Gastric cancer increases transmigratory potential of peripheral blood monocytes by upregulation of β1- and β2-integrins

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Introduction: Immune responses within the tumor depend on the ability of leukocytes to migrate from peripheral circulation into the local microenvironment. This process is controlled by mechanisms that guide leukocytes to the side of inflammation, allowing them to cross vascular endothelial barrier. Monocytes/macrophages are the predominant population of leukocyte infiltrate of many tumors, including, gastric cancer. However, to date mechanisms that control monocyte trafficking to the side of tumor growth are not fully elucidated.
Aim of the study: It this study we aimed to evaluate transmigratory potential of peripheral blood monocytes from gastric cancer patients.
Material and methods: By using multicolor flow cytometry we assessed expression of β1- and β2-integrins on peripheral blood monocytes from gastric cancer patients.
Results: We found increased frequencies of VLA-4 and VLA-6 expressing monocytes and increased expression of analyzed β2-integrins in gastric cancer patients when compared to age matched controls.
Conclusions: In summary, this study revealed that gastric cancer increases transmigratory potential of peripheral blood monocytes.

Key words: gastric cancer, monocytes, β2-integrins, VLA-4, VLA6.

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Introduction

Macrophages represent predominant component of leukocyte infiltrate in many tumors, including gastric cancer [1, 2]. Due to their pleiotropic biological activities they act as orchestrators of immune response within tumor. They may play pivotal role in tumor development acting as tumor suppressors (M1 phenotype – classically activated cells) or tumor supporters (M2 phenotype – alternatively activated cells) [1, 3]. Unfortunately, majority of macrophages within tumor microenvironment acquire the latter phenotype and are referred to as tumor associated macrophages (TAMs). Macrophage polarization depends on the immune modulatory properties of tumor and stroma cells, that can interact locally within tumor tissue or affect peripheral precursors (monocytes) [4].

Recruitment of monocytes into tumor microenvironment is a hallmark of cancer development and progression [5, 6]. Notably, migration of peripheral blood cells to the side of tumor growth is controlled by different soluble factors, namely cytokines, chemokines, growth factors and metabolites [7, 8]. On the other hand, vascular and epithelial junctions represent a barrier for leucocyte migration [9]. Interestingly, monocyte transmigration through vessel wall is possible due to the presence of membrane adhesion molecules, including proteins belonging to β1 and β2-integrin family [10]. Leukocyte adhesion molecules interact with their ligands expressed on cytokine activated endothelium and allows monocytes to avoid forces exerted by the rapid blood flow in vasculature. Consequently, monocytes start to roll along apical endothelial surface until complete immobilization and transmigration [11]. Unfortunately, to date the molecular, humoral and cellular mechanisms that control monocyte trafficking in cancer are not fully elucidated. Therefore, here we aimed to evaluate whether systemic activation of peripheral blood monocytes observed in gastric cancer patients increases transmigratory potential of these cells.

Material and methods

Patients

15 normal donors and 40 gastric cancer patients, succsesive qualified to stomach resection at Chair of Surgical Oncology, Prof. F. Lukaszczyk Memo-

Table 1. Clinical characteristics of study population

Parameter	Gastric cancer	Normal
n	40	17
Mean age (range)	62,79 (30-86)	58,35 (49-68)
Gender (female/male)	13/27	8/9
Stage according to AJCC (frequencies of all)		
Stage I	12 (30%)	
Stage II	2 (5%)	
Stage III	14 (35%)	
Stage IV	12 (30%)	

AJCC – American Joint Committee on Cancer

rial Centre of Oncology in Bydgoszcz (Poland), were enrolled to the study (Table 1). None of the patients received chemotherapy and radiotherapy before or was subjected to surgery or blood transfusion for at least six month before blood acquisition. Furthermore, none of the patients showed any clinical or cellular sings on ongoing infection. Peripheral blood was collected upon the approval of the Bioethical Committee of the Collegium Medicum in Bydgoszcz. Each participant was familiarized with the objectives of the study and expressed written consent.

Flow cytometry

100 µl of fresh heparin-anticoagulated blood was stained with panel of mouse anti-human monoclonal antibodies (Table 2). Stain-then-lyse protocol was used as previously described [12]. Appropriate fluorescence-minus-one (FMO) and isotype controls were used for every staining for setting compensation and to assure correct gating. Samples were analyzed by using FACScan flow cytometer (Becton Dickinson) and at least 40 000 events were collected. Next, collected data were analyzed by using FlowJo version 7.6.1. (TreeStar). Used gating strategy is presented on Fig. 1.

Statistics

Statistical analysis was carried out using GraphPad Prism 6 software (GraphPad Software). U Mann-Whitney test was used. The differences were considered statistically significant at $p < 0.05$. The results are presented as median (interquartile range).

Results

Due to the constitutive expression of analyzed β_2-integrins on the surface of monocytes we analyzed their expression levels. First we found significant increase of CD11a and CD11b expression in gastric cancer patients when compared to normal donors. Additionally, we found no differences in CD11c and CD18 expression level. Next, we found that gastric cancer increase frequencies of CD49d (α4 subunit of VLA-4 integrin) and CD49f (α6 subunit of VLA-6 integrin) expressing monocytes. Interestingly, we did not observed any differences in expression level of above mentioned molecules.

Discussion

Integrins are membrane glycoproteins controlling numerous physiological processes, including cell adhesion, chemotaxis, and phagocytosis [13]. β2-integrins are heterodimeric receptor proteins consisting of α and β subunits linked by sulphide bridges. All receptors shear common β2-chain (CD18) and differ in α chain variants, namely αL – CD11a; αM – CD11b; αX – CD11c. They are involved in direct adhesion of leukocytes to endothelial cells [14, 15]. Similarly, β1-integrins shear common β1 chain (CD29) and differ in α subunits. To date, at least 10 different α chains were discovered including CD49d and CD49f expressed on monocytes. In contrast to β2-integrins, the latter represent a group of protein receptor responsible for cell interactions with extracellular matrix [13]. Here, we found that gastric cancer increase frequencies of both VLA-4 and VLA-6 expressing monocytes. Interestingly, Jin *et al.* showed that VLA-4 is playing leading role in monocyte transmigration to tumor microenvironment [16]. However, in some contrast to previous studies by Zhang *et al*., they found that this process occurred in the αMβ2 (CD11b/CD18) integrin independent manner [16, 17]. It seems, that β2-integrins may play supportive role in monocyte transmigration process and observed upregulation of CD11a and CD11b expression is a consequence of monocyte activation and their inflammatory phenotype [18]. Notably, in our previous report we found that gastric cancer patients showed increased frequencies of inflammatory (activated) monocytes, namely intermediate (CD14++CD16+) and non-classical (CD14+CD16++) cells [12]. Interestingly, VLA-4 support not only transmigration of inflammatory monocytes but also

Table 2. Characteristics of monoclonal antibodies used in the study

Name	Clone	Isotype	Format	Manufacturer
CD14	MoP9	IgG2b	PerCP	Becton Dickinson
CD49d (VLA-4 α subunit)	9F10	IgG1	FITC	Becton Dickinson
CD49f (VLA-6 α subunit)	1.BB.460	IgG2b	FITC	Becton Dickinson
CD11a	G43-25B	IgG2a	FITC	Becton Dickinson
CD11b	1.BB.189	IgG1	FITC	Santa Cruz Biotechnology
CD11c	B-Ly6	IgG1	PE	Becton Dickinson
CD18	6.7	IgG1	PE	Becton Dickinson

PerCp – peridinin chlorophyll protein complex; FITC – fluorescein isothiocyanate; PE – phycoerythrin

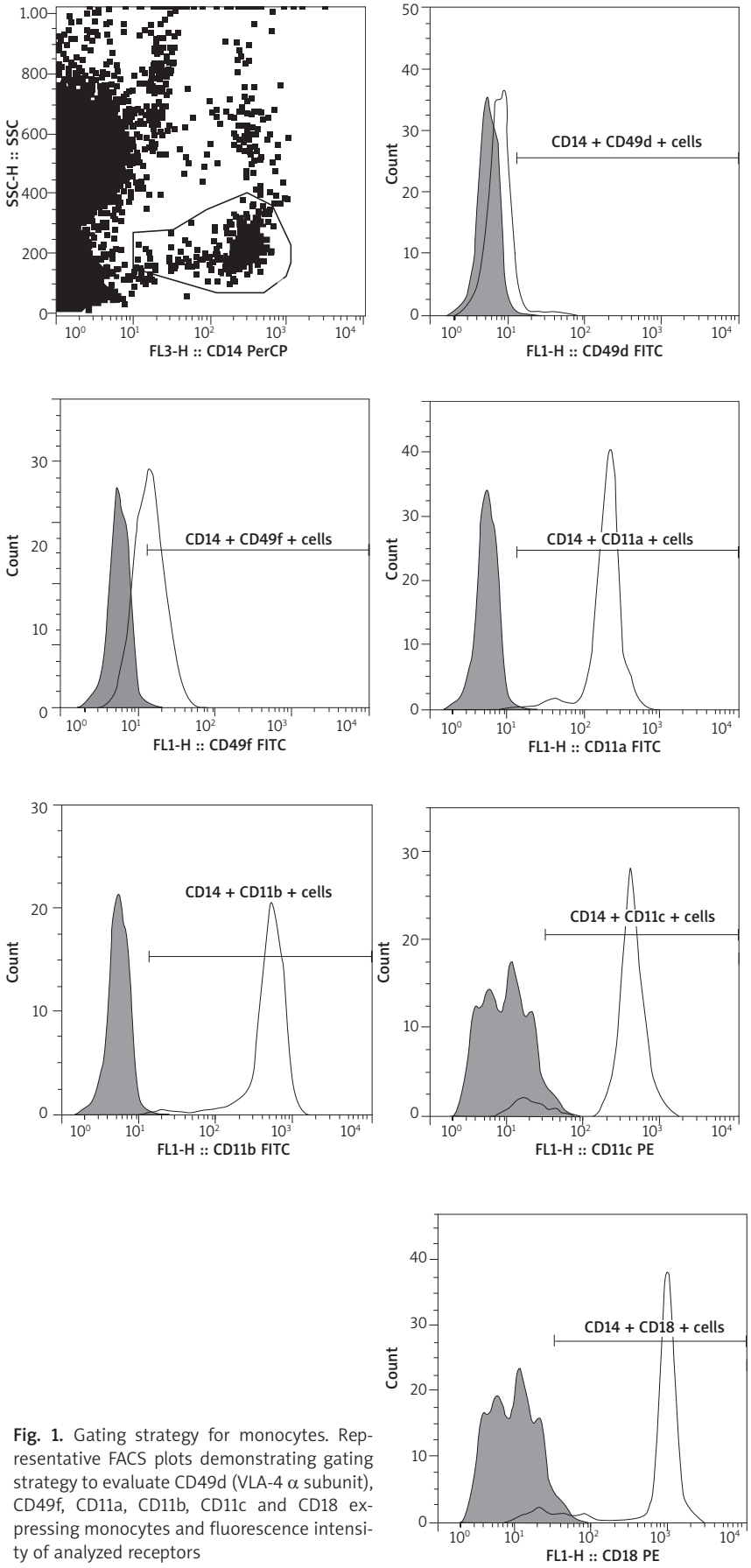

Fig. 1. Gating strategy for monocytes. Representative FACS plots demonstrating gating strategy to evaluate CD49d (VLA-4 α subunit), CD49f, CD11a, CD11b, CD11c and CD18 expressing monocytes and fluorescence intensity of analyzed receptors

the transition of macrophages from classically activated (M1) to pro-tumoral (M2) cells, by Rac2 activation [19]. Therefore, increased frequencies of VLA-4 expressing monocytes may be a consequence of their non-classical activation. Furthermore, VLA-6 was shown to support tumor growth and angiogenesis by promoting tumor infiltration of Tie-2 expressing monocytes and macrophages (TEMs) [20]. In summary, we showed here that peripheral blood monocytes from gastric cancer patients possess high transmigratory potential and are sensitive for non-classical polarization.

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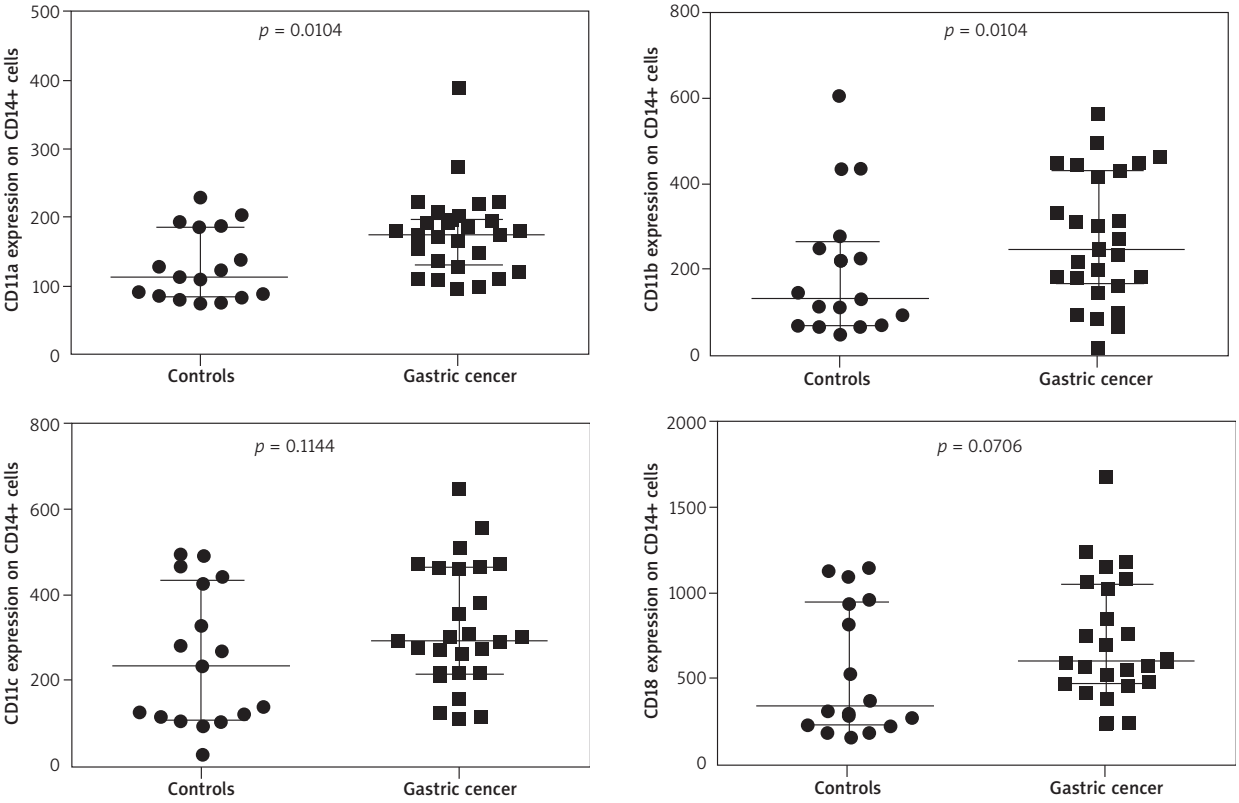

Fig. 2. β2-integrin expression in peripheral blood monocytes. Summary of analyses of (A) CD11a, (B) CD11b, (C) CD11c and (D) CD18 expression on CD14+ cells. Data are presented as median and interquartile range

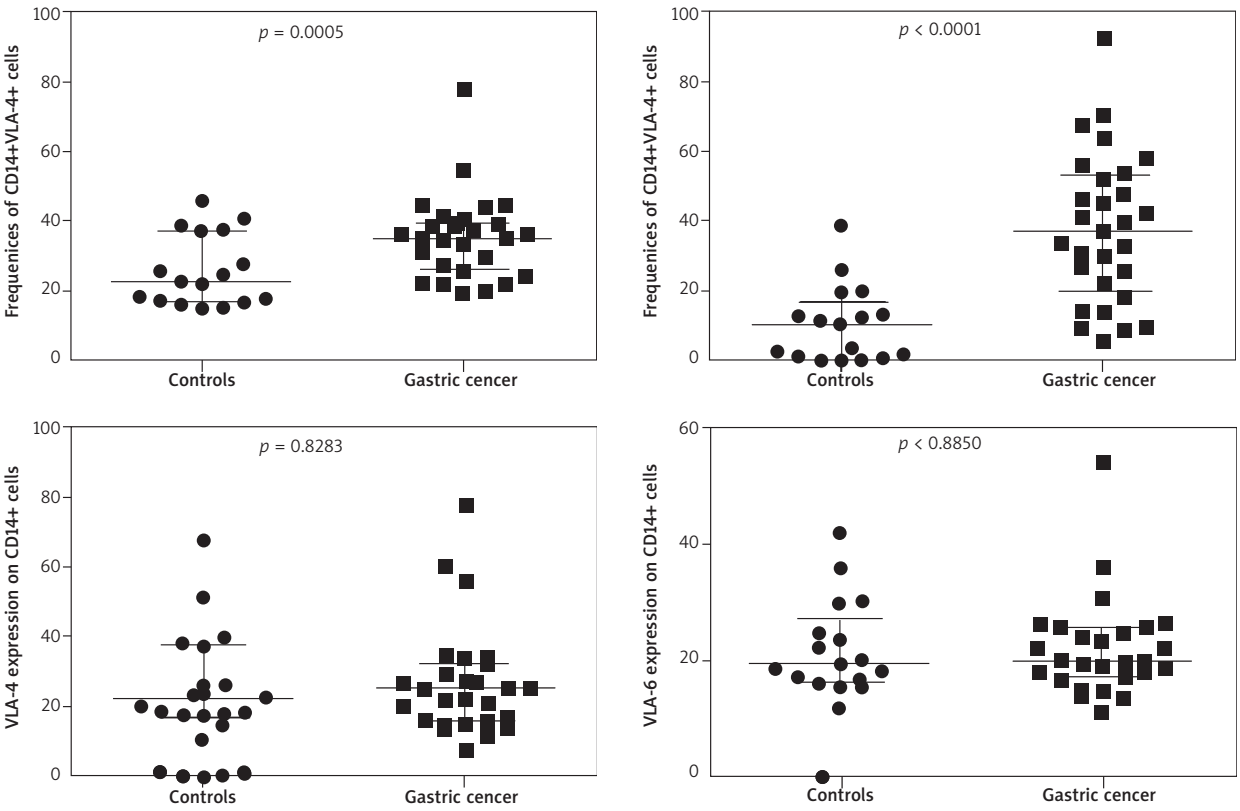

Fig. 3. VLA-4 and VLA-6 expression in peripheral blood monocytes. Summary of analyses of (A) VLA-4 and (B) VLA-6 expressing monocytes and (C) VLA-4 and (D) VLA-6 expression on CD14+ cells. Data are presented as median and interquartile range

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