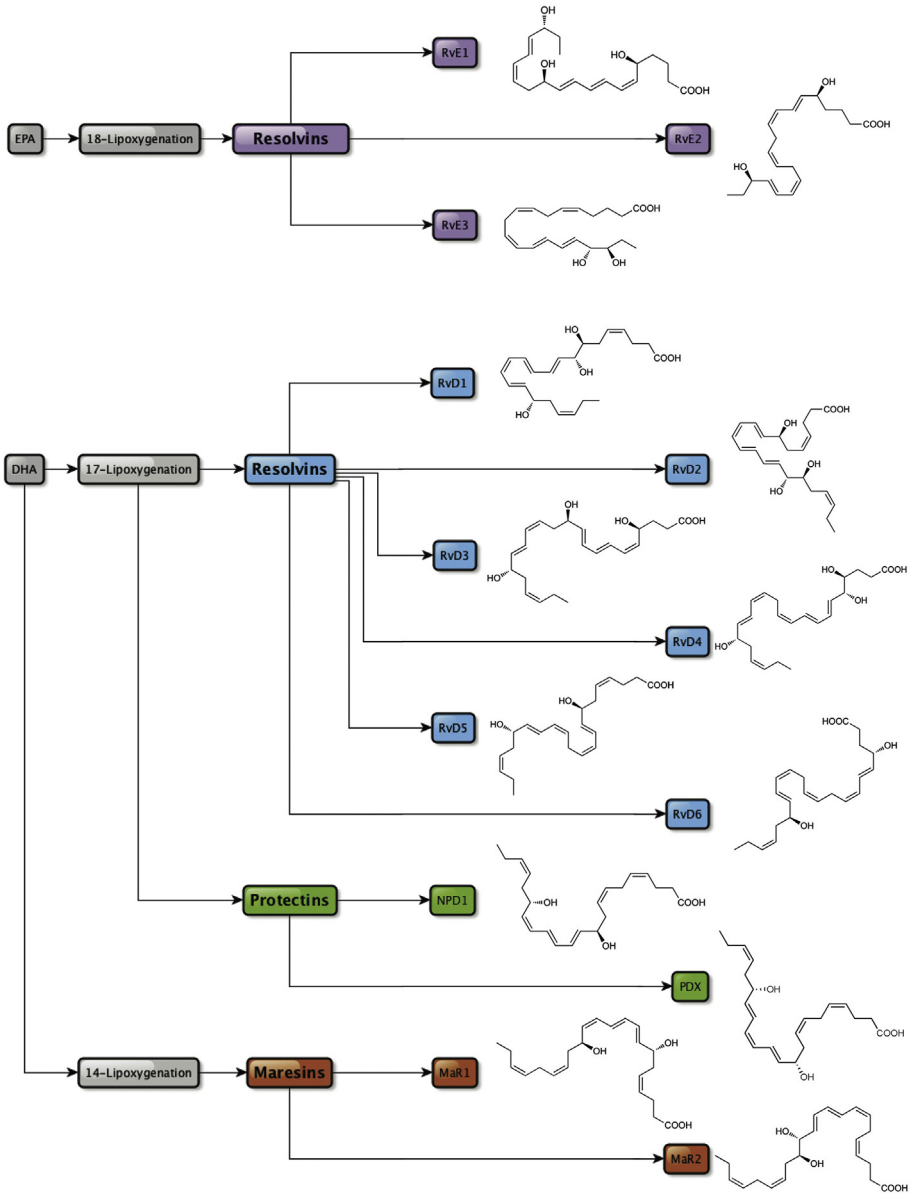

Fig. 1. Network of Specialized pro-resolving mediators and biosynthetic pathways.

mediators (SPMs; see Fig. 1), a process that is pivotal for the transition from inflammation to resolution [2,7–9]. SPMs, in turn, counter-regulate the proinflammatory mediators (for example, cytokines and most eicosanoids), accelerate efferocytosis (the phagocytosis of dying and dead cells) and wound healing, as well as reduce antibiotic requirements, in part, by enhancing phagocytosis without immune suppression [2,10]. SPMs are considered immunoresolvents because they activate, as agonists, the resolution of inflammation and infections [11].

We review herein the protocols and approach for LM-SPM-based profiling [12–15] and a recently devised total lipid mediator (LM) capture approach coupled with solid-phase extraction (SPE) and LM-SPM liquid chromatography-tandem mass spectrometry (LC-MS/MS)–based metabololipidomics to assess endogenous production of LMs and elucidate the temporal production and relationships between specific eicosanoids and SPMs in human tissues and SPM functions (Fig. 2A and Fig. 2B) [3]. These procedures update the earlier introduced lipidomics approach to identify these potent local mediators [13].

To study the relationship(s) between blood coagulation, innate immune phagocytic function, and LM, it was essential to obtain the complete LM profile by targeted monitoring of D-series resolvins, E-series resolvins, protectins, maresins, lipoxins, prostaglandins, and leukotrienes and their biosynthetic pathway markers during the coagulation time course of human blood. To this end, fresh human whole blood was subjected to coagulation through the intrinsic pathway [1] and monitored over time (0–24 h) to confirm clot formation and its contraction. Each sample was rapidly snap-frozen and freeze-thawed to lyse the cells and extract total eicosanoids and SPMs from the supernatants for SPE-LC-MS/MS metabololipidomics (Fig. 2) [3]. Blood clots formed between 8 and 15 min after the onset of coagulation, which was followed by clot retraction and serum formation, which increased rapidly between 4 and 8 h based on increased 750-nm light transmission through the fluid phase that formed above retracted clots (Figs. 2 and 3A). The percentages of specific leukocyte populations (neutrophils, lymphocytes, and monocytes) and their viability were determined throughout the time course [3].

Please cite this article in press as: P.C. Norris, C.N. Serhan, Metabololipidomic profiling of functional immunoresolvent clusters and eicosanoids in mammalian tissues, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.037