CASE STUDY
OPIS PRZYPADKU

TLR EXPRESSION ON PERIPHERAL BLOOD MONOCYTES IN PATIENTS WITH PSORIASIS

DOI: 10.36740/WLek202002137

Valeriia V. Pochernina, Andrey M. Daschuk
KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE

ABSTRACT

Toll-like receptors (TLRs) are signalling pattern-recognition receptors, which play an important part in initiating the immune response in psoriasis. The available literature has little information about study of these receptors in blood. The purpose of the present work was to study the level of expression of TLR2 and TLR4 types on blood monocytes in a psoriasis patient. Within 2016-2018, TLR2 and TLR4 were examined thrice in the blood of a patient with psoriasis during its exacerbation before the beginning of his treatment. The expression of surface receptors CD282 (TLR2) and CD284 (TLR4) on peripheral blood monocytes was studied by the method of flow cytometry with use of monoclonal antibodies. The obtained data show that the expression of TLR2 on peripheral blood monocytes was high, while that of TLR4 was mostly within its reference values of 3.3±0.1 mfi. Hence, the conducted studies have shown that the expression of TLR2 on peripheral blood monocytes in all three studies was high, thereby demonstrating involvement of this factor into the pathogenesis of the above disease. During all three blood examinations, monocytes revealed an extremely high intensity of TLR2 fluorescence on studied cells that exceeded the same intensity in healthy subjects by 1.5-2 times. The expression of TLR4 on peripheral blood monocytes during all three studies was within its reference values, this fact demonstrating absence of any information value of the above factor in the pathogenesis of psoriasis.

KEY WORDS: psoriasis, Toll-like receptors, monocytes

Wiad Lek. 2020;73(2):401-404

INTRODUCTION

Toll-like receptors (TLRs) are an important class of pattern-recognition receptors, which are present in the skin. Having bound to a ligand, TLRs undergo conformation changes and form a molecular cascade of a signal transmission to the cell nucleus with the resultant transcription of proinflammatory cytokine genes, adhesion molecules and co-stimulating molecules that initiate development of the adaptive immune response. Defence reactions in barrier tissues are aimed at recognition of pathogens, their destruction and elimination from the organism. That is the result of contact with pathogens depends upon a rapid and effective work of immunity components in these tissues. TLRs are the primary sensors of microbial products that register an alarm signal from pathogens and mobilize the immune system of the organism to fight infectious agents [1-4].

There are many different types of TLR-expressing cells in the human skin. It has been shown in the epidermis that keratinocytes express functional TLRs. Besides, the skin has resident and carrying cells of the immune system that express TLRs, including Langerhans cells, monocytes/macrophages, dendrite cells, T and B lymphocytes, and mast cells. Finally, endothelial cells of the microvasculature and stromal cells, such as fibroblasts and adipocytes, express TRLs too. Each of the above types of cells has different patterns of expression of TLRs and may facilitate the skin immune response [1-4].

The literature is accumulating data that TLRs and their ligands play an important part in the pathogenesis of autoimmune diseases by their contribution to development of the inflammatory response rather than only provide anti-infectious defence. At present, TLRs are the subject of an active study both at norm and in pathologies. In recent years the interest to study TLRs in psoriasis has been growing, this fact being caused by inclusion of these receptors in general mechanisms of defence as well as by their presence on keratinocytes [5-8].

The participation of TLRs in pathogenetic mechanisms in psoriasis is also confirmed by researches aimed at study of the efficacy of anti-TNF therapy, which shows an inhibiting effect on the above factors [9].

A certain part in the pathogenesis of psoriasis is played by the microbial factor, which complicates the course of the disease. TLR2 and TLR4 are able to activate immune cells in response to gram-positive and gram-negative bacteria, respectively. In particular, an important part of stimulation of TLR2 in psoriatic arthritis is assigned to gram-positive streptococci [10].

On the other hand, the activation of TLRs is caused by resistance to pathogenic microorganisms. The resistance to skin bacterial infections, e.g. Staphylococcus aureus (S. aureus), is based on the function of intact congenital immune mechanisms. TLR2 recognizes components of S. aureus and is known to be expressed on monocytes. Staphylococcal exotoxins, such as staphylococcal enterotoxin B or α-toxin, are produced by many strains of S. aureus. α-toxin significantly increased the expression of TLR2. TLR2-medicated secretion of IL-1β, IL-6 and IL-8 was considerably higher after activation with help of staphylococcal

exotoxins. But no differences were observed in monocytes of psoriasis patients versus healthy controls [11, 12].

Psoriasis demonstrates a high expression and activity of antimicrobial peptides that cause inhibition of TLR4 function on dendrite cells and lead to dysmaturity of dendrite cells and release of anti-inflammatory cytokines, thereby suppressing reactions of hypersensitivity and inhibiting inflammation. On the one hand, TLRs can make their contribution to raise the level of antimicrobial peptides and skin immune reactions in psoriatic lesions. On the other hand, it has been demonstrated that the cathelicidin antimicrobial peptide (LL37), which is found in large amounts in psoriatic skin, can transform the non-stimulating affine DNA into a powerful activator of TLR9 in dendrite cells, thereby causing production of interferon α with the resultant production of cytokines of Th1 profile and may support skin inflammation in psoriasis. This can be an important mechanism, by which TLRs can stimulate the autoimmune response in psoriasis [6, 13-15].

Besides, a high expression of heat-shock proteins (HSPs) in psoriatic lesions by keratinocytes has been revealed. These proteins can stimulate secretion of TLR4 on antigen-presenting cells, mainly Langerhans cells, playing a crucial part in the maturation and secretion of TNF and IL-12 and thereby participating in immunopathology of psoriasis [6, 16].

The vast majority of scientific papers show expression of TLRs on keratinocytes, though TLRs play an important part on peripheral blood monocytes too. Such studies are very rare.

THE AIM

Purpose of the research to investigate the level of expression of TLR2 and TLR4 on blood monocytes in a psoriasis patient and reveal their influence on the development of exacerbation in psoriasis.

CLINICAL CASE

Male patient F. consulted a dermatologist on a regular basis in November of 2016; he was followed up from 2010 when at first the following diagnosis was made: extensive plaque psoriasis vulgaris with a moderate degree of infiltration, the steady stage, a moderately recurrent course. He related the onset of his disease and subsequent exacerbations of the skin pathological process to frequent nerve overstrains. His family history contained matrilineal inheritance for psoriasis. He had exacerbations once a year in winters. He received several courses of out- and in-patient treatment in compliance with protocols. Within 2016-2019 patient F. was admitted to hospital 3 times with complaints about extensive eruptions on the skin of his scalp, trunk, upper and lower extremities. The periods of exacerbation lasted from 2 to 3 months.

17.11.2016. On examination, the skin of the patient's scalp, his anterior trunk surface and upper extremities revealed infiltrative erythematous foci. The whole surface of eruptions was covered with silver-white scales, which easily desquamated. PASI index = 38.3. By results of the clinical-laboratory examination the following diagnosis was made: extensive psoriasis vulgaris, the progressive stage.

1.11.2017. On examination, the skin of the patient's scalp (in the occipital region), his anterior and posterior trunk surfaces and some places on his upper and lower extremities revealed oedematous-infiltrative erythematous foci. The whole surface of eruptions was covered with silver-white scales, which easily desquamated. The psoriatic triad was positive. The nail plates of his first and second fingers on both hands had pin-point depressions ("pitting"). PASI index = 45.8. By results of the clinical-laboratory examination the following diagnosis was made: extensive psoriasis vulgaris with a torpid course, the progressive stage, a moderately recurrent course; psoriatic onychodystrophy.

30.10.2018. On examination, the skin of the patient's scalp and his upper and lower extremities as well as all his trunk surfaces revealed extensive oedematous-infiltrative erythematous foci. The whole surface of eruptions was covered with silver-white scales. The psoriatic triad was positive. The nail plates had numerous pin-point depressions ("pitting") and transverse fissures. PASI index = 53.6. By results of the clinical-laboratory examination the following diagnosis was made: extensive psoriasis vulgaris with a torpid course and a sharply manifested degree of infiltration, the progressive stage, a moderately recurrent course; psoriatic onychodystrophy. The course of psoriasis was classified as severe.

During each exacerbation of his disease, when patient F. underwent in-patient treatment, the beginning of therapy was preceded by studies of the level of expression of TLR2 and TLR4.

Blood was taken from the cubital vein. In order to reduce traumatization of the patient, the procedure of blood taking for the study coincided in time with blood taking for routine examinations. The venous blood for the study was taken aseptically after an overnight fast. As a stabilizer, 3.8% sodium citrate solution was used.

The study of the expression of superficial receptors CD282 (TLR2) and CD284 (TLR4) on peripheral blood monocytes was conducted by the method of flow cytometry with use of monoclonal antibodies (Becton Dickinson, USA) (Table I). Stained samples were cytofluorimetrically analysed on a Navious flow cytometer (BeckmanCoulter, CIIIA). The expression of superficial receptors CD282 (TLR2) and CD284 (TLR4) on monocytes was assessed by the median intensity of fluorescence (MIF), which corresponds to MIF for a separated group of cells. Actually MIF characterizes the level of expression of the above molecules on the cell surface.

The obtained data demonstrate that the expression of TLR2 on peripheral blood monocytes was high, while TLR4 was mostly within the limits of its reference values of 3.3±0.1 mfi.

This fact may point to participation of TLR2 in exacerbation of the disease.

Table I. Dynamics in the level of expression of Toll-like receptors

Indices	Norm	Date		
		17.11.2016	1.11.2017	30.10.2018
TLR2	52.0-73.0	99.5	101.0	125.0
TLR4	2.6-3.6	3.2	3.4	3.18

Table II. Results of the clinical blood analysis.

Indices	Norm	Date		
		17.11.2016	1.11.2017	30.10.2018
Erythrocytes	4.0-5.0*10^{12}/l	4.96*10^{12}/l	5.0*10^{12}/l	4.7*10^{12}/l
Haemoglobin	130-160 g/l	162 g/l	158 g/l	150 g/l
Colour index	0.85-1.15	0.93	0.9	0.9
Leukocytes	4.0-9.0*10^{9}/l	8.98*10^{9}/l	9.1*10^{9}/l	12.1*10^{9}/l
Eosinophils	0.5-5.0%	1%	5%	2%
Stab neutrophils	1.0-6.0%	5%	7%	4%
Segmented neutrophils	47-72%	52%	59%	77%
Monocytes	3.0-11.0%	4%	4%	12%
Lymphocytes	19.0-37.0%	38%	25%	5%
ESR	1-10 mm/h	12 mm/h	10 mm/h	20 mm/h

An area of eruptions is one of the factors that determine the degree of severity of psoriasis. A reliable difference in the area of eruptions in patients depending upon the level of expression of TLR2 on peripheral blood monocytes was revealed. The area of eruptions was larger in case of a high level of expression of TLR2.

The picture of peripheral blood, represented by the absolute amount of immunocompetent cells, is characterized by an increased content of leukocytes because of a more or less increased count of all formed elements (Table II).

MIF of TLR4 on peripheral blood monocytes does not differ.

CONCLUSIONS

1. The expression of TLR2 on peripheral blood monocytes in all three studies was high, thereby demonstrating involvement of this factor into the pathogenesis of the above disease.
2. In all three studies, monocytes revealed an extremely high intensity of TLR2 fluorescence on studied cells that exceeded the same intensity in healthy subjects by 1.5-2 times.
3. The expression of TLR4 on peripheral blood monocytes during all three studies was within its reference values, this fact demonstrating absence of any information value of the above factor in the pathogenesis of psoriasis.

REFERENCES

1. Miller L.S. Toll-like receptors in skin. Adv Dermatol. 2008; 24: 71-87.
2. Chen J.Q., Szodoray P., Zeher M. Toll-Like Receptor Pathways in Autoimmune Diseases. Clin Rev Allergy Immunol. 2016; 50(1): 1-17.
3. Ermertcan A.T., Öztürk F., Gündüz K. Toll-like receptors and skin. J Eur Acad Dermatol Venereol. 2011; 25(9): 997-1006.
4. Spivak N.J., Bogdanova I.M., Martirosova N.I. et al. Rol` Toll-podobnih receptorov v regulytsii immunogo otveta v norme i pri patologii [The role of Toll-like receptors in the regulation of the immune response in normal and pathological conditions] Physiology journal. 2008; 54 (6): 87-99 (In Russian).
5. Daschuk A.M. Rol` Toll-podobnih receptorov v regulytsii I initsiatsii immunogo otveta pri psoriase [The role of Toll-like receptors in the regulation and initiation of the immune response in psoriasis]. In: Plotnikova V.V., Daschuk A.M. Actual problems of dermatology, venereology and AID-infections: materials of scientific-practical conference. Kharkiv: SAM. 2015, p. 71-76 (In Russian).
6. Sorokina I.V. Toll-podobnie receptori i pervichnoe raspoznavanie patogena pri dermatozah infekcionnoi i neinfekcionnoi etiologii [Toll-like receptors and primary pathogen recognition in dermatoses of infectious and non-infectious etiology], Immunopathology, allergology, infectology. 2012; 2: 6-15 (In Russian).
7. Kulczycka L., Sysa-Jędrzejowska A., Robak E. Udział receptorów Toll-like w patogenezie wybranych chorób skóry // Postepy Hig Med Dosw. 2010; 64: 364-371.
8. Shi G., Wang T., Li S. et al. TLR2 and TLR4 polymorphisms in Southern Chinese Psoriasis Vulgaris patients. 2016; 83(2): 145-147.
9. Vagelid P., Exarchou A., Zafiriou E., et al. Effect of TNF-α inhibitors on transcriptional levels of pro-inflammatory interleukin-33 and Toll-like receptors-2 and -9 in psoriatic plaques // Exp Ther Med. 2015; 10: 1573-1577.
10. Carrasco S., Neves F.S., Fonseca M.H. et al. Toll-like receptor (TLR) 2 is upregulated on peripheral blood monocytes of patients with psoriatic arthritis: a role for a gram-positive inflammatory trigger? Clin Exp Rheumatol. 2011; 29(6): 958-962.
11. Valins W., Amini S., Berman B. The Expression of Toll-like Receptors in Dermatological Diseases and the Therapeutic Effect of Current and Newer Topical Toll-like Receptor Modulators. J Clin Aesthet Dermatol. 2010; 3(9): 20-29.

12. Niebuhr M., Schorling K., Heratizadeh A. et al. Staphylococcal α-toxin induces a functional upregulation of TLR-2 on human peripheral blood monocytes. Exp Detmatol. 2015; 24(5): 321-400.
13. Lande R., Gregorio J., Facchinetti V. et al. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature. 2007; 449(7162): 564–569.
14. Büchau A.S., Gallo R.L. Immunity and antimicrobial defense systems in psoriasis. Clin Dermatol. 2007; 25(6): 616-624.
15. Gilliet M., Lande R. Antimicrobial peptides and self-DNA in autoimmune skin inflammation. Curr Opin Immunol. 2008; 20(4): 401-407.
16. Seung N.R., Park E.J., Kim C.W. et al. Comparison of expression of heat-shock protein 60, Toll-like receptors 2 and 4, and T-cell receptor gammadelta in plaque and guttate psoriasis. J Cutan Pathol. 2007; 34(12): 903-911.

The article was written within the framework of the thesis research for a degree of Doctor of Philosophy in the subject "The role of Toll-like receptors in the pathogenesis of psoriasis".

ORCID and contributionship:
Valeriia V. Pochernina – 0000-0003-2701-5125[B,C,D,F]
Andrey M. Daschuk – 0000-0003-2401-9809[A,E,F]

Conflicts of interest:
Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Valeriia V. Pochernina
Kharkiv National Medical University
Kharkiv, Ukraine
tel: +380939730359
e-mail: valeriapochernina@gmail.com

Received: 06.09.2019
Accepted: 27.12.2019

A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article