Advances in Clinical Medicine 临床医学进展, 2022, 12(3), 1671-1678
Published Online March 2022 in Hans. http://www.hanspub.org/journal/acm
https://doi.org/10.12677/acm.2022.123240

Hans汉斯

NETs在痛风中的研究进展

李秀青，苏　娟*

青海大学，青海　西宁

收稿日期：2022年2月6日；录用日期：2022年2月28日；发布日期：2022年3月9日

摘　要

痛风是一种常见的具有自限性的自身炎症性疾病，急性发作时以关节的红肿热痛为主。近年来，研究发现中性粒细胞胞外诱捕网(neutrophils extracellular traps, NETs)可能在痛风的炎症机制起着重要作用，一方面有促进痛风性关节炎的急性发作作用，另一方面有促进痛风炎症缓解的作用。血小板与机体的炎症有着密切关系，同时活化的血小板具有促进NETs的形成作用。本篇综述将概述痛风、NETs及血小板相互之间的影响。

关键词

痛风，NETs，血小板，炎症

Research Progress of NETs in Gout

Xiuqing Li, Juan Su*

Qinghai University, Xining Qinghai

Received: Feb. 6th, 2022; accepted: Feb. 28th, 2022; published: Mar. 9th, 2022

Abstract

Gout is a common self-limited inflammatory disease. The acute attack is mainly caused by redness, swelling, heat and pain of joints. In recent years, it has been found that neutrophils extracellular traps (NETs) may play an important role in the inflammatory mechanism of gout. On the one hand, they can promote the acute attack of gout arthritis, and on the other hand, they can promote the inflammatory relief of gout. Platelets are closely related to inflammation, and activated platelets can promote the formation of NETs. This review will provide an overview of the interactions between gout, NETs, and platelets.

*通讯作者 Email: sujuanqh@163.com

文章引用: 李秀青, 苏娟. NETs 在痛风中的研究进展[J]. 临床医学进展, 2022, 12(3): 1671-1678.
DOI: 10.12677/acm.2022.123240

Keywords

Gout, NETs, Platelet, Inflammation

Copyright © 2022 by author(s) and Hans Publishers Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/
Open Access

1. 引言

痛风(Gout)是嘌呤代谢紊乱及(或)尿酸排出减少所引发的一种疾病，由于体内的血尿酸水平不断增加，造成单钠尿酸盐(MSU)晶体的沉积，可沉积于关节以及周边的组织和其他脏器中，从而导致炎症反应。临床上，该疾病的特点为急性发作的关节部位炎症，通常影响单个关节，间或有不同持续时间的无症状期。如果不治疗，痛风通常发展为软组织中尿酸盐沉积形成痛风石，导致关节炎反复发作，影响多个关节和渐进性关节破坏，久而久之形成痛风石性的慢性关节炎。MSU 晶体也可沉积于肾脏，造成机体肾衰竭及肾结石形成。痛风是一种分布在世界各地的疾病，患病率受多种因素影响。近年来，痛风性关节炎(gouty arthritis, GA)的发生率持续上升。据统计，在英国，痛风的患病率从 1997 年的 1.5%上升到 2012 年的 2.5% [1]，在美国为 3.9%，而且仍在不断上升[2]。我国痛风总患病率为 1.1%，痛风患病率男性明显超过女性，农村高于城市，沿海地区高于内陆地区，内陆高寒地区高于其他地区[3]。痛风的发生在年龄上高原地区较沿海提早五年，并呈现出显著的年轻化趋向[4]。血清尿酸水平随着海拔高度的上升也呈现出升高趋向[5]。

痛风主要包括原发性和继发性两大类，在原发性痛风病人中只有不足百分之一由酶缺陷所致，而且大多数病因仍然未知，临床以痛风性关节炎为主要症状。痛风已不再被看作一种孤立的疾病了，现更多的研究已将痛风与高血压、心血管疾病、肾脏疾病、高血糖、代谢综合征、高脂血症、癌症发病率增加和过早衰老等疾病联系起来[6] [7] [8] [9]。继发性痛风则可因肾脏疾患、血液系统疾病或用药等因素所致，痛风为其并发症。除高尿酸血症外，痛风性关节炎尚有一些不十分明了的因素诱导炎症发生、发展及炎症消失的基本过程，其中中性粒细胞胞外诱捕网(neutrophils extracellular traps, NETs)在炎症调控中的作用逐渐受到关注。

2. 中性粒细胞胞外诱捕网(Neutrophils Extracellular Traps, NETs)

2.1. 中性粒细胞

中性粒细胞在机体对抗病菌侵袭的第一道免疫防御中具有重要作用[10]，是最先到达炎症部位的免疫细胞，而中性粒细胞的主要防御机制则包括对病菌的吞噬、脱颗粒、细胞因子的产生和 NETs 的形成[10] [11]。本篇综述主要叙述 NETs 的形成。

2.2. NETs 的定义

1996 年，Takei 等[12]阐述了多形核中性粒细胞(polymorphonucler neutrophil, PMN)在经佛波醇活化后，会产生一个不同于典型细胞凋亡或坏死过程的死亡方式。2004 年，Brinkmann 等[13]进一步发现，PMN 在杀伤细菌的过程中会出现相似的死亡方式，并命名为 NETs。2011 年，Mitroulis 等[14]人研究发现，急性痛风病人活化的中性粒细胞也会产生 NETs，并与细胞因子 IL-1β 有关。NETs 是由中性粒细胞

所释放的一个以 DNA 为基础骨架表面上附着有组蛋白、胞质蛋白、蛋白酶，以及一些小颗粒蛋白的纤维网状结构。由中性粒细胞形成 NETs，并伴随着细胞裂解死亡的一个过程被叫做 NETosis。适量的 NETs 可以清除病原体，过量的 NETs 会造成组织病理损伤。

2.3. Nets 与痛风性关节炎

痛风性关节炎是指当尿酸浓度超过溶解度值时，MSU 被组织内的巨噬细胞识别，进而启动炎症反应并招募大量中性粒细胞。在这一过程中，大量的炎性因子和细胞内容物被释放出来，被认为是急性痛风性关节炎发病的关键[15]。MSU 晶体沉积在组织中导致 NLRP3 炎症小体的激活[16] [17]。NLRP3 炎症小体信号通道的活化，引起了促炎细胞因子白介素-1β (IL-1β)的释放，从而诱发了各种促炎因子的表达，如肿瘤坏死因子 α (TNF-α)、IL-6 和 IL-8。这些细胞因子还能招募大量单核细胞和中性粒细胞进入关节滑膜，进而引起关节局部的炎症反应(如毛细血管通透性增加、血浆渗出、发热、水肿)，导致严重的关节疼痛、肿胀和功能障碍。一系列研究结果证明，MSU 晶体能够刺激中性粒细胞，使得中性粒细胞活化，进而诱发了 NETs 的产生[18] [19]，这可能进一步阐述了痛风性关节炎的发生机制。同时，当巨噬细胞在吞噬 MSU 晶体后可激活 NLPR3 炎性小体，促进了 IL-1β 的释放，可诱发中性粒细胞形成更多的 NETs [20] [21]。在痛风病人中分离出的中性粒细胞也可以自发性地释放 NETs，而痛风病人中的血清也可以诱发健康捐赠者的 NETs 的生成。由 MSU 晶体诱发 NETs 生成的主要过程:中性粒细胞在接受 MSU 晶体刺激之后，激活蛋白激酶 C (protein kinase C, PKC)，进一步激活烟酰胺腺嘌啶二核苷酸磷酸(nicotinamide adenine dinucleotide phosphate, NADPH)氧化酶，促进活性氧(reactive oxygen species, ROS)的生成。之后激活髓过氧化物酶(myeloperoxidase, MPO)，中性粒细胞弹性蛋白酶(neutrophil elastase, NE)迁移到细胞核，水解组蛋白，NE 与 MPO 共同协助染色质解聚[22] [23]。与此同时，蛋白精氨酸脱亚胺酶(protein-arginine deiminase type, PAD) 4 促进组蛋白瓜氨酸化，从而进一步促进核染色质去致密化[24] [25]。最后，核膜破裂，染色质和颗粒蛋白在细胞质中混合，通过 GSDMD 孔或 GSDMD 驱动的膜撕裂从细胞中排出，中性粒细胞死亡[26]。但值得注意的是，虽然 MSU 可以诱导血清 NETs 的形成，但是，临床有部分患者在急性发作期，血尿酸水平正常或低下，而血清 NETs 水平增多，说明除 MSU 外还有其他调控 NETs 的因素。且不同刺激物刺激 NETs 形成的形式不同。过多的 NETs 产生和随之排出的各种颗粒蛋白质和各种蛋白酶等化学成份，对炎症反应的加重具有很大影响[27]，且 NETs 可诱发粒细胞和单核细胞产生各种的促炎细胞因子和趋化作用因子[28]，因此也可增强机体产生的发炎反应。但也有研究报道 NETs 参与了痛风性关节炎的缓解，由于 NETs 形成的数量逐渐增加，可能导致了 NETs 的大规模积聚，而高浓度的 NETs 黏附形成了一种特殊聚合网状结构 aggregated NETs (aggNETs)。Schauer 等人[29]的研究证实，aggNETs 能够快速捕获促炎细胞因子和趋化因子，并且其能够分解促炎介质的水平及阻止中性粒细胞的聚集，控制了急性阶段炎症反应的加剧。此外，aggNETs 还能够紧密的包绕在 MSU 晶体表面并对 MSU 晶体表面进行包埋，使之与周围炎症介质隔离，从而参与痛风性关节炎的缓解。另外，aggNETs 也可导致形成成熟的痛风石，而痛风石也是痛风慢性期的特征性症状，可导致骨质侵蚀，故 aggNETs 对痛风的慢性炎症起着重要作用。且最新研究表明，MSU 晶体嵌入 aggNETs 中可能是痛风石沉积的基础，并且可能影响疾病演变[30]。

3. NETs 与血小板

3.1. 血小板

血小板是由骨髓中成熟的巨核细胞胞质经裂解后脱落下来的有生物学活性的小的循环无核细胞，分为外囊泡和内囊泡，内囊泡为血小板颗粒，包括 α-颗粒、致密颗粒和溶酶体。血小板的激活是影响血小板功能的关键因素。而血小板也可通过结合成可溶性血小板激动剂(如 ADP 或凝血酶)或暴露于内皮下细

胞外基质成分(如胶原)被激活[31] [32]。相关配体能与血小板结合从而启动了细胞内信息通道，并引起血小板形状变化和细胞骨架的重新排列，血小板颗粒内容物的释放以及细胞表面黏附分子的活化。血小板颗粒是许多促炎因子和促凝介质的来源。包括趋化因子和促凝因子，允许血小板在原发性止血和炎症过程中积极发挥作用。选择素是大量出现在各种细胞类型上的黏附分子，如内皮细胞、白细胞和血小板等[33]。在静止的血小板中，p 选择素贮存于血小板 α 粒中。在被活化后，血小板将 P 选择素合并到质膜的表面，并和其他细胞发生相互作用，比如在中性粒细胞和单核细胞上的 p-选择素糖蛋白配体-1 (PSGL-1) [34]-[39]。科学研究已经证明，p-选择素糖蛋白配体-1 和 p-选择素的结合，是启动血小板和中性粒细胞之间第一次相互作用的必要条件[40] [41]。除 p-选择素外，血小板上表达的黏附分子中还含有连接黏附分子(JAMA, JAM-C)、细胞间黏附分子(ICAM)-2 和血小板内皮细胞黏附分子-1 (PECAM-1) [42]。

除了粘附分子外，血小板细胞表面还表达不同的受体，如补体受体、toll 样受体和免疫球蛋白检测受体(FcR) [34] [43] [44]。这些受体为血小板能够感知内源性促凝剂和促炎介质、外源性病原体并对其作出反应的能力，而使血小板激活[43]。TLR 是一种高度保守的模式识别受体，能够感知外源病原菌的共同基序，又称为病原体相关分子模式(pathogen associated molecular patterns, PAMPs)。TLRs 检测 PAMPs 导致免疫反应的启动[45]。功能性 TLR4 在血小板上表达[43]，其主要功能是识别脂多糖(LPS) [46]。

3.2. NETs 与活化的血小板

血小板在 NETosis 过程中起着重要的作用，TLR4 是关键的介质之一。脓毒症病人的血浆能够诱导 TLR4 依赖的血小板－中性粒细胞相互作用，导致 NET 的形成。此外，中性粒细胞在脓毒症期间迁移到肝窦，释放 NETs 以防止进一步的细菌传播[47]。血小板－中性粒细胞的作用主要是由两种细胞上的一些受体相互作用而引起的。他们最重要的作用之一就是通过血小板膜表面 p-选择素与中性粒细胞上的 PSGL-1 相结合。另外，血小板 GPIb 也可与中性粒细胞表达的 Mac-1 (CD11b/CD18) [48]相结合，且可能影响 NETs 形成。研究表明，阻断 Mac-1 (CD11b/CD18)显著减少了 NETs 的形成，有研究者表明 LFA-1 (CD11a/CD18)在这一过程中可能发挥作用，也有表示否定的[49]。此外，血小板中的多种成分均可刺激 NETs 的形成，但中性粒细胞和血小板之间直接或间接的分子相互作用仍不清楚。

3.3. NETs 与血小板共同促进血栓形成

众所周知，血小板有着促进血栓生成的重要功能。但是，在多种促使血栓形成的炎症细胞和凝血介质中，多形核中性粒细胞(PMNs)和 NETs 也被证实在炎症相关血栓性疾病中有重要作用[50] [51] [52]。有研究报道，在许多血栓性疾病中，血小板(PLTs)是通过细胞－细胞接触或可溶性介质形成的 NETs [53] [54] [55]。而 NET 结构包含组织因子[56]，von Willebrand 因子(vWF)和组蛋白[57]，作为血小板黏附、激活和聚集的支架。在缺血性血管病变中，局部的中性粒细胞与血小板相互作用可能是 NET 形成的关键因素[53] [56] [58]。现研究者已经发现 NETs 通过包括 TF、Factor XII、MPs、vWF 和纤维蛋白原在内的前血栓介质参与血栓形成[51] [52] [56] [59]。且已有研究者证实在颈内动脉血栓栓塞患者颈动脉病变部位周围的 NETs 与血小板源性微粒复合物可诱导凝血酶和纤维蛋白的生成[60]。近期有研究表明，中性粒细胞和 NETs 有助于局部动脉内皮细胞激活，触发组织因子活性和凝血酶生成，并促进血栓形成[61]。然而，其潜在机制仍不完全清楚。

3.4. 血小板与炎症

研究表明活化的血小板具有调节免疫及炎症的重要作用，参与多种疾病的发生与发展。近期，血小板被认为是炎症损伤的驱动因素，与循环中性粒细胞一起作用，使炎症持续存在[62] [63] [64]。粘附的血

小板也被证明可以将中性粒细胞引导到炎症部位[65] [66]或作为锚点二次捕获循环中性粒细胞[67] [68] [69] [70]，这在动脉中尤其明显。活化的血小板和血小板衍生的可溶性因子如细胞外囊泡[71]除了促进捕获外，还可以作为中性粒细胞和内皮细胞活化的驱动因子[70] [72] [73] [74] [75] [76]，并影响它们的迁移行为[77]。p-选择素和 vWF 可募集更多的循环中性粒细胞和血小板[78]，导致血小板和中性粒细胞募集更多，进一步加重内皮。中性粒细胞沿着活化的内皮细胞迁移是解决急性炎症的第一步；然而，中性粒细胞积聚若不适当清除，则会导致持续的慢性炎症。

近期，研究表明多种疾病中 NETs 与血小板活化具有相关性，值得思考的是在痛风患者中，MUS 晶体虽可刺激 NETs 的形成，但关于血小板这方面的研究甚少，且痛风属自身炎性疾病，NETs 与血小板均与炎症息息相关，但两者在痛风中的相关性未见国内外报道。

基金项目

第三批青海省“高端创新人才千人计划”引进培养人才项目(编号：青人才字<2018>2 号)。

参考文献

[1] Kuo, C.-F., Grainge, M.J., Mallen, C., *et al*. (2015) Rising Burden of Gout in the UK but Continuing Suboptimal Management: A Nationwide Population Study. *Annals of the Rheumatic Diseases*, **74**, 661-667. https://doi.org/10.1136/annrheumdis-2013-204463

[2] Singh, G., Lingala, B. and Mithal, A. (2019) Gout and Hyperuricaemia in the USA: Prevalence and Trends. *Rheumatology* (*Oxford*), **58**, 2177-2180. https://doi.org/10.1093/rheumatology/kez196

[3] Chen, Y.Z., Tang, Z.Z., Huang, Z.Y., *et al*. (2017) The Prevalence of Gout in Mainland China from 2000 to 2016: A Systematic Review and Meta-Analysis. *Journal of Public Health*, **25**, 521-529. https://doi.org/10.1007/s10389-017-0812-5

[4] 张珂珂, 张晓坤, 李长贵. 青藏高原地区痛风的临床特点[J]. 青岛大学医学院学报, 2013, 49(4): 249-296.

[5] 阿祥仁, 周健武. 高原地区体检人群血尿酸水平和高尿酸血症调查研究[J]. 中华检验医学杂志, 2018, 41(6): 462-465.

[6] Krishnan, E. (2010) Inflammation, Oxidative Stress and Lipids: The Risk Triad for Atherosclerosis in Gout. *Rheumatology*, **49**, 1229-1238. https://doi.org/10.1093/rheumatology/keq037

[7] Ichikawa, N., Taniguchi, A., Urano, W., *et al*. (2011) Comorbidities in Patients with Gout. *Nucleosides Nucleotides Nucleic Acids*, **30**, 1045-1050. https://doi.org/10.1080/15257770.2011.596499

[8] Feldman, N., Rotter-Maskowitz, A. and Okun, E. (2015) DAMPs as Mediators of Sterile Inflammation in Aging-Related Pathologies. *Ageing Research Reviews*, **24**, 29-39. https://doi.org/10.1016/j.arr.2015.01.003

[9] Eisenbacher, J.L., Schrezenmeier, H., Jahrsdörfer, B., *et al*. (2014) S100A4 and Uric Acid Promote Mesenchymal Stromal Cell Induction of IL-10+/IDO+ Lymphocytes. *The Journal of Immunology*, **192**, 6102-6110. https://doi.org/10.4049/jimmunol.1303144

[10] Borregaard, N. (2010) Neutrophils, from Marrow to Microbes. *Immunity*, **33**, 657-670. https://doi.org/10.1016/j.immuni.2010.11.011

[11] Mantovani, A., Cassatella, M.A., Costantini, C. and Jaillon, S. (2011) Neutrophils in the Activation and Regulation of Innate and Adaptive Immunity. *Nature Reviews Immunology*, **11**, 519-531. https://doi.org/10.1038/nri3024

[12] Takei, H., Araki, A., Watanabe, H., *et al*. (1996) Rapid Killing of Human Neutrophils by the Potent Cxtivator Phorbol 12-Myristate 13-Acetate (PMA) Accompanied by Changes Different from Typical Apoptosis. *Journal of Leukocyte Biology*, **59**, 229-240. https://doi.org/10.1002/jlb.59.2.229

[13] Brinkmann, V., Reichard, U., Goosmann, C., *et al*. (2004) Neutrophil Extracellular Traps Kill Bacteria. *Science*, **303**, 1532-1535. https://doi.org/10.1126/science.1092385

[14] Mitroulis, I., Kambas, K., Chrysanthopoulou, A., *et al*. (2011) Neutrophil Extracelluar Trap Formation Is Associated with IL-1*β* and Autophagy-Related Signaling in Gout. *PLos ONE*, **6**, e29318. https://doi.org/10.1371/journal.pone.0029318

[15] McDermott, M.F., Kingsbury, S.R. and Conaghan, P.G. (2011) Terole of the NLRP3 Inflammasome in Gout. *Journal of Inflammation Research*, **4**, 39-49. https://doi.org/10.2147/JIR.S11330

[16] Liu-Bryan, R. (2010) Intracellular Innate Immunity in Gouty Arthritis: Role of NALP3 Inflammasome. *Immunology and Cell Biology*, **88**, 20-23. https://doi.org/10.1038/icb.2009.93

[17] Wei, H., Hu, C., Xie, J., *et al*. (2014) Doliroside A Attenuates Monosodium Urate Crystals-Induced Inflammation by Targeting NLRP3 Inflammasome. *European Journal of Pharmacology*, **740**, 321-328. https://doi.org/10.1016/j.ejphar.2014.07.023

[18] Jorch, S.K. and Kubes, P. (2017) An Emerging Role for Neutrophil Extracellular Traps in Noninfectious Disease. *Nature Medicine*, **23**, 279-287. https://doi.org/10.1038/nm.4294

[19] Pruchniak, M.P., Kotula, I. and Manda-Handzlik, A. (2015) Neutrophil Extracellular Traps (NETs) Impact upon Autoimmune Disorders. *Central European Journal of Immunology*, **40**, 217-224. https://doi.org/10.5114/ceji.2015.52836

[20] 戚明珠, 苏晓慧, 林娜, 等. 中性粒细胞胞外诱捕网对缺血性脑卒中的影响及中药干预研究进展[J]. 中国中药杂志, 2021, 46(1): 1-5.

[21] 王玉婷, 顾兵, 李华南, 等. 中性粒细胞胞外诱捕网检测方法综述[J]. 中国药理学通报, 2019, 35(12): 1646-1649.

[22] Burgener, S.S. and Schroder, K. (2020) Neutrophil Extracellular Traps in Host Defense. *Cold Spring Harbor Perspectives in Biology*, **12**, a037028. https://doi.org/10.1101/cshperspect.a037028

[23] Bruschi, M., Moroni, G., Sinico, R.A., *et al*. (2021) Neutrophil Extracellular Traps in the Autoimmunity Context. *Frontiers in Medicine*, **8**, Article ID: 614829. https://doi.org/10.3389/fmed.2021.614829

[24] Gupta, S. and Kaplan, M.J. (2016) The Role of Neutrophils and NETosis in Autoimmune and Renal Diseases. *Nature Reviews Nephrology*, **12**, 402-413. https://doi.org/10.1038/nrneph.2016.71

[25] Cahilog, Z., Zhao, H., Wu, L., *et al*. (2020) The Role of Neutrophil NETosis in Organ Injury: Novel Inflammatory Cell Death Mechanisms. *Inflammation*, **43**, 2021-2032. https://doi.org/10.1007/s10753-020-01294-x

[26] Song, W., Ye, J., Pan, N., Tan, C., *et al*. (2021) Neutrophil Extracellular Traps Tied to Rheumatoid Arthritis: Points to Ponder. *Frontiers in Immunology*, **11**, Article ID: 578129. https://doi.org/10.3389/fimmu.2020.578129

[27] Sil, P., Wicklum, H., Surell, C., *et al*. (2017) Macrophage-Derived IL-1*β* Enhances Monosodium Urate Crystal-Triggered NET Formation. *Inflammation Research*, **66**, 227-237. https://doi.org/10.1007/s00011-016-1008-0

[28] Schorn, C., Strysio, M., Janko, C., *et al*. (2010) The Uptake by Bloodborne Phagocytes of Monosodium Urate Is Dependent on Heat Labile Serum Factor and Divalent Cations. *Autoimmunity*, **43**, 236-238. https://doi.org/10.3109/08916930903510948

[29] Schauer, C., Janko, C., Munoz, L.E., *et al*. (2014) Aggregated Neutrophil Extracellular Traps Limit Inflammation by Degrading Cytokines and Chemokines. *Nature Medicine*, **20**, 511-517. https://doi.org/10.1038/nm.3547

[30] Garcia-Gonzalez, E., Gamberucci, A., Lucherini, O.M., *et al*. (2021) Neutrophil Extracellular Traps Release in Gout and Pseudogout Depends on the Number of Crystals Regardless of Leukocyte Count. *Rheumatology* (*Oxford*), **6**, 4920-4928. https://doi.org/10.1093/rheumatology/keab087

[31] Watson, S.P. (2009) Platelet Activation by Extracellular Matrix Proteins in Haemostasis and Thrombosis. *Current Pharmaceutical Design*, **15**, 1358-1372. https://doi.org/10.2174/138161209787846702

[32] De Candia, E. (2012) Mechanisms of Platelet Activation by Thrombin: A Short History. *Thrombosis Research*, **129**, 250-256. https://doi.org/10.1016/j.thromres.2011.11.001

[33] Zarbock, A., Ley, K., McEver, R.P., *et al*. (2011) Leukocyte Ligands for Endothelial Selectins: Specialized Glycoconjugates That Mediate Rolling and Signaling under Flow. *Blood*, **118**, 6743-6751. https://doi.org/10.1182/blood-2011-07-343566

[34] Semple, J.W., Italiano, J.E. and Freedman, J. (2011) Platelets and the Immune Continuum. *Nature Reviews Immunology*, **11**, 264-274. https://doi.org/10.1038/nri2956

[35] Hamburger, S.A. and McEver, R.P. (1990) GMP-140 Mediates Adhesion of Stimulated Platelets to Neutrophils. *Blood*, **75**, 550-554. https://doi.org/10.1182/blood.V75.3.550.550

[36] Larsen, E., Palabrica, T., Sajer, S., *et al*. (1990) PADGEM-Dependent Adhesion of Platelets to Monocytes and Neutrophils Is Mediated by a Lineage-Specific Carbohydrate, LNF III (CD15). *Cell*, **63**, 467-474. https://doi.org/10.1016/0092-8674(90)90443-I

[37] Moore, K.L., Varki, A. and McEver, R.P. (1991) GMP-140 Binds to a Glycoprotein Receptor on Human Neutrophils: Evidence for a Lectin-Like Interaction. *Journal of Cell Biology*, **112**, 491-499. https://doi.org/10.1083/jcb.112.3.491

[38] Moore, K.L., Patel, K.D., Bruehl, R.E., *et al*. (1995) P-Selectin Glycoprotein Ligand-1 Mediates Rolling of Human Neutrophils on P-Selectin. *Journal of Cell Biology*, **128**, 661-671. https://doi.org/10.1083/jcb.128.4.661

[39] Von Hundelshausen, P. and Weber, C. (2007) Platelets as Immune Cells: Bridging Inflammation and Cardiovascular Disease. *Circulation Research*, **100**, 27-40. https://doi.org/10.1161/01.RES.0000252802.25497.b7

[40] Evangelista, V., Manarini, S., Sideri, R., *et al*. (1999) Platelet/Polymorphonuclear Leukocyte Interaction: P-Selectin Triggers Protein-Tyrosine Phosphorylation-Dependent CD11b/CD18 Adhesion: Role of PSGL-1 as a Signaling Molecule. *Blood*, **93**, 876-885. https://doi.org/10.1182/blood.V93.3.876

[41] Yang, J., Furie, B.C. and Furie, B. (1999) The Biology of P-Selectin Glycoprotein Ligand-1: Its Role as a Selectin Counterreceptor in Leukocyte-Endothelial and Leukocyte-Platelet Interaction. *Thrombosis and Haemostasis*, **81**, 1-7. https://doi.org/10.1055/s-0037-1614407

[42] van Gils, J.M., Zwaginga, J.J. and Hordijk, P.L. (2009) Molecular and Functional Interactions among Monocytes, Platelets, and Endothelial Cells and Their Relevance for Cardiovascular Diseases. *Journal of Leukocyte Biology*, **85**, 195-204. https://doi.org/10.1189/jlb.0708400

[43] Andonegui, G., Kerfoot, S.M., McNagny, K., *et al*. (2005) Platelets Express Functional Toll-Like Receptor-4. *Blood*, **106**, 2417-2423. https://doi.org/10.1182/blood-2005-03-0916

[44] Semple, J.W. and Freedman, J. (2010) Platelets and Innate Immunity. *Cellular and Molecular Life Sciences*, **67**, 499-511. https://doi.org/10.1007/s00018-009-0205-1

[45] Albiger, B., Dahlberg, S., Henriques-Normark, B., *et al*. (2007) Role of the Innate Immune System in Host Defence against Bacterial Infections: Focus on the Toll-Like Receptors. *Journal of Internal Medicine*, **261**, 511-528. https://doi.org/10.1111/j.1365-2796.2007.01821.x

[46] Andonegui, G., Bonder, C.S., Green, F., *et al*. (2003) Endothelium-Derived Toll-Like Receptor-4 Is the Key Molecule in LPS-Induced Neutrophil Sequestration into Lungs. *Journal of Clinical Investigation*, **111**, 1011-1020. https://doi.org/10.1172/JCI16510

[47] McDonald, B., Urrutia, R., Yipp, B.G., *et al*. (2012) Intravascular Neutrophil Extracellular Traps Capture Bacteria from the Bloodstream during Sepsis. *Cell Host & Microbe*, **12**, 324-333. https://doi.org/10.1016/j.chom.2012.06.011

[48] Simon, D.I., Chen, Z., Xu, H., *et al*. (2000) Platelet Glycoprotein Ibalpha Is a Counter Receptor for the Leukocyte Integrin Mac-1 (CD11b/CD18). *Journal of Experimental Medicine*, **192**, 193-204. https://doi.org/10.1084/jem.192.2.193

[49] Rossaint, J., Herter, J.M., Van Aken, H., *et al*. (2014) Synchronized Integrin Engagement and Chemokine Activation Is Crucial in Neutrophil Extracellular Trap-Mediated Sterile Inflammation. *Blood*, **123**, 2573-2584. https://doi.org/10.1182/blood-2013-07-516484

[50] de Boer, O.J., Li, X., Teeling, P., *et al*. (2013) Neutrophils, Neutrophil Extracellular Traps and Interleukin-17 Associate with the Organisation of Thrombi In Acute Myocardial Infarction. *Thrombosis and Haemostasis*, **109**, 290-297. https://doi.org/10.1160/TH12-06-0425

[51] Stakos, D.A., Kambas, K., Konstantinidis, T., *et al*. (2015) Expression of Functional Tissue Factor by Neutrophil Extracellular Traps in Culprit Artery of Acute Myocardial Infarction. *European Heart Journal*, **36**, 1405-1414. https://doi.org/10.1093/eurheartj/ehv007

[52] Fuchs, T.A., Brill, A., Duerschmied, D., *et al*. (2010) Extracellular DNA Traps Promote Thrombosis. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 15880-15885. https://doi.org/10.1073/pnas.1005743107

[53] Maugeri, N., Campana, L., Gavina, M., *et al*. (2014) Activated Platelets Present High Mobility Group Box 1 to Neutrophils, Inducing Autophagy and Promoting the Extrusion of Neutrophil Extracellular Traps. *Journal of Thrombosis and Haemostasis*, **12**, 2074-2088. https://doi.org/10.1111/jth.12710

[54] McDonald, B., Davis, R.P., Kim, S.J., *et al*. (2017) Platelets and Neutrophil Extracellular Traps Collaborate to Promote Intravascular Coagulation during Sepsis in Mice. *Blood*, **129**, 1357-1367. https://doi.org/10.1182/blood-2016-09-741298

[55] Perdomo, J., Leung, H.H.L., Ahmadi, Z., *et al*. (2019) Neutrophil Activation and NETosis Are the Major Drivers of Thrombosis in Heparin-Induced Thrombocytopenia. *Nature Communications*, **10**, Article No. 1322. https://doi.org/10.1038/s41467-019-09160-7

[56] Wvon Bruhl, M.L., Stark, K., Steinhart, A., *et al*. (2012) Monocytes, Neutrophils, and Platelets € Cooperate to Initiate and Propagate Venous Thrombosis in Mice *in Vivo*. *Journal of Experimental Medicine*, **209**, 819-835. https://doi.org/10.1084/jem.20112322

[57] Staessens, S., Denorme, F., Francois, O., *et al*. (2020) Structural Analysis of Ischemic Stroke Thrombi: Histological Indications for Therapy Resistance. *Haematologica*, **105**, 498-507. https://doi.org/10.3324/haematol.2019.219881

[58] Mangold, A., Alias, S., Scherz, T., *et al*. (2015) Coronary Neutrophil Extracellular Trap Burden and Deoxyribonuclease Activity in ST-Elevation Acute Coronary Syndrome Are Predictors of ST-Segment Resolution and Infarct Size. *Circulation Research*, **116**, 1182-1192. https://doi.org/10.1161/CIRCRESAHA.116.304944

[59] Wang, Y., Luo, L., Braun, O.O., *et al*. (2018) Neutrophil Extracellular Trap-Microparticle Complexes Enhance Thrombin Generation via the Intrinsic Pathway of Coagulation in Mice. *Scientific Reports*, **8**, Article No. 4020. https://doi.org/10.1038/s41598-018-22156-5

[60] Zhou, P., Li, T., Jin, J.Q., *et al*. (2020) Interactions between Neutrophil Extracellular Traps and Activated Platelets Enhance Procoagulant Activity in Acute Stroke Patients with ICA Occlusion. *EBOI Medicine*, **53**, Article ID: 102671. https://doi.org/10.1016/j.ebiom.2020.102671

[61] Folco, E.J., Mawson, T.L., Vromman, A., *et al*. (2018) Neutrophil Extracellular Traps Induce Endothelial Cell Activation and Tissue Factor Production through Interleukin-1alpha and Cathepsin G. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **38**, 1901-1912. https://doi.org/10.1161/ATVBAHA.118.311150

[62] Ramirez, G.A., Manfredi, A.A. and Maugeri, N. (2019) Misunderstandings between Platelets and Neutrophils Build in Chronic Inflammation. *Frontiers in Immunology*, **10**, Article No. 2491. https://doi.org/10.3389/fimmu.2019.02491

[63] Gros, A., Ollivier, V. and Ho-Tin-Noe, B. (2015) Platelets in Inflammation: Regulation of Leukocyte Activities and Vascular Repair. *Frontiers in Immunology*, **5**, Article No. 678. https://doi.org/10.3389/fimmu.2014.00678

[64] Lindberg, U., Svensson, L., Hellmark, T., *et al*. (2018) Increased Platelet Activation Occurs in Cystic Fibrosis Patients and Correlates to Clinical Status. *Thrombosis Research*, **162**, 32-37. https://doi.org/10.1016/j.thromres.2017.12.012

[65] Zuchtriegel, G., Uhl, B., Puhr-Westerheide, D., *et al*. (2016) Platelets Guide Leukocytes to Their Sites of Extravasation. *PLOS Biology*, **14**, e1002459. https://doi.org/10.1371/journal.pbio.1002459

[66] Pitchford, S., Pan, D. and Welch, H.C.E. (2017) Platelets in Neutrophil Recruitment to Sites of Inflammation. *Current Opinion in Hematology*, **24**, 23-31. https://doi.org/10.1097/MOH.0000000000000297

[67] Kim, K.H., Barazia, A. and Cho, J. (2014) Real-Time Imaging of Heterotypic Platelet-Neutrophil Interactions on the Activated Endothelium during Vascular Inflammation and Thrombus Formation in Live Mice. *Journal of Visualized Experiments*, **74**, Article ID: 50329. https://doi.org/10.3791/50329

[68] Zwaginga, J.J., Torres, H.I.G., Lammers, J.-W.J., *et al*. (1999) Minimal Platelet Deposition and Activation in Models of Injured Vessel Wall Ensure Optimal Neutrophil Adhesion under Flow Conditions. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **19**, 1549-1554. https://doi.org/10.1161/01.ATV.19.6.1549

[69] Totani, L., Piccoli, A., Dell'Elba, G., *et al*. (2014) Phosphodiesterase Type 4 Blockade Prevents Platelet-Mediated Neutrophil Recruitment at the Site of Vascular Injury. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **34**, 1689-1696. https://doi.org/10.1161/ATVBAHA.114.303939

[70] Evangelista, V., Pamuklar, Z., Piccoli, A., *et al*. (2007) SRC Family Kinases Mediate Neutrophil Adhesion to Adherent Platelets. *Blood*, **109**, 2461-2469. https://doi.org/10.1182/blood-2006-06-029082

[71] Kuravi, S.J., Harrison, P., Rainger, G.E., *et al*. (2019) Ability of Platelet-Derived Extracellular Vesicles to Promote Neutrophil-Endothelial Cell Interactions. *Inflammation*, **42**, 290-305. https://doi.org/10.1007/s10753-018-0893-5

[72] Etulain, J., Martinod, K., Wong, S.L., *et al*. (2015) P-Selectin Promotes Neutrophil Extracellular Trap Formation in Mice. *Blood*, **126**, 242-246. https://doi.org/10.1182/blood-2015-01-624023

[73] Maugeri, N., Rovere-Querini, P., Baldini, M., *et al*. (2014) Oxidative Stress Elicits Platelet/Leukocyte Inflammatory Interactions via HMGB1: A Candidate for Microvessel Injury in Systemic Sclerosis. *Antioxidants & Redox Signaling*, **20**, 1060-1074. https://doi.org/10.1089/ars.2013.5298

[74] Dole, V.S., Bergmeier, W., Mitchell, H.A., *et al*. (2005) Activated Platelets Induce Weibel-Palade-Body Secretion and Leukocyte Rolling *in Vivo*: Role of P-Selectin. *Blood*, **106**, 2334-2339. https://doi.org/10.1182/blood-2005-04-1530

[75] Petri, B., Broermann, A., Li, H., *et al*. (2010) von Willebrand Factor Promotes Leukocyte Extravasation. *Blood*, **116**, 4712-4719. https://doi.org/10.1182/blood-2010-03-276311

[76] Abdulla, A., Awla, D., Hartman, H., *et al*. (2012) Platelets Regulate P-Selectin Expression and Leukocyte Rolling in Inflamed Venules of the Pancreas. *European Journal of Pharmacology*, **682**, 153-160. https://doi.org/10.1016/j.ejphar.2012.02.014

[77] Frydman, G.H., Le, A., Ellett, F., *et al*. (2017) Technical Advance: Changes in Neutrophil Migration Patterns upon Contact with Platelets in a Microfluidic Assay. *Journal of Leukocyte Biology*, **101**, 797-806. https://doi.org/10.1189/jlb.1TA1115-517RR

[78] Gremmel, T., Koppensteiner, R., Kaider, A., *et al*. (2015) Impact of Variables of the P-Selectin—P-Selectin Glycoprotein Ligand-1 Axis on Leukocyte-Platelet Interactions in Cardiovascular Disease. *Thrombosis and Haemostasis*, **113**, 806-812. https://doi.org/10.1160/TH14-08-0690