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Activation of the CD147 Signaling Pathway by Platelets Promotes the Proinflammatory Phenotype of Monocytes in Rheumatoid Arthritis

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Resume: *Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint inflammation and damage. Recent studies have highlighted the crucial role of platelets in the pathogenesis of RA through their interaction with immune cells and contribution to inflammatory processes. In this study, we investigated the role of the surface protein CD147 on platelets in inducing a proinflammatory monocyte phenotype in RA. We found that platelet–monocyte interactions mediated by CD147 lead to phenotypic changes in monocytes, particularly within the CD14++CD16+CCR2+ (Mon2) subset, which is associated with increased production of proinflammatory cytokines and activation of transcription factors such as NF-κB. These findings underscore the important role of platelets in shaping the inflammatory response in RA and propose CD147 as a potential therapeutic target for novel treatment strategies.*

Keywords: Rheumatoid arthritis, platelets, monocytes, CD147, proinflammatory phenotype, inflammation, cytokines.

Relevance

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by immune system activation within the synovial membrane of the joints, accompanied by systemic manifestations. Monocytes and macrophages play a crucial role in RA pathogenesis by releasing pro-inflammatory cytokines such as TNF-α and IL-6. Previously considered a homogeneous population, monocytes are now known to consist of three distinct subsets: classical CD14++CD16–CCR2+ (Mon1), intermediate CD14++CD16+CCR2+ (Mon2), and non-classical CD14+CD16++CCR2– (Mon3). In RA patients, an increased number of CD16+ monocytes—particularly the Mon2 subset—has been observed, which correlates with inflammatory activity.

Recent studies have demonstrated that platelets also play a key role in the pathophysiology of inflammatory arthritis. The expression of CD147, which is upregulated upon platelet activation, can trigger signaling pathways such as NF-κB, promoting the production of MMPs, TNF-α, and IL-6. However, the role of monocyte–platelet interactions in RA pathogenesis and the mechanisms underlying monocyte phenotypic changes remain insufficiently understood.

The aim of this study is to assess platelet activation, monitor changes in monocyte subsets, and analyze their association with monocyte–platelet aggregates (MPAs) in RA patients. A secondary objective is to identify the mechanisms by which interactions between monocytes and platelets influence monocyte phenotype and function.

Materials and Methods

The study included 30 patients with active rheumatoid arthritis (RA) who met the classification criteria of the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) [1]. The mean age of the patients was 44 years (ranging from 20 to 72 years), and the average disease duration was 8 years. All patients exhibited active disease, meeting the following criteria: (1) swelling in at least six joints; (2) tenderness in at least six joints; and (3) fulfillment of at least two of the following three conditions: (a) morning stiffness lasting more than 45 minutes on the day of visit; (b) erythrocyte sedimentation rate (ESR) ≥28 mm/h; (c) C-reactive protein (CRP) level ≥20 mg/dL. None of the patients were receiving disease-modifying therapy at the time of enrollment; they were either untreated or taking only nonsteroidal anti-inflammatory drugs (NSAIDs). Disease activity was assessed in all patients using the Disease Activity Score based on the evaluation of 28 joints (DAS28) [24]. A DAS >3.6 was considered indicative of active disease, whereas a DAS <2.6 was considered remission. Control peripheral blood samples were obtained from 10 age- and sex-matched healthy donors.

The study protocol was approved by the Ethics Committee of the Fourth Military Medical University. Written informed consent was obtained from all participants (patients and controls). Pre-conjugated fluorescent antibodies were purchased from BD Biosciences (San Jose, CA, USA): CD14–phycoerythrin (PE), P-selectin (CD62P)–PE, immunoglobulin G (IgG1)–PE, CD16–PE-Cy5, CD40L–PE, CD42a–fluorescein isothiocyanate (FITC), PAC-1–FITC, CD147–FITC, TNF-α–FITC, IL-6–FITC, CD61–PerCP, CCR2–Alexa Fluor, and the BrdU Flow Kit. The anti-CD147 antibody was kindly provided by the Department of Cell Biology, Fourth Military Medical University. Additionally, an antibody against IKK-beta was used. Fasting blood samples were collected in tubes containing the ACD (acid–citrate–dextrose) anticoagulant. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Ficoll, followed by flow cytometric analysis [2]. Monocytes were further purified

by positive selection using antibody-coated magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's protocol. The purity of the isolated monocytes was assessed by flow cytometry based on forward and side scatter (FSC and SSC), and monocytes comprised 96% of the total cell population. Monocytes were resuspended at a concentration of 10^6 cells/mL in complete culture medium (RPMI 1640 supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM glutamine, and 10% heat-inactivated fetal bovine serum; Gibco BRL/Life Technologies, Grand Island, NY, USA). Purified platelets were added to the monocyte suspension (final volume 1 mL; monocyte:platelet ratio = 1:100) and incubated at 37 °C in a humidified 5% CO_2 atmosphere for 48 hours.

Методика окрашивания поверхностных белков клеток была ранее описана [3]. Моноциты изначально отбирались (гейтировались) на основе их характеристик по прямому и боковому рассеянию света, после чего в пределах гейта измеряли трехцветную флуоресценцию. Подтипирование моноцитов проводили по экспрессии CD14 и CD16. Клетки CD14+CD16– (Mon1) определялись как моноциты, экспрессирующие CD14, но не CD16. Далее CD14+CD16+ моноциты подразделялись по экспрессии CCR2 на CD14++CD16+CCR2+ (Mon2) и CD14+CD16++CCR2– (Mon3). В каждом эксперименте анализировали не менее 20 000 событий в гейте моноцитов. Результаты представлялись в виде процента положительных клеток. Циркулирующие моноцит-тромбоцитарные агрегаты (MPA) определялись по двойной экспрессии CD14 и CD42a. Внутриклеточное окрашивание цитокинов выполнялось с использованием набора фиксации и проницаемости BD Cytofix/Cytoperm (BD Biosciences). Соответствующие антитела IgG использовались в качестве изотипических контролей. После промывки в фосфатно-солевом буфере (PBS), окрашенные клетки анализировались на проточном цитофлуориметре FACS Calibur с использованием программного обеспечения CellQuest (BD Biosciences). Monocytes (1×10^6 cells/mL) isolated from healthy donors were pre-incubated with lipopolysaccharide (LPS), a monoclonal anti-CD147 antibody [4], or an IKK inhibitor (Cell Signaling Technology, Danvers, MA, USA) for 2 hours prior to initiating co-culture or indirect co-culture experiments. For direct co-culture, purified platelets were added to monocytes at a ratio of 1:100 and incubated at 37°C in 5% CO_2 for 48 hours. For indirect co-culture, Transwell chambers (Merck Millipore, Billerica, MA, USA) with polycarbonate membranes (pore size 0.4 μm) were used to allow exchange of soluble factors while preventing direct cell contact. Briefly, monocytes (1×10^6 cells/mL) were placed in the upper chamber, while platelets (1×10^8 cells/mL) or culture medium alone were added to the lower chamber.
Monocyte subset proliferation was assessed using the BrdU Flow Kit according to the manufacturer's instructions.

All results presented are representative of at least three independent experiments and are expressed as mean ± standard deviation (SD). Statistical analysis was performed using either Student's *t*-test or the Mann–Whitney *U* test, depending on data distribution. For multiple comparisons against a single control group, one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was used. All statistical analyses were conducted using GraphPad software. *P* values < 0.05 were considered statistically significant.

Results and Discussion

Flow cytometry revealed significantly elevated expression of platelet activation markers CD147, PAC-1, and P-selectin (CD62P) in RA patients, particularly in those with active disease. The proportions of positive platelets for CD147, PAC-1, and CD62P were $47.63 \pm 3.1\%$, $41.73 \pm 2.89\%$, and $10.18 \pm 1.15\%$ in active RA, compared to $12.26 \pm 0.83\%$, $3.8 \pm 0.44\%$, and $0.39 \pm 0.07\%$ in healthy controls ($P < 0.05$). CD40L expression was also higher in active RA ($6.11 \pm 0.44\%$) vs. controls ($3.3 \pm 0.56\%$; $P < 0.05$), with no significant difference in inactive RA. CD147 expression on platelets was consistently higher than other activation markers and showed strong positive correlations with disease activity (DAS28) and the expression of PAC-1 and CD62P. These findings suggest that CD147 may serve as a constitutive marker of platelet activation and disease severity in RA. Monocyte subtypes were defined as classical (Mon1: CD14++CD16–CCR2+), intermediate (Mon2: CD14++CD16+CCR2+), and non-classical (Mon3: CD14+CD16++CCR2–). Mon2 and Mon3 frequencies were significantly increased in RA patients, especially Mon2 in active disease. CD147 expression was upregulated in all monocyte subsets in RA, with the highest levels observed in Mon2. CD147 expression in Mon2 correlated more strongly with monocyte–platelet aggregate (MPA) formation than its frequency or expression in Mon1/Mon3. MPA levels were markedly elevated in RA patients (active: $55.36 \pm 2.53\%$, inactive: $37.32 \pm 1.22\%$) vs. controls ($15.69 \pm 3.33\%$; $P < 0.0001$). Mon2 showed the greatest propensity to form aggregates with platelets, and MPA levels correlated positively with Mon2 frequency and CD147 expression on both Mon2 and platelets.

Functional co-culture assays demonstrated that direct contact between platelets and monocytes (but not indirect co-culture) led to increased Mon2 frequency, CD147 expression, and inflammatory cytokine production (IL-6, TNF-α). These effects were abolished by CD147 blockade, implicating CD147–CD147 interactions in mediating these responses. CD147 was shown to activate NF-κB signaling, enhancing the secretion of MMP-9 and proinflammatory cytokines, with the most pronounced effects observed in Mon2 cells. Our findings suggest that CD147 plays a critical regulatory role in monocyte–platelet interactions, particularly in Mon2-driven inflammation in RA. Given its contribution to cytokine production, MMP activity, and disease activity, CD147 represents a promising therapeutic target for modulating systemic inflammation in RA.

Conclusion

Overall, our findings expand current understanding of CD147 expression on platelets in the context of rheumatoid arthritis (RA). The study highlights the pivotal role of Mon2 monocytes in sustaining a proinflammatory cytokine milieu, closely associated

with platelet activation. These results underscore the importance of platelet–monocyte interactions in RA pathophysiology, with CD147 emerging as a central mediator, potentially via NF-κB signaling. Targeting CD147 may represent a promising therapeutic strategy for RA management.

Despite advances in understanding the roles of monocytes and platelets in RA, the underlying mechanisms of their interaction and impact on monocyte phenotype and inflammation remain incompletely understood. Further research is needed to elucidate these complex pathways, which may pave the way for novel and more effective treatment approaches for RA.

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