DERMATOLOGY

A.M. Dashchuk, V.V. Pochernina

TOLL-LIKE RECEPTORS AND THEIR ROLE IN THE DEVELOPMENT OF PSORIASIS

Kharkiv National Medical University, Ukraine

Abstract: The study deals with determination of the role of TOLL-like receptors 4 and 9 inthe development of psoriasis. Patients with psoriasis were shown to have an increase in production and hypersecretion of pro-inflammatory biomarkers, namely TLR4 and TLR9-positive cellsby epithelial cells. Respective TLR4 and TLR9-positive cells were detectedboth in the areas of the skin affected by psoriatic rash and in the intact skin. However, the number of the respective cells in the affected skin areas is greater than in the intact skin.

Key Words: psoriasis, TOLL-like receptors, chronic dermatoses.

INTRODUCTION

Psoriasis is a common, chronic skin disease, affecting approximately 2% of the population. Most scientists refer to the common clinical variant termed psoriasis vulgaris, which affects approximately 85 to 90% of all patients with the disease. Psoriasis is associated with a high degree of morbidity; patients are embarrassed about the appearance of their skin, and there are side effects of medications. Moreover, patients with psoriasis, like those with other major medical disorders, have reduced levels of employment and income as well as a decreased quality of life.
The combined costs of long-term therapy and social costs of the disease have a major impact on health care systems and on the society in general.
According to the International Federation of Psoriasis Association the prevalence of psoriasis in the world is not the same, it depends on the region and ranges from 1.2% to 5%, and the average incidence is approximately 3% of the general population [13-15].

• ***Corresponding Author:***
***Andrey Dashchuk**, MD, PhD, Professor, Head of the Department of Dermatology, Venereology and AIDS, Kharkiv National Medical University, Ukraine. E-mail: kafedraderma@i.ua*

The interest in the study has been stimulated by theidentification of increased amount of Toll-receptor in the skin of patients with psoriasis.
TOLL-like receptors (TLRs) are a class of conserved receptors that recognize pathogen-associated microbial patterns. These receptors are also expressed in the skin cells including keratinocytes, melanocytes and Langerhans cells. The system of innate recognition formed during vertebrate evolution, implemented using effector cells involved in the first line of defense against all antigenically foreign compounds. These include the following types: epithelial cells, macrophages, dendritic cells, granulocytes, mast cells, NK cells, and others. These effectors have phagocytic and killer activity, provide network signals, activating and directing antigen-specific response by cells of the adaptive immune system. These cells serve as a bridge between pathogen-associated molecular structures (PAMPs) and antigen-specific cells of the adaptive immune response, broadcast signals of specific genetically encoded receptors (PRRs) in the soluble mediators that bind to the T and B cells via specific cytokine / chemokine receptors. One of the key events is the synthesis of complex pro-inflammatory cytokines stimulating most stages of inflam-

mation and providing the activation of various cell types involved in the maintenance and regulation of inflammation. Because of several functionally different classes of PRRs most well-characterized Toll-like receptors (TLRs) relate to the signaling PRRs and are an important component of the innate immune system. Numerous experimental studies, as well as accumulated findings of clinical practice of compelling evidence suggest the key role of Toll-like receptors in the development of immune diseases [1,2,3].

Table 1

Classification TLRs of human depending on the chromosomal localization of the genomic structure and amino acid sequences.

SubfamilyTLRs	*The members of the subfamilies*
TLR2	TLR1, TLR2, TKR6, TLR10
TLR3	TLR3
TLR4	TLR4
TLR5	TLR5
TLR9	TLR7, TLR8, TLR9

The role and function of TLRs in human skin has become a subject of study relatively recently. In the foreign literature there are few data on the presence of different TLRs in the different layers of the epidermis keratinocytes in healthy individuals [4-6]. According to B. Baker et al., TLRsexpressed on cells of the epidermis are subject to change as we move from the basal keratinocytes to horny layer of the epidermis [7]. According to E. James et al., keratinocytes of the skin of healthy individuals express TLR1, TLR2, TLR4 and TLR5. A. Pivarcsi established the presence of TLR2 and TLR4 in all layers of the epidermis of healthy individuals [6]. Mempel M. et al. showed that the culture of primary keratinocytes in healthy person produces TLR1, TLR2, TLR3, TLR5 and TLR9. Simultaneously, TLR4, TLR6, TLR7 and TLR8 were found in the same culture [8]. Some authors [5] believe that TLRs activated keratinocytes are able to initiate the adaptive immune response. In particular, S. Akira found that supernatant TLR-stimulated keratinocytes triggered dendritic cell maturation [9]

The role of TLRs in psoriasis is not well understood. E. Begone et al.[10] identified marked TLR1 expression on keratinocytes of the basal layer of the epidermis in patientswith psoriasis. B. Baker determined marked expression of TLR2 in the upper ranks of the spinous layer of the epidermisin the affected skin of patients with psoriasis, while in the skin of healthy subjects, and the unaffected skin of patients with psoriasis TLR2 expression was detected in the lower ranks of the thorny layer, located above the basal layer. J. Curry et al. found a decrease in TLR5expression in keratinocytes of the basal layer of the epidermis of the skin affected with psoriasis compared with the skin of healthy individuals [11]. Katunina et al. described patients with psoriasis who were shown to have dermis expression of TLR2 and TLR4 in the endothelium of blood vessels, cells and histiocytic macrophage number of inflammatory infiltrates, epithelial cells in the sweat glands and the outer root sheath of hair follicles [12].

2 PURPOSES, SUBJECTS AND METHODS:

2.1 Purpose

The purpose of the present study was to examine the role of toll-like receptor in psoriasis

2.2 Subjects

The study involved 25 patients with ordinary psoriasis. Patients underwent biopsy study of the skin affected by psoriatic rash, as well as areas with intact skin. Comparison of immunohistochemical findings was followed by the assessment of biopsy samples taken from 5 healthy individuals.

2.3 Methods

Biopsy samples were evaluated by standard histological processing: fixed in 10% formalin solution exposed to histological wiring by dehydration in ethanol and pouring paraffin.

The study of the number and distribution of TOLL-like receptor of Class 4 (CD284) and 9 (SD289) (TLR4, TLR9) in the structures of the skin was performed by immunohistochemistry using monoclonal antibodies to TLR4 and TLR9

produced by “Abbiotec” (USA), streptavidin-biotinylated secondary antibodies Novocastra Peroxidase Detection System Production “Leica Microsystems” (United Kingdom). Paraffin sections were spread on slides coated with polylysine. The reaction was assessed according to the protocols attached to the employed monoclonal antibodies. High temperature antigen unmasking was performed by boiling sections in citrate buffer (pH 6.0) in a microwave oven at the maximum power of 900 W for three cycles of 5 minuteswith one-minute break. Cooled preparations were washed in Tris buffer solution (pH 7.54-7.58), treated with 0.3% peroxide solution conduit to methanol (1: 1) to prevent endogenous peroxidase activity. Incubations with primary antibodies were performed for 60 min at 23°C and30 min for secondary antibodies.

Background staining was performed in an incubator at 37°C with Mayer's hematoxylin contrasting sections. Immunohistochemicalagentswere obtained with a coverslip and examined using a light microscope Leica DM4000V (Germany). Calculation of the area of expression of toll-like receptors in the epidermis and dermis was carried out using a computer image analysis program ImageJ. In the epidermis cell area was determined with a positive response, which is expressed in square pixels. The content of the dermis toll-like receptor was determined by calculating the area of vascular endothelium where receptor expression was observed.

Conflict of interests

There is no conflict of interests.

3 RESULTS AND DISCUSSION

The surface layers of the epidermis, as in patients with psoriasis and healthy subjects from the control group, had only some cells with weakly positive staining. Also there were areas in the skin with no signs of edema and adverse reactions to TLR4 and TLR9.

Single TLR4 and TLR9-positive cells were observed in the dermis in small clusters of inflammatory cells. In positive substrate reaction there were fine granules with light to moderate staining in the nucleus and in the cytoplasm of positive cells.

In psoriatic plaques epidermis layeris significantly thickened with an increased number of TLR4 and TLR9-positive cells. The study identified the following pattern: TLR4 and TLR9-positive cells in the epidermis wereobserved in the areas of edema with a considerably smaller amount in the sites of compact settlement of epithelial cells.

The intact skin of patients with psoriasis was found to have more active focal expression of TLR4 and TLR9in the epidermis. The topography of such sites contained increased papillae or formed papillae. In the epidermis they were determined by positive staining of epithelial cells of the entire epidermis.

Active expression of the marker was observed in other areas involving the epidermis. Moderate positive staining was used as in the few cells of prevascular inflammatory infiltrates papillary dermis.

TLR4 and TLR9 expression in the epidermis of healthy individuals in the control group was most significant in the basal and prickle layerof the skin.

Patients with psoriasis were found to have an increase in production and hypersecretion of pro-inflammatory biomarkers, such as TLR4 and TLR9-positive cellsby epithelial cells of the skin. Respective TLR4 and TLR9-positive cells were identifiedboth in the areas of the skin affected by psoriatic rash and in the intact skin. However, the number of the respective cells in the affected skin areas was greater than in the intact skin.

4 CONCLUSIONS

Expression of TLR-4 and TLR9-positive cells suggests that an important link in the pathogenesis of the dermatosis is antigenic stimulation of immune cells that leads to the development of the inflammatory process in the superficial layers of the skin.

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РЕЗЮМЕ

Дащук А.М., Почерніна В.В.

TOLL-ПОДІБНІ РЕЦЕПТОРИ І ЇХ РОЛЬ У ПАТОГЕНЕЗІ ПСОРІАЗУ

Харківськийнаціональниймедичнийуніверситет

Обговорюється вивчення TOLL-подібнихрецепторів 4 і 9 типу в шкірі хворих на псоріаз (в інтактній та в ураженій шкірі). У хворих на псоріаз встановлена гіперпродукція і гіперсекреція епітеліоцитами шкіри прозапальних біологічних маркерів, зокрема TLR4 і TLR9- позитивних клітин. Відповідні TLR4 і TLR9-позитивні клітини визначаються як в уражених псоріатичними висипаннями ділянках шкіри, так і в інтактнній шкірі. Але кількість відповідних клітин в ділянках ураженої шкіри більше, ніж інтатктной.

Ключові слова: псоріаз, TOLL-подібні рецептори, хронічні дерматози.

РЕЗЮМЕ

Дащук А.М., Почернина В.В.

TOLL-ПОДОБНЫЕ РЕЦЕПТОРЫ И ИХ РОЛЬ В ПАТОГЕНЕЗЕ ПСОРИАЗА

Харьковский национальный медицинский университет

Обсуждается изучение TOLL-подобных рецепторов 4 и 9 типа в коже больных псориазом (в интактной коже и в пораженной коже). У больных псориазом установлена

гиперпродукция и гиперсекреция эпителиоцитами кожи провоспалительных биологических маркеров, в частности TLR4 и TLR9-положительных клеток. Соответствующие TLR4 и TLR9-положительные клетки определяются как в пораженных псориатическими высыпаниями участках кожи, так и в интактной коже. Но количество соответствующих клеток в участках пораженной кожи больше, чем в интатктной.

Ключевые слова: псориаз, TOLL-подобные рецепторы, хронические дерматозы

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