

Clinical and Experimental Evidences on the Prothrombotic Properties of Neutrophils

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SUMMARY

Epidemiologic studies have shown that the neutrophil count correlates with the risk of myocardial infarction and stroke and identify patients more susceptible to reinfarction and in-hospital death. In particular, neutrophils action was initially associated to blood rheological changes, or to the effect of neutrophil-derived eicosanoids or proteases. Animal models indicate that platelet-leukocyte P-selectin dependent cross-talk contributes to fibrin deposition during in vivo thrombus formation. In fact, platelet P-selectin, through its leukocyte counter-receptor PSGL-1, determines the activation of leukocyte $\beta 2$ integrins, the binding of fibrinogen and the expression of tissue factor on leukocyte surface. Monocytes stimulated in vitro with LPS, PMA and P-selectin synthesize and express tissue factor. fMLP, P-selectin, TNF α and C5a are effective stimuli that trigger the synthesis and expression of biologically active tissue factor in neutrophils. The experimental evidence well agrees with clinical observations: patients with acute coronary syndromes, acute respiratory distress syndrome, antiphospholipid syndromes, giant cell arteritis and myeloproliferative syndromes have increased the expression of tissue factor on leukocyte surface. Moreover circulating neutrophils express mRNA codifying for full-length and/or alternatively spliced tissue factor, suggesting a new important link between thrombosis and inflammation. All together, clinical and experimental evidence suggest that the leukocyte thrombogenic profile is a relevant player in patients with a high risk of thromboembolic events and possibly represents a suitable target for molecular intervention.

Keywords: neutrophils; tissue factor; thrombosis; inflammation; arterial thromboembolism

INTRODUCTION

Epidemiologic studies in the last two decades have revealed the correlation between the leukocyte count and the risk of myocardial infarction and stroke. More recent data, obtained in various independent experimental models, convincingly indicated that, besides the increased neutrophil counts in peripheral blood, neutrophil activation status per se reveals a higher risk of thromboembolism.

ARE LEUKOCYTES INVOLVED IN THROMBOSIS?

The first experimental evidence was published by Palabrica and co-workers [1, 2] in a study carried out in baboons that described the requirement of leukocyte for fibrin deposition during experimental thrombus formation. This pioneering study, based on the similar number of neutrophils in baboons and humans, demonstrated that leukocytes (in particular granulocytes in this animal model) were responsible for fibrin formation. Actually, a P-selectin blocking antibody showed to be able to curb the fibrin deposition, indicating that the recognition of P-selectin, possibly expressed by activated platelets, played a key role.

This experimental evidence was confirmed by the analysis of coronary thrombi obtained from patients with segment T elevated acute myocardial infarction. In this study, neutrophils were identified as the major thrombus component. In contrast monocytes and lymphocytes were poorly represented [3-6].

P-SELECTIN AS LEUKOCYTE AGONIST

Platelet P-selectin, through its leukocyte counter-receptor PSGL-1, determines the activation of $\beta 2$ integrins, with firm platelet-neutrophil adhesion. Several studies have established that the $\alpha M\beta 2$ integrin (CD11b/CD18, Mac-1) is required for i) leukocyte recruitment by activated platelets, and ii) for trans-platelet migration of leukocytes and iii) for thrombus formation after arterial damage in mice models. In particular, studies by Evangelista and co-workers [7-10] have demonstrated that platelet P-selectin interaction with the leukocyte counter-receptor PSGL-1 triggers activation of $\alpha M\beta 2$ integrin. The underlying mechanism involves Src-family tyrosine kinases. Kinase activation regulates $\alpha M\beta 2$ -dependent adhesion of neutrophils to activated platelets and induces the conformation shift of this integrin to an active form, with creation of binding sites for the relevant ligands (including in particular fibrinogen and von Willebrand factor).

P-selectin recognition also results in: i) the release of neutrophil primary (azurophilic) granules content, including cathepsin G [11], elastase and myeloperoxidase [12, 13] and ii) the release of tissue factor bearing microparticles [14] that are able to accelerate the fibrin formation and deposition [15]. In addition, membrane-to-membrane contact between platelets and neutrophils promotes thromboxane A₂ and leukotriene C₄ formation [16, 17], a potent vasoconstrictor. These events critically depend on effective P-selectin/PSGL-1 interaction [17, 18]. In turn, these bioactive molecules favour platelet and endo-

thelial activation, jeopardize vascular integrity and affect the coagulation system by mechanism not prevented by aspirin [19].

NEUTROPHILS EXPRESS TISSUE FACTOR

Tissue factor is a key molecule in the activation of the extrinsic coagulation cascade. The rate of the reactions is critically dependent on the exposure of phosphatidylserine on the surface of cells or other particulate substrates, i.e. on the availability of a template where coagulation factors assemble [15].

In addition to monocytes, endothelial cells and platelets, neutrophils express the tissue factor. Originally, the presence of the mRNA codifying for TF (TFmRNA) was demonstrated in infected and inflamed tissues of rabbits and monkeys [20, 21]. Besides TFmRNA expression, neutrophil tissue factor abilities to promote plasma coagulation and effective fibrin deposition were also demonstrated.

Later, *in vitro* observations with human purified neutrophils indicated that all stimuli are not equally effective at inducing tissue factor expression and the *de novo* synthesis of the molecule [22] by a JAK2-dependent mechanism [23]. PMA and LPS fail to induce any procoagulant activity in neutrophils [23]. Importantly, resting human neutrophils do not effectively react to LPS. This lack of response is at least partially explained by a relative absence of relevant toll-like receptors.

P-selectin [22], C5a [24], TNF α [25] (all physiological stimuli) and fMLP [22] share the ability to induce TFmRNA, as well as the *ex-novo* synthesis and up-regulation of tissue factor on the neutrophil surface.

The tissue factor expressed after the recognition of each of these stimuli is biologically active, and efficiently induces the thrombin production [22-26]. Ritis and co-workers [24, 25, 26] demonstrated that neutrophils also produce an alternatively spliced tissue factor after stimulation with C5a and TNF. Results obtained in patients undergoing thrombosis or in those with high risk of thromboembolic events, such as myeloproliferative syndromes [25, 27], antiphospholipid antibody syndrome [24], acute distress respira-

tory syndromes [26], acute coronary syndromes [28] and giant cell arteritis [29] have further highlighted the relevance of the link between inflammatory stimuli, including P-selectin, C5a and TNF α , and the generation of bioactive tissue factor.

MONOCYTE DEPENDENT NEUTROPHIL TISSUE FACTOR EXPRESSION

Monocytes produce the tissue factor after stimulation with P-selectin, LPS and PMA [19, 22, 30]. In contrast, the neutrophil agonist, fMLP is unable to induce the TFmRNA production and the expression of the molecule on the monocyte surface [22]. Actually, the co-culture of autologous human neutrophils and monocytes in the presence of fMLP do not influence TFmRNA expression in neutrophils. Some transfer of tissue factor from activated monocytes to neutrophils has been demonstrated by Egorina and co-workers [30]. In healthy subjects, neutrophil:monocytes ratio is usually 17:1 (around 5,000 neutrophils and 300 monocytes per each μ L of blood). In particular conditions, including some in which tissue factor biological action is crucial, such as acute myocardial infarction, polycythemia vera, essential thrombocytemia, giant cell arteritis, polymyalgia rheumatica and acute lung injury, neutrophils count easily reach 10,000/ μ L of blood, while the number of monocytes is not influenced. Considering the relative ratio between neutrophils and monocytes and the different half-life of neutrophils and monocytes, the actual relevance of tissue factor transfer from monocytes and the relative contribution to the overall expression of the molecule by neutrophils remains to be established.

CONCLUSION

Altogether, clinical and experimental evidences suggest that the neutrophil thrombogenic profile contributes to the risk of thromboembolic events and possibly represents a suitable target for molecular intervention. Studies in the next years will shed further light on this exciting issue.

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Клинички и експериментални показатељи протромботских својстава неутрофила

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КРАТАК САДРЖАЈ

Епидемиолошка истраживања су показала да је број неутрофила у корелацији с ризиком од инфаркта миокарда и можданог удара и да указује на болеснике који су подложнији рецидиву инфаркта и болничкој смрти. Активност неутрофила се у почетку повезивала с реолошким променама крви или ефектом еикосаноида или протеаза неутрофилног порекла. Код експерименталних животиња је показано да интеракција тромбоцита и леукоцита зависна од *P*-селектина доприноси депоновању фибрина током формирања тромба *in vivo*. У ствари, тромбоцитни *P*-селектин преко леукоцитног рецептора *PSGL-1* одређује активацију леукоцитних β_2 интегрина, везивање фибриногена и експресију ткивног фактора на површини леукоцита. Моноцити стимулирани *in vitro* са *LPS*, *PMA* и *P*-селектином синтетички и врше експресију ткивног фактора. *fMLP*, *P*-селектин, *TNF- α* и *C5a* су ефикасни стимулатори који иницирају синтезу и експресију биолошки активног тки-

вног фактора у неутрофилима. Експериментални показатељи су у потпуном складу с клиничким запажањима; болесници с акутним коронарним синдромом, синдромом акутног респираторног дистреса, антифосфолипидним синдромом, артеријским гигантских ћелија и мијелопролиферативним синдромима показују повећану експресију ткивног фактора на површини леукоцита. Штавише, циркулишући неутрофили експримирају информациону РНК, која кодира ткивни фактор пуне дужине, односно ткивни фактор који је настао алтернативним спајањем егзона, што указује на нову, значајну повезаност тромбозе и запаљењског процеса. Све у свему, клинички и експериментални показатељи сугеришу да тромбогени профил неутрофила има релевантну улогу код болесника који су под великим ризиком за развој тромбоемболије и вероватно представља погодан циљ за молекуларну интервенцију.

Кључне речи: неутрофили; ткивни фактор; тромбоза; запаљење; артеријски тромбоемболизам

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