The TLRs family expression in lesions and peripheral blood of patients with acne and their regulating effect on the synthesis of inflammatory mediators

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ABSTRACT
Objective: To study the TLRs family expression in lesions and peripheral blood of patients with acne and their regulating effect on the synthesis of inflammatory mediators. Methods: A total of 60 patients with acne vulgaris who were treated in our hospital between June 2013 and July 2016 were selected as the acne group, and 48 patients with trauma who accepted debridement and suturing in our hospital during the same period were selected as the control group. The acne focus tissue and the skin tissue after debridement were collected to determine the expression of TLRs and inflammatory mediators, and the peripheral blood mononuclear cells were collected to determine the expression of TLRs. Results: TLR2 and TLR4 protein expression in skin tissue and peripheral blood mononuclear cells of acne group were significantly higher than those of control group while the TLR3, TLR5, TLR7 and TLR9 protein expression were not significantly different from those of control group; IL-1α, IL-1β, IL-8 and TNF-α levels in acne tissue with high TLR2 and TLR4 expression were significantly higher than those in acne tissue with low TLR2 and TLR4 expression. Conclusion: TLR2 and TLR4 are highly expressed in lesions and peripheral blood of patients with acne and have promoting effect on the synthesis of inflammatory mediators.

1. Introduction

Acne is a chronic skin disease that trends to occur in forehead and face, chest and back, and sebaceous gland-abundant parts, it’s common in puberty, and it is manifested as comedo, inflammatory papules and cyst, pustules, etc. The pathologic features in acne lesions include propionibacterium colonization, excessive epithelial cell keratinization, excessive sebaceous glands secretion and excessive inflammatory reaction[1,2]. Inflammation is the key pathological link to cause acne formation and development, propionibacterium is considered as an important factor to start inflammatory response, but the mechanism that starts and regulates the inflammatory response remains to be further confirmed[3,4]. Toll-like receptors (TLRs) are the pattern recognition receptors regulating inflammation in the body, and they can recognize the pathogen-associated molecular patterns and adjust the synthesis and secretion of a variety of inflammatory mediators. Propionibacterium, as the pathogen-associated molecular pattern, can start the inflammatory response in acne lesions after recognized by TLRs[5]. In order to make clear the relation between TLRs and acne illness progression, the TLRs family expression in lesions and peripheral blood of patients with acne and their regulating effect on the synthesis of inflammatory mediators were analyzed in the following study.
2. Subjects and methods

2.1 Research subjects

A total of 60 patients with acne vulgaris who were treated in our hospital between June 2013 and July 2016 were selected as the acne group of the study, all patients were in accordance with the diagnostic criteria for acne vulgaris, and patients associated with autoimmune disease and severe local infection, and those who took glucocorticoid and retinoic acid drugs in the past two months were ruled out. 48 patients with trauma who accepted debridement and suturing in our hospital during the same period were selected as the control group of the study, all patients accepted debridement and suturing for trauma, and they were without history of skin diseases or autoimmune diseases. Acne group included 39 male cases and 21 female cases that were 19-31 years old; control group included 29 male cases and 19 female cases that were 22-38 years old. The two groups of patients were not significantly different in general data.

2.2 Research methods

2.2.1 Detection methods of TLRs expression and inflammatory mediator levels in lesions

Lesion tissue of acne group and skin tissue of control group after debridement were collected and added in protein lysis buffer to extract total protein from the tissue, the tissue was centrifuged at 12 000 r/min and 4 °C for 20 min to get clear protein suspension, enzyme-linked immunosorbent assay kits were used to determine TLR2, TLR3, TLR4, TLR5, TLR7, TLR9, IL-1α, IL-1β, IL-8 and TNF-α contents, BCA kits were used to determine total protein content, and the TLR2, TLR3, TLR4, TLR5, TLR7, TLR9, IL-1α, IL-1β, IL-8 and TNF-α protein content per unit mass total protein were calculated.

2.2.2 Detection methods of TLRs expression in peripheral blood mononuclear cells

5 mL peripheral venous blood was collected from acne group and control group, added in lymphocyte separation medium and centrifuged to separate the suspended mononuclear cells, peripheral blood mononuclear cells were washed with PBS and centrifuged again, cell precipitation was kept and added in protein lysis buffer to extract the total protein in the cells, the cells were centrifuged at 12 000 r/min and 4 °C for 20 min to get clear protein suspension, enzyme-linked immunosorbent assay kits were used to determine TLR2, TLR3, TLR4, TLR5, TLR7 and TLR9 contents, BCA kits were used to determine total protein content, and the TLR2, TLR3, TLR4, TLR5, TLR7 and TLR9 protein content per unit mass total protein were calculated.

2.3 Statistical methods

SPSS 20.0 software was used to input the data for TLRs expression and inflammatory mediator levels, the median of TLR2 and TLR4 expression in lesion tissue of acne group was calculated, the acne patients with expression > median were judged as those with high expression and the acne patients with expression < median were judged as those with low expression; measurement data analysis between two groups was by t test and P<0.05 indicated statistical significance in differences.

3. Results

3.1 TLRs protein expression in skin tissue

Analysis of TLR2, TLR3, TLR4, TLR5, TLR7 and TLR9 protein expression in skin tissue between acne group and control group was as follows: TLR2 and TLR4 protein expression in skin tissue of acne group were significantly higher than those of control group while the TLR3, TLR5, TLR7 and TLR9 protein expression were not significantly different from those of control group. Differences in TLR2 and TLR4 protein expression in skin tissue were statistically significant between acne group and control group (P<0.05).

3.2 TLRs protein expression in peripheral blood mononuclear cells

Analysis of TLR2, TLR3, TLR4, TLR5, TLR7 and TLR9 protein expression in peripheral blood mononuclear cells between acne group and control group was as follows: TLR2 and TLR4 protein expression in peripheral blood mononuclear cells of acne group were significantly higher than those of control group while the TLR3, TLR5, TLR7 and TLR9 protein expression were not significantly different from those of control group. Differences in TLR2 and TLR4 protein expression in peripheral blood mononuclear cells were statistically significant between acne group and control group (P<0.05).

Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>TLR2 (ng/mg protein)</th>
<th>TLR3 (ng/mg protein)</th>
<th>TLR4 (ng/mg protein)</th>
<th>TLR5 (ng/mg protein)</th>
<th>TLR7 (ng/mg protein)</th>
<th>TLR9 (ng/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acne group</td>
<td>60</td>
<td>2.85±0.35</td>
<td>1.44±0.17</td>
<td>4.62±0.68</td>
<td>1.05±0.15</td>
<td>1.35±0.18</td>
<td>1.13±0.15</td>
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<tr>
<td>Control group</td>
<td>48</td>
<td>1.04±0.17</td>
<td>1.51±0.19</td>
<td>2.15±0.31</td>
<td>1.08±0.17</td>
<td>1.41±0.20</td>
<td>1.09±0.13</td>
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<tr>
<td>T</td>
<td>17.548</td>
<td>0.38±0.05</td>
<td>11.485</td>
<td>0.185</td>
<td>0.205</td>
<td>0.126</td>
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</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
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</tbody>
</table>

Table 2.

<table>
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<tr>
<th>Groups</th>
<th>n</th>
<th>TLR2 (ng/mg protein)</th>
<th>TLR3 (ng/mg protein)</th>
<th>TLR4 (ng/mg protein)</th>
<th>TLR5 (ng/mg protein)</th>
<th>TLR7 (ng/mg protein)</th>
<th>TLR9 (ng/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acne group</td>
<td>60</td>
<td>0.85±0.11</td>
<td>0.65±0.09</td>
<td>2.16±0.35</td>
<td>0.76±0.09</td>
<td>0.42±0.06</td>
<td>0.62±0.08</td>
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<tr>
<td>Control group</td>
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<td>0.38±0.05</td>
<td>0.68±0.08</td>
<td>0.95±0.12</td>
<td>0.79±0.10</td>
<td>0.41±0.05</td>
<td>0.65±0.09</td>
</tr>
<tr>
<td>T</td>
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<td>0.271</td>
<td>11.495</td>
<td>0.105</td>
<td>0.093</td>
<td>0.058</td>
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<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
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<td>&gt;0.05</td>
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</tbody>
</table>
TNF-α expression was as follows: (1) IL-1β, IL-8 and TNF-α levels in acne tissue with high TLR2 expression were significantly higher than those in acne tissue with low TLR2 expression; (2) IL-1β, IL-8 and TNF-α levels in acne tissue with high TLR4 expression were significantly higher than those in acne tissue with low TLR4 expression. The differences were statistically significant in IL-1α, IL-1β, IL-8 and TNF-α levels in acne tissue with high and low TLR2 and TLR4 expression (P<0.05).

### 3.3 Inflammatory mediator levels in skin tissue

Analysis of inflammatory mediators IL-1α, IL-1β, IL-8 and TNF-α levels in acne tissue with different TLR2 and TLR4 expression was as follows: (1) IL-1α, IL-1β, IL-8 and TNF-α levels in acne tissue with high TLR2 expression were significantly higher than those in acne tissue with low TLR2 expression; (2) IL-1α, IL-1β, IL-8 and TNF-α levels in acne tissue with high TLR4 expression were significantly higher than those in acne tissue with low TLR4 expression. The differences were statistically significant in IL-1α, IL-1β, IL-8 and TNF-α levels in acne tissue with high and low TLR2 and TLR4 expression (P<0.05).

### 4. Discussion

Acne is a common skin disease in adolescence, and the propionibacterium colonization in the hair follicles and excessive inflammatory reaction activation are the important pathological characteristics of patients with acne.[6-8] and the mechanism of inflammatory reaction activation after propionibacterium colonization has not been fully elucidated. TLRs are the transmembrane receptor family expressed in a variety of immune cells and inflammatory cells, there are a total of 13 members TLR1-13, and they can specifically recognize pathogen-associated molecular patterns and adjust the immune response and inflammatory response. Bacterial endotoxin, DNA, ssRNA, dsRNA, fungi products and viral products are all pathogen-associated molecular patterns that can be recognized by TLRs. It has been confirmed that the TLRs family members such as TLR2, TLR3, TLR4, TLR5, TLR7 and TLR9 are associated with skin diseases such as systemic lupus erythematosus, psoriasis, atopic dermatitis and condyloma acuminatum. Highly expressed TLR4 and TLR9 are associated with the occurrence of skin lesions in patients with systemic lupus erythematosus.[9], the high expression of TLR2, TLR4 and TLR5 is related to the skin lesions of psoriasis and atopic dermatitis.[10], and TLR3, TLR4 and TLR9 can identify the virus and participate in the pathological process of condyloma acuminatum.[11].

In recent years, the relationship between TLRs and acne has received more and more attention. The study of domestic FANG Jing-jing[12] confirms that the expression of TLR2 increases significantly in the peripheral blood mononuclear cells of patients with acne; study of foreign Ozlu E[13] confirms that the TLR2 and TLR4 expression increase significantly in acne lesions. Nonetheless, there is no clear report at present about the relationship between other members in TLRs family and acne. In the study, in order to define the TLRs family members involved in acne condition changes, the TLR2, TLR3, TLR4, TLR5, TLR7 and TLR9 protein expression in lesions tissue and peripheral blood mononuclear cells were analyzed, and the results showed that TLR2 and TLR4 protein expression in skin tissue and peripheral blood mononuclear cells of acne group were significantly higher than those of control group while the TLR3, TLR5, TLR7 and TLR9 protein expression were not significantly different from those of control group. The results match the studies of domestic FANG Jing-jing[12] and foreign Ozlu E[13], and indicate that the abnormally high expression of TLR2 and TLR4 in TLRs family is involved in the occurrence and development of the acne, and other TLRs members do not participate in acne development.

TLR2 and TLR4 regulation on the inflammatory response is to conduct signal transduction mainly through MyD88-dependent pathway and MyD88-nondependent pathway, activate transcription factors NF-kB, AP-1 and IRF-3, and thus adjust the expression of IL-1α, IL-1β, IL-8, TNF-α and other inflammatory mediators. In the acne development process, the increased expression of inflammatory mediators in the lesions is closely related to the excessive epithelial cell keratinization and excessive sebaceous glands secretion. IL-1α and IL-1β are the two subtypes of IL-1, and they have significant promoting effect on the keratinization.
of pilosebaceous\cite{14,15}; IL-8 can act on the infected granulocyte group, promote the differentiation and activation of mononuclear macrophages, and thus increase the expression and secretion of other inflammatory mediators\cite{16}; TNF-α is the inflammatory cytokine secreted by mononuclear macrophages, and is involved in the cascade activation of the inflammatory response in the acne lesions\cite{17}. In order to define the regulating effect of TLR2 and TLR4 in acne in acne lesions on these inflammatory mediator expression, the inflammatory mediator levels in acne tissue with different TLR2 and TLR4 expression were analyzed in the study, and the result showed that IL-1α, IL-1β, IL-8 and TNF-α levels in acne tissue with high TLR2 and TLR4 expression were significantly higher than those in acne tissue with low TLR2 and TLR4 expression. This means that the highly expressed TLR2 and TLR4 in acne tissue could promote the expression and secretion of IL-1α, IL-1β, IL-8 and TNF-α through MyD88-dependent pathway and MyD88-nondependent pathway.

**References**


