3625
Topic Category: 4066-ASIP Resolution Phase Mediators and Mechanisms

First Author: Juliana Priscila Vago
The Scripps Research Institute 3030 Science Park San Diego, CA 92037
United States
Phone: 8584059045
julypri@gmail.com

First Author is a: Postdoctoral Fellow
First Author is a member of: American Society for Investigative Pathology
First Author Degree: PhD, DSc, or equivalent

Presentation Preference: Oral

Sponsor: Juliana Priscila Vago
Sponsor Phone: 8584059045
julypri@gmail.com
Sponsor's Society: Pathology - American Society for Investigative Pathology (ASIP) - Host Society
Keywords: 1. macrophage reprogramming 2. plasminogen system
Awards: ASIP Trainee Travel Award, ASIP Promoting Diversity in Science Trainee Travel Award, HCS-Sponsored Trainee Travel Award

Plasminogen and the Plasminogen receptor, Plg-R_{KT}, regulate efferocytosis and macrophage reprogramming

Juliana Priscila Vago[1], Michelle Amantea Sugimoto[2], Nagyung Baik[1], Robert J Parmer[3], Lindsey A Miles[1], Lirlandia Pires Sousa[1,2]. [1]The Scripps Research Institute, San Diego, CA, [2]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [3]University of California, San Diego, CA

Inflammation resolution is an active process that functions to restore tissue homeostasis. Phagocytic clearance of apoptotic leukocytes (efferocytosis) at sites of inflammation plays an important role in inflammation resolution by inducing remarkable macrophage phenotypic plasticity. The M1/M2 nomenclature provides a basic classification system to distinguish proinflammatory (M1) from anti-inflammatory/resolving macrophages (M2/Mres). Plasminogen (Plg) is synthesized by the liver and is the proenzyme of the broad-spectrum serine protease, plasmin (Pla), which is the major enzyme responsible for fibrinolysis. The participation of the Plg system in the productive phase of inflammation is well known, but the involvement of the Plg system in the resolving phase of inflammation is just emerging. Here we investigated the effects of deletion of Plg and the Plg receptor, Plg-R_{KT}, separately, in the context of resolution of inflammation *in vivo*. $Plg^{-/-}$ and Plg-$R_{KT}^{-/-}$ mice and their respective wild type littermates were challenged by intrapleural injection of LPS (250ng/mouse, i.pl.) or PBS and cells present in the pleural cavity were harvested at 4, 8, 24 and 48 hr and processed for total and differential leukocyte counts and flow cytometric analyses. In this model of pleurisy there is a time-dependent influx of leukocytes into the pleural cavity. The number of neutrophils and M1 macrophages peaks at 8 hr, and decreases at 48 hr. There is a significant increase in mononuclear cell recruitment into the pleural cavity that coincides with the resolution phase of inflammation and with prevalence of M2 and Mres macrophages at 48hr. In the current study, in the setting of LPS-induced inflammation, increased numbers of M1 macrophages were present in $Plg^{-/-}$ mice 8h after LPS challenge without changes in number of M2 or Mres macrophages, as compared to $Plg^{+/+}$ littermates [$Plg^{+/+}$ = 155 ± 39 and $Plg^{-/-}$ = 301 ± 57 x 10^3 M1 macrophages/cavity ($P < 0.05$, n=6)]. There were no genotype-dependent effects on the number of neutrophils recruited to the pleural cavity at all time points evaluated. At 48 hr the total number of mononuclear cells recruited to the pleural cavity was significantly decreased in both $Plg^{-/-}$ and Plg-$R_{KT}^{-/-}$ mice, relative to their respective wild type littermate controls [$Plg^{+/+}$ = 3,432 ± 335 and $Plg^{-/-}$ = 2,377 ± 242 ($P < 0.01$, n=5); Plg-$R_{KT}^{+/+}$ = 2,780 ± 341 and Plg-$R_{KT}^{-/-}$ = 1,725 ± 95 x 10^3 mononuclear cells/cavity ($P < 0.05$, n=6)]. Additionally, the frequency of the M2 marker, CD206, and Annexin A1 (both important for clearance of apoptotic cells) was decreased in $Plg^{-/-}$ macrophages 48 hr after LPS injection. To explore the roles of Plg and Plg-R_{KT} in efferocytosis, mice were injected with zymosan intraperitoneally, followed by an intraperitoneal injection of apoptotic human neutrophils labeled with carboxyfluorescein succinimidyl ester (CFSE). Significantly decreased efferocytosis of apoptotic neutrophils by $Plg^{-/-}$ macrophages and by Plg-$R_{KT}^{-/-}$ macrophages, compared with their wild type littermate controls was observed [$Plg^{+/+}$ = 20.16 ± 3.57 and $Plg^{-/-}$ = 9.04 ± 3.50 ($P < 0.05$, n=5); Plg-$R_{KT}^{+/+}$ = 18.11 ± 4.57 and Plg-$R_{KT}^{-/-}$ = 5.80 ± 2.90 frequency of total cells/cavity ($P < 0.05$, n=6)]. These results suggest that Plg regulates macrophage polarization and efferocytosis of apoptotic cells, thereby contributing to the resolution of inflammation and that these effects are mediated by the Plg receptor, Plg-R_{KT}.

Support or Funding Information
CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil). National Institutes of Health (grant HL 081046 to L.A.M.) and by Merit Review Award #5I01BX002026 from the U.S. Department of Veterans Affairs (to R.J.P.).