

# Evaluation of CD15s and PSGL-1 expression on peripheral blood neutrophils and sPSGL-1 and sP-selectins form concentration in the serum of patients with Lyme borreliosis

Aneta Iżycka<sup>1</sup>, Ewa Jabłońska<sup>1</sup>, Joanna Zajkowska<sup>2</sup>,  
Sławomir Pancewicz<sup>2</sup>, Sławomir Ziarko<sup>3</sup>, Piotr Radziwon<sup>3</sup>,  
Teresa Hermanowska-Szpakowicz<sup>2</sup>

<sup>1</sup>Department of Immunology, Medical University of Białystok; <sup>2</sup>Department of Infectious Diseases and Neuroinfection, Medical University of Białystok; <sup>3</sup>Regional Blood Center for Transfusion Medicine Białystok

## SUMMARY

*Evaluation of CD15s and PSGL-1 expression on peripheral blood neutrophils and sPSGL-1 and sP-selectins form concentration in the serum of patients with Lyme borreliosis*

<sup>1</sup>Iżycka A., <sup>1</sup>Jabłońska E., <sup>2</sup>Zajkowska J., <sup>2</sup>Pancewicz S.,  
<sup>3</sup>Ziarko S., <sup>3</sup>Radziwon P., <sup>2</sup>Hermanowska-Szpakowicz T.

<sup>1</sup>Department of Immunology, Medical University of Białystok; <sup>2</sup>Department of Infectious Diseases and Neuroinfection, Medical University of Białystok; <sup>3</sup>Regional Blood Center for Transfusion Medicine Białystok

Int. Rev. Allergol. Clin. Immunol., 2010; Vol. 16, No. 1-2

*This study evaluates the expression of CD15s and PSGL-1 on neutrophils in peripheral blood and serum soluble form of PSGL-1 and P-selectin in serum of patients with Lyme borreliosis. In studies in patients with Lyme borreliosis demonstrated no significant differences in mRNA expression of both PSGL-1 mRNA and CD 15 on neutrophils, but we found a significant increase sPSGL-1 and sP-selectin in the serum of patients with Lyme borreliosis. Observed in our study increased levels of soluble P-selectin and PSGL-1 by blocking a specific ligand on neutrophils may lead to inhibition of the adhesion process, which may be one reason for the continuation of long-term inflammation.*

**Key words:** Lyme borreliosis, neutrophils, adhesion molecules, CD15s, PSGL-1, P-selectin

The change of adhesion molecule expression on the neutrophils (PMN) may have significant influence on defense reactions of the organism in response to the activity of inflammatory agents and may cause an impaired immune response to different pathogens,

## STRESZCZENIE

*Ocena ekspresji CD15s i PSGL-1 na neutrofilach krwi obwodowej oraz stężenia rozpuszczalnej formy sPSGL-1 i sP-selektyny w surowicy pacjentów z boreliozą z Lyme*

<sup>1</sup>Iżycka A., <sup>1</sup>Jabłońska E., <sup>2</sup>Zajkowska J., <sup>2</sup>Pancewicz S.,  
<sup>3</sup>Ziarko S., <sup>3</sup>Radziwon P., <sup>2</sup>Hermanowska-Szpakowicz T.

<sup>1</sup>Zakład Immunologii, Uniwersytet Medyczny w Białymstoku; <sup>2</sup>Klinika Chorób Zakaźnych i Neuroinfekcji, Uniwersytet Medyczny w Białymstoku; <sup>3</sup>Regionalne Centrum Krwiodawstwa i Krwiolęcznictwa w Białymstoku

Int. Rev. Allergol. Clin. Immunol., 2010; Vol. 16, No. 1-2

*Celem pracy była ocena ekspresji CD15s i PSGL-1 na neutrofilach krwi obwodowej oraz stężenia rozpuszczalnej formy PSGL-1 i P-selektyny w surowicy pacjentów z boreliozą z Lyme. W przeprowadzonych badaniach u pacjentów z boreliozą z Lyme nie wykazaliśmy istotnych różnic w ekspresji zarówno mRNA PSGL-1 jak i mRNA CD 15 na neutrofilach, natomiast stwierdziliśmy istotny wzrost sPSGL-1 i sP-selektyny w surowicy krwi pacjentów z boreliozą z Lyme. Obserwowane w badaniach własnych zwiększenie stężenia rozpuszczalnych form P-selektyn i PSGL-1 poprzez blokowanie specyficznych ligandów na neutrofilach może prowadzić do zahamowania procesu adhezji, co może być jedną z przyczyn utrzymywania się długotrwałego procesu zapalnego.*

**Słowa kluczowe:** borelioza z Lyme, neutrofile, cząsteczki adhezyjne, CD15s, PSGL-1, P-selektyna

*Borrelia burgdorferi* spirochete causing Lyme borreliosis [7,20].

It is known that *Borrelia burgdorferi* spreads in the organism via lymphatic and blood vessels. Coburn et al. proved that one of the mechanisms

enabling spirochetes to penetrate tissues is binding a pathogen with blood platelets by means of  $\lambda_5\beta_3$ , combining with endothelial cells and further penetration of certain tissues and organs [2]. Ma et al. demonstrated that *Borrelia burgdorferi* spirochete location inside the epithelial cells enabled it to survive in the host's infected organism [14].

*Borrelia burgdorferi* spirochetes' invasion needs also the interaction and penetration of leukocytes through the vascular endothelium. It has been proved that leukocytes directly and actively take part in the development of *Lyme disease*.

The studies performed so far proved that defense mechanisms, associated with a host's nonspecific response, got impaired in the course of *Lyme borreliosis*. A decrease in the percentage of phagocytosing cells, as well as weakening of chemotaxis process and migration were observed in our earlier studies [9, 10]. The impaired phagocytic activity can be associated with the changed expression of adhesion cells on the PMN. In our study, we evaluated  $\beta$  integrin (LFA-1, p-150 and Mac-1) and L-selectin expression on the neutrophils and the concentration of a soluble form of ICAM-1 and L-selectin in the blood serum of patients with *Lyme borreliosis*. The study results proved the increase in the expression of these adhesion molecules on the neutrophils, which may intensify the PMN adhesion to endothelial cells and their transfer towards the inflammatory focus. Thus, the process of the PMN adhesion to endothelial cells with L-selectin and LFA-1 contribution seems to be normal in patients with *Lyme borreliosis*. The question arises whether the further stages of adhesion processes are not impaired and whether the PMN may move transfer effortlessly towards the inflammatory focus? Adhesion and migration of neutrophils depend not only on L-selectin and  $\beta_2$  integrin, but also on other adhesion structures influencing significantly adhesion of leukocytes.

Recognition of endothelial selectins, initiating adhesion process by leukocytes, requires the presence and superficial expression of their ligands, specific oligosaccharide structures such as: sialo-Lewis X (CD15s, sLeX) and PSGL-1. The expression of these molecules has been proved on monocytes, neutrophils and macrophages. They take part in the initial stage of adhesion.

It has been proved that binding of PSGL-1 with L-selectin causes the mutual binding of leukocytes, which strengthens the recruitment of leukocytes to the blood vessel wall. Similarly, Picker et al. proved that L-selectins present on the leukocytes recognized and bound oligosaccharide X(sLeX) sialo-Lewis determinants, and due to this process L-selectins could mediate in the homotype adhesion of neutrophils

during leukocyte rolling on the endothelial blood vessels of the inflammatory changed tissues [17]. Mutual interactions of selectins and their ligands have to follow fast to facilitate leukocyte rolling and binding. More et al. observed that anti-PSGL-1 antibodies inhibited margination and rolling of human neutrophils, monocytes, eosinophils to P-selectins of endothelial cells. PSGL-1 blocking indicates a significant role of this molecule in the process of adhesion [16].

PSGL-1 and P-selectin are also present in a soluble form, which is released due to enzymatic proteolysis. Following the competitive binding of PSGL-1 soluble forms with P-selectin it may inhibit the regulation of leukocyte adhesion, which may be one of the reasons for weakening of an immune response in patients with *Lyme borreliosis*.

Taking into consideration a significant role of neutrophils and adhesion process in controlling the development of *Borrelia burgdorferi* spirochete infection, the attempt has been made to evaluate CD15s and PSGL-1 expression on neutrophils and the concentration of their ligands – soluble forms of sPSGL-1 and sP-selectin in the serum of patients with *Lyme borreliosis*. This examination will enable to gain knowledge on the mechanisms responsible for the inhibition of a nonspecific response in this disease.

## MATERIALS AND METHODS

### Materials

The study included 32 patients diagnosed and treated in the Department of Infectious Diseases and Neuroinfection of the Medical University of Białystok (25 men and 7 women) with *Lyme borreliosis*. The mean age of patients was 60 years.

The diagnosis of *Lyme borreliosis* was made basing on the serological tests showing the presence of IgM antibodies against p 41 flagellum antigens of *Borrelia burgdorferi*.

The blood samples were taken twice: before treatment and after 4-week – antibiotic therapy with  $\beta$ -lactam antibiotics and cephalosporins. Patients with *Lyme borreliosis*: the group with erythema migrans – amoxycycline, the group with *Lyme arthritis* – cefotaxym.

The control group consisted of 15 healthy volunteers.

### Isolation PMN

The blood samples were taken for EDTA to determine the expression of CD15s and PSGL-1. Neutrophils were isolated by the Zeman et al. method. The neutrophils obtained were suspended in PBS to obtain the concentration of  $5 \times 10^6$  cells/ml.

### RNA isolation

RNA was extracted with a silica gel-based membrane system, the RNeasy Mini Kit (Qiagen), according to the manufacturer's specifications, and following the recommended protocol adjustments for fresh samples or viably frozen cells, as appropriate.

The quantity of RNA was defined in the spectrophotometer at the wave length of 260 nm.

The expression of CD15s and PSGL-1 was determined by means of RT-PCR method Real-time RT-PCR was performed with a Light Cycler (Roche Molecular Biochemicals, Mannheim, Germany) in Light Cycler capillaries using a commercially available master mix containing Taq DNA polymerase and SYBR-Green I deoxyribonucleoside triphosphates (Light Cycler RNA master SYBR-Green I, Roche Molecular Biochemicals).

In this method, the initial matrix is RNA, which in the process of reverse transcription is rewritten on the complementary DNA, undergoing the amplification. The RT-PCR reaction enables to determine quantitatively the amplified products in the factual time thanks to the fluorescent dyes. After activation, these dyes send a signal, which grows directly to the amount of the amplified product. This method is used to measure the quantity of gene copy or the expression level of RNA in the cells and tissues. The quantity of the copies of the nucleonic acid molecule is monitored in each cycle of the amplification reaction. The number of PCR reaction cycles after which the fluorescent level exceeds the defined threshold is used to calculate the quantity of the molecules present in the mixture at the beginning of the reaction. 50 ng of RNA was used to the reaction RT-PCR

The following set of primers was used:

CD15 sens 5'- GCTATGGAGATACAGACCACTCA -3'  
CD15 antisens

5' CAGATGGCAGAGTGAGCTAAG - -3'

PSGL-1 5'- CACCTCCGAGGCATCTTCAACTG -3',  
PSGL-1 antisens

5- CGTTGGTATCGGCTCTCATTCATG -3'.

The analysis was performed using the kit of OneStep SybrGreen Master Kit (Roche) in the apparatus Lightcycler (Roche). For DNA sequencing, the following PCR parameters were used: 95°C for 5s followed by 45 cycles of 68°C for 20s, 72°C for 15s. The protocol of the RT-PCR reaction was as follows: the amplification conditions for LightCycler consisted of an initial 1,5 min denaturation at the temperature of 95°C for 10 min, duplication at the temperature of 78°C for 20s and elongation at the temperature of 72°C for 25s (45 cycles). The specificity of the PCR products obtained was proved via their electrophoresis in the 2% agarose gel with ethydine bromide. The reaction generated Mac-1 316

bp, p150 406 and GPDH 540 bp, respectively. The quantitative analysis of the copies was carried out based on the calculated number of the copies in the standard RNA (Applied Biosystem) using the formula:

$$\text{Xg/mRNA} / [\text{length of the transcript in nucleotides} \times 340] \times 6, 022 \times 10^{23} = Y/\mu\text{l}$$

The concentrations of soluble forms of sPSGL-1 and sP-selectin were determined by ELISA method using the kits of BenderMedSystem company.

Statistical analysis was performed using Statistica 6.0 software. The compatibility test of Kolomagorow-Smirnow was used for measurable features consistent with normal distribution. Student's t – test was applied to compare the chosen groups and Student's t - test for pairs to compare features in two time spans. Mann-Whitney's test was taken for the features inconsistent with this distribution and Wilcoxon's test for pairs. The significance level of  $p < 0.05$  was regarded as statistically significant in calculations.

## RESULTS

No significant differences were proved in the expression of mRNA PSGL-1 on the neutrophils in patients with *Lyme borreliosis* before treatment in comparison with the values obtained in controls (table 1). Similarly, no marked differences were observed in the expression of this molecule after antibiotic therapy compared to the results obtained before treatment. The expression of mRNA CD15s on the neutrophils behaved likewise in patients with *Lyme borreliosis* both before and after treatment. The value was not different from the obtained in controls (table 2).

The expression of mRNA PSGL-1 and CD15s on the PMN of the peripheral blood was compared in patients with different forms of *Lyme borreliosis*: Ery-

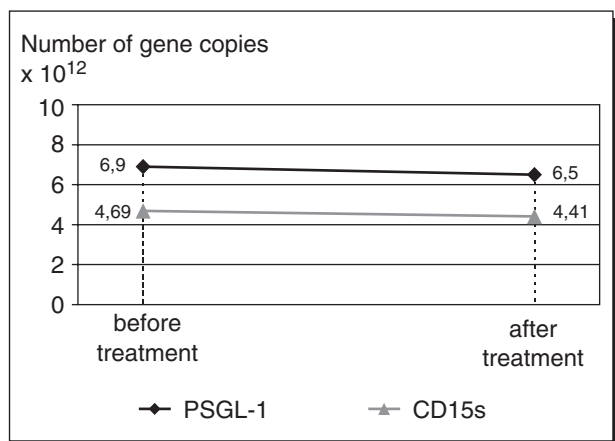
**Table 1.** Number of gene copies PSGL-1 on PMN in patients with *Lyme borreliosis*

Group	PSGL-1 (μl)	
	X	SD
Control group	$6,58 \times 10^{12}$	1,4
Before treatment	$7,21 \times 10^{12}$	1,2
After treatment	$7,82 \times 10^{12}$	1,8

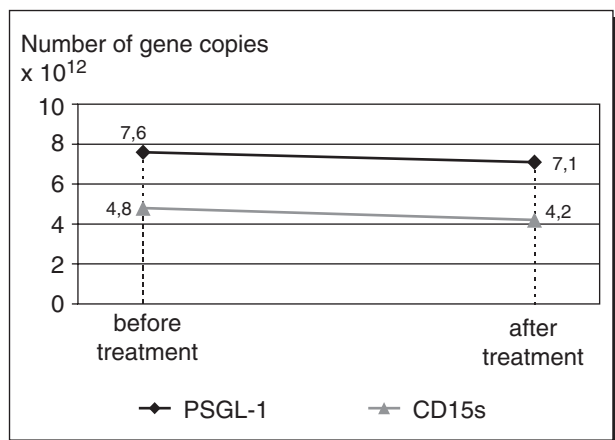
**Table 2.** Number of gene copies CD 15s on PMN in patients with *Lyme borreliosis*

Group	CD15s (μl)	
	X	SD
Control group	$3,77 \times 10^{12}$	0,59
Before treatment	$4,21 \times 10^{12}$	0,98
After treatment	$4,32 \times 10^{12}$	1,1

thema migrans and Lyme arthritis (fig. 1,2). No statistically significant differences were observed in the expression of these molecules between Erythema migrans and *Lyme arthritis*. Similarly, no differences were found in the values obtained before and after treatment in both forms of *Lyme borreliosis*.



**Fig. 1.** Dynamics of expressions changes of mRNA PSGL-1 and CD15s on PMN in patients with Erythema migrans before and after treatment



**Fig. 2.** Dynamics of expressions changes of mRNA PSGL-1 and CD15s on PMN in patients with *Lyme arthritis* before and after treatment

The percentages of the cases of specific forms of *Lyme borreliosis* with expression of mRNA PSGL-1 and CD15s on the neutrophils higher than the mean value of controls were calculated. The higher expression of PSGL-1 was observed in 57% of patients with Erythema migrans, whereas after treatment in 42%. In *Lyme arthritis*, the higher expression of mRNA PSGL-1 was revealed in 56% of patients, whereas after treatment in 45%.

In patients with Erythema migrans before treatment, high expression of mRNA of CD15 molecule was demonstrated in 58% of patients and after treatment in 51%. In patients with *Lyme arthritis* before treatment, higher expression of mRNA CD15s was

revealed in 53% of patients, whereas after treatment in 49%.

An increase in the concentration of a soluble form of sPSGL-1 was found in the serum of patients with *Lyme borreliosis* in comparison with the values obtained in controls. In the examination after treatment the level of soluble sPSGL-1 did not differ significantly in comparison with the level before treatment (table 3). The serum sP-selectin concentration increased markedly in patients with *Lyme borreliosis* before treatment when compared to controls, whereas after treatment the level of adhesion molecule decreased in comparison with the level before treatment (table 4).

**Table 3.** Level of sP-selectin in serum of patients with *Lyme boreliosis*

Group	sP-selektyna (ng/ml)	
	X	SD
Control group	54,8	9,83
Before treatment	67,4*	15,8
After treatment	62,8	12,6

\*statistically significant difference at  $p < 0.05$

**Table 4.** Level of sPSGL-1 in serum of patients with *Lyme boreliosis*

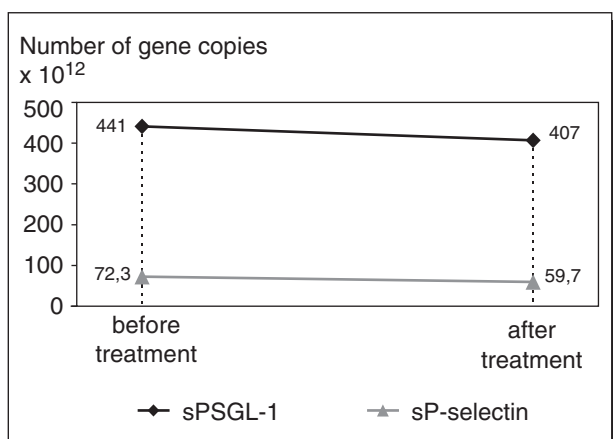
Group	sPSGL-1 (ng/ml)	
	X	SD
Control group	319,9	34,2
Before treatment	425,4*	41,7
After treatment	398,2	31,4

\*statistically significant difference at  $p < 0.05$

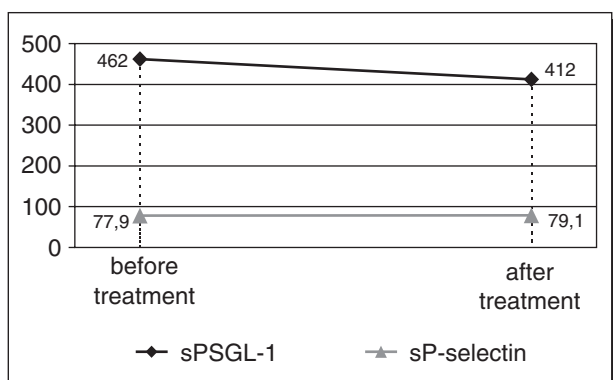
The levels of soluble sP-selectin and sE-selectin were analyzed with regard to a clinical form of *Lyme borreliosis* (fig. 3, 4). No statistically significant differences were observed in the expression of these molecules between patients with Erythema migrans and *Lyme arthritis*. Similarly, no differences were found in the levels before and after treatment in both forms of *Lyme borreliosis*.

No correlation was proved between CD15s, PSGL-1 and the concentration of sPSGL-1 and sP selectin in patients with *Lyme borreliosis*.

The percentages of the cases before treatment with the elevated levels of soluble sP-selectin and sE-selectin in comparison with the mean values of controls were also calculated. The increase in the percentage of serum soluble sP-selectin higher than the mean values of controls was revealed in 53.6% of patients, whereas the increase in sE-selectin concentration higher than the mean values of controls was demonstrated in 67.8% of patients.



**Fig. 3.** Level of sP-selectins and sPSGL-1 in serum of patients with in patients with Erythema migrans before and after treatment



**Fig. 4.** Level of sP-selectins and sPSGL-1 in serum of patients with in patients with Lyme arthritis before and after treatment

In patients after treatment, the percentage of cases with the elevated level of soluble sP-selectin in comparison with the mean value of controls equaled 47.2%, whereas the level of soluble sE-selectin decreased to 47.7%.

## DISCUSSION

The process of adhesion is a key stage of migration of cell neutrophils to the inflamed tissues. An initial interaction between endothelial cells and leukocytes takes place with the participation of endothelial selectins – selectin E, P and leukocytic selectin-L, binding loosely with PSGL-1 neutrophils. Improper binding of selectins with neutrophils may reduce markedly further stages of adhesion, leading to an impaired anti-inflammatory response.

In the studies carried out in patients with *Lyme borreliosis*, no differences were found in the expression of both mRNA PSGL-1 and mRNA CD 15 on the neutrophils. However, the significant increase in sPSGL-1 and sP-selectin was revealed in the blood serum of patients with *Lyme borreliosis*.

Proteolytic enzymes of neutrophils may influence the increase in the concentration of a soluble form of PSGL-1 *Gardiner et al.* [5] examined the effect of elastasis and G cathepsin on the binding of P-selectin with PSGL-1 present on the neutrophils. They incubated neutrophils with purified cathepsin G and elastasis, which caused shelling of PSGL-1 and the inhibition of neutrophils binding with P-selectin. These examinations were confirmed using elastasis inhibitors and G cathepsin, which prevented from the loss of membrane PSGL-1. G cathepsin and elastasis were reported to mediate in proteolytic shelling of PSGL-1, which may decrease the function of this adhesin in the activation of neutrophils in an anti-inflammatory response. There are reports showing the influence of *Borrelia burgdorferi* spirochetes on elastasis of neutrophils. *Lusitani, Garcia* [4, 13] incubated human neutrophils with *Borrelia burgdorferi* spirochetes and proved that these cells released high amounts of elastasis. The release of elastasis by neutrophils infected with *Borrelia burgdorferi* spirochetes, may have an influence on shelling the membrane form of PSGL-1 from the surface of the leukocytes leading to an increase in the soluble form of this adhesion, which was confirmed in our study.

The increase in the concentration of the soluble form of selectin-P was demonstrated in the blood serum in our study. Selectin-P is expressed on the endothelial cells and blood platelets from the cytoplasmic granules induced by thrombin, histamine. The expression of selectin-P on the cell surface is not only associated with the fusion of granules with the cell membrane, but also regulated at the transcription level. A transcription gen is reduced by TNF- $\alpha$ , IL-1, leading to the expression of this molecule that is impermanent and undergoes fast shelling from the cell surface [7, 8].

The high concentration of sP-selectin can be explained by the activation of endothelium by *Borrelia burgdorferi* spirochete [2, 14, 16]. It has been proved that the vascular endothelium may be activated via the surface antigens of *Borrelia burgdorferi* spirochete. *Sellati et al.* examined the ability to activate endothelial cells by both synthesized and natural OspA antigen [19]. The activation of endothelial cells via the surface protein of *Borrelia burgdorferi* was confirmed in our study, in which the increase in a soluble form of P-selectin was revealed before treatment in patients both with Erythema migrans and *Lyme arthritis* in comparison with controls.

*Wooten et al.* proved the capability of direct activation of NF- $\kappa$ B agent by the surface antigen OspA of *B. burgdorferi* spirochete [22]. Due to the activity of OspA of *B. burgdorferi* spirochete, NF- $\kappa$ B is released from I $\kappa$ B, which is able to transfer to the cell

nucleus, activating the expression of many genes, including genes responsible for the synthesis of cytokines and adhesion molecules [13, 14, 15]. Activation of endothelium cells seems to be one of the pathogenetic mechanisms induced by *Borrelia burgdorferi* spirochete. The increase in P-selectin expression takes place on the activated endothelial cells. The membrane form of this adhesin may get shelled via the activity of metalloproteinases, due to which a soluble form is formed [5, 6, 8, 15]. This was confirmed by Behr's et al. studies, which proved that *Borrelia burgdorferi* spirochete induced the formation of metalloproteinases by human chondrocytes Gebbia et al. incubated human neutrophils, monocytes, keratinocytes with *Borrelia burgdorferi* spirochetes and proved the increase in the production of MMP-9, MMP-1, MMP-2 by these cells [1, 6]. The increase in the concentrations of metalloproteinases, caused by the cells stimulated by *B.b* spirochetes, may be one of the causes of the increased concentration of sP-selectin in the serum of patients with *Lyme borreliosis*.

The increase in the concentration of sP-selectin and PSGL-1 may cause the impaired migration of neutrophils to the site of an on-going inflammatory process [3, 7, 11, 17, 18]. The soluble forms of P-selectin can bind with their ligands on the neutrophils, that is, with PSGL-1 and CD15, and in this way, margination of PMN to the endothelium of the blood vessels becomes impossible. Similarly, it was found that sPSGL-1 might block neutrophils binding with the membrane receptor of P-selectin present on the endothelium cells or on the blood platelets. It was proved that binding of neutrophil PSGL-1 with P-selectin activated the integrin-dependent adhesion. However, Gardinere et al. demonstrated that soluble forms of P-selectin inhibited integrin-dependent adhesion of neutrophils stimulated by TNF $\alpha$  to the resting endothelium [5].

Another effect of blocking the bindings between PSGL-1 on the neutrophils and P-selectin on the blood platelets may be the inhibited production and secretion of chemotactic factors by macrophages (IL-8 and MCP-1).

The enhanced concentrations of sP-selectin in the blood serum of patients with *Lyme borreliosis* may weaken the capability of phagocytosis and oxygen-dependent intracellular killing [9, 10]. There are known findings indicating that P-selectins present on the surface of the endothelial cells and blood platelets recognize specific sialo Le(x) (CD15s) ligands present on the leukocytes, causing the formation of platelet-monocytic or platelet-granulocytic complexes [18, 20, 21]. These complexes make neutrophils and monocytes produce reactive oxygen forms (ROF). The inhibition of binding P-selectin with CD15s may influence negatively the production of ROF, in con-

sequence, leading to the disturbed elimination of spirochetes.

Another negative effect of the blockage of PSGL-1 binding with ligands may be the inhibition of further stages of adhesion. Green et al. [8] proved that PSGL-1 binding with  $\alpha$ L-selectin might increase Ca<sup>2+</sup> secretion and activation of protein kinases of MAPK, change the shape of neutrophil, cause the degranulation and fast increase in  $\beta_2$  integrin growth, which leads to the firm adhesion of cells to the vascular endothelium and afterwards migration of leukocytes to the site of the on-going inflammatory process.

## CONCLUSIONS

The increase in the concentration of soluble forms of P-selectin and PSGL-1 via blocking specific ligands on neutrophils may inhibit adhesion, which may be one of the reasons for a long-lasting inflammatory process.

## REFERENCES

1. Behera Aruna K., Cheleste M. Thorpe, J. Michael Kidder, Wendy Smith, Ethan Hildebrand, and Linden T. Hu: *Borrelia burgdorferi*-Induced Expression of Matrix Metalloproteinases from Human Chondrocytes Infect. Immun. 2004; 72, 2864.
2. Coburn J., Leong J.M., Erban J.K.: *Integrin  $\lambda_5\beta_3$  mediated binding of the Lyme disease agent Borrelia burgdorferi to human platelets*. Proc Natl Acad Sci USA 1993, 90, 7059-63.
3. Etzioni M.: *Leukocyte adhesion deficiency II: a group of integrin activation defects in hematopoietic lineage cells*. Curr Opin Allergy Clin Immunol. 2004 Dec, 4, 485-90.
4. Garcia R.C, Muriner R.: *Complement receptor 3 binds the Borrelia burgdorferi outer surface proteins OspA and OspB in an C3b-independent manner*. Infect Immun. 2005; 73: 6138-42.
5. Gardiner E., De Luca M, McNally T., D. Michelson A.: *Regulation of P-selectin binding to the neutrophil P-selectin counter-receptor P-selectin glycoprotein ligand-1 by neutrophil elastase and cathepsin G*. Blood, 2001; 98: 1440-1447.
6. Gebbia J, Coleman A.: *Borrelia spirochetes upregulate release and activation of matrix metalloproteinase gelatinase B (MMP-9) and collagenase 1 (MMP-1) in human cells*. Infect Immun. 2001, 69, 456-62.
7. Górski A.: *The role of cell adhesion molecules in immunopathology*. Immunol. Today 1994, 15, 251.
8. Green C.E., Pearson D.E.: *Shear-dependent capping of L-selectin and P-selectin glycoprotein ligand 1 by E-selectin signals activation of high-avidity beta2-integrin on neutrophils*. J Immunol. 2004, 15; 172: 7780-90.
9. Iżycka A, Jabłońska E, Zajkowska J, i in. *Bakteriobójcze właściwości granulocytów obojętnochłonnych (PMN) krwi obwodowej u chorych z boreliozą z Lyme*. Med Dośw Mikrobiol 2000; 52: 165.
10. Iżycka A, Jabłońska E, Zajkowska J: *Expression of LFA - 1 and L-selectin on peripheral blood neutrophils and concentrations of soluble forms of sICAM-1 and sL-selectin in blood serum of patients with Lyme borreliosis* praca wystana do druku Acta Microbiologica.
11. Ley E.: *Molecular mechanisms of leukocyte rolling and adhesion to microvascular endothelium*. Eur. Heart J. 1993, 14 suppl. II 68-73.
12. Lund-Jochansen F., Olweus J., Horejsi V.: *Activation of human phagocyte through carbohydrate antigens (CD15, sialyl-15 CDw17)* J. Immunol. 1992, 148, 3221.
13. Lusitani D., Malavista A.: *Calprotectin, an abundant cytosolic protein from human polymorphonuclear leukocytes, inhibits the growth of Borrelia burgdorferi*. Infect Immun. 2003 71, 4711-6.

14. Ma Y, Seiler KP, Tai KF, Yang L, Woods M.: *Outer surface lipoproteins of Borrelia burgdorferi stimulate nitric oxide production by the cytokine-inducible pathway*. Infect. Immun., Sep 1994; 62: 3663-3671.
15. Mc Ever M.: *P-selectin and PSGL-1: exploiting connections between inflammation and venous thrombosis*. Thromb Haemost. 2002 Mar; 87(3): 364-5.
16. Moore A.: *Structure and function of P-selectin glycoprotein ligand-1*. Leuk Lymphoma. 1998, 29, 1-15.
17. Picker L.J., Warnock R., Burns A.R.: *The neutrophil selectin presents carbohydrate ligands to the vascular selectins ELAM-1 and GMP-140*. Cell 1991, 66, 921-923.
18. Schumacher M., Siebers A.: *P-selectin glycoprotein ligand-1 (PSGL-1) is up-regulated on leucocytes from patients with chronic obstructive pulmonary disease*. Clin Exp Immunol. 2005; 142, 370-6.
19. Sellati TJ, LD Abrescia, JD Radolf, and MB Furie: *Outer surface lipoproteins of Borrelia burgdorferi activate vascular endothelium in vitro*. Infect. Immun., Aug 1996; 64: 3180-3187.
20. Stocks C., Albrechtsen M.: *Expression of the CD15 differentiation antigen (3-fucosyl - N-acetyl-lactosamine, Lex) on putative neutrophil adhesion molecules CR3 and NCA-160*. J. Biochem. 1990, 268, 275-280.
21. Welpy J.K., Keene J.L., Schmuke J.J. i wsp.: *Selectin as potential targets of therapeutic intervention in inflammatory diseases*. Biochim. Biophys. Acta 1994, 1197, 215.
22. Wooten RM, Modur VR, McIntyre TM.: *Borrelia burgdorferi outer membrane protein A induces nuclear translocation of nuclear factor-kappa B and inflammatory activation in human endothelial cells* J. Immunol., 1996; 157: 4584-45.

---

**Correspondence:** Aneta Iżycka  
15-204 Białystok, Sybiraków str. 7/84  
e-mail: anetaiz@wp.pl

---