Immunogenetic regulation of HLA-DR, DQ, DP and CD1a positive cells in the pathogenesis of keloid and hypertrophic scar

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Recent studies have shown that HLA-DR, DQ, DP and CD1a positive cells were related to keloid and hypertrophic scar. Therefore, this study was aimed at investigating the role of immunogenetic factors in the pathogenesis of keloid and hypertrophic scar. The expression levels and distribution patterns of HLA-DR, -DQ, -DP and CD1a molecules in peripheral blood mononuclear cells were determined in 10 samples of keloid, hypertrophic scar, flat scar and normal people with the stain of streptavidin-peroxidase (SP) method. The results show that although the amounts of HLA-DR+, -DQ+, -DP+ and CD1a+ cells had no significant differences among the keloid, hypertrophic scar, flat scar and normal people groups (P>0.05), the integrated optical density (IOD) of HLA-DR+, -DQ+, -DP+ and CD1a+ cells in the keloid and hypertrophic scar were higher respectively, than that in the flat scar and normal people groups (P<0.05). The increased content of HLA-DR, -DQ, -DP and CD1a proteins in peripheral blood of the keloid and hypertrophic scar groups therefore suggest that the over-hyperplasia of scars may be associated with immunogenetic factors.

Key words: Keloids, hypertrophic scars, human leukocyte antigen (HLA), CD1a.

INTRODUCTION

HLA-DR, -DQ, -DP molecules are in the category of human leukocyte antigen (HLA) –II, participating in the processing and presenting of antigens, playing a role in the induction of T-cell activation and are associated with the immunoglobulin E (IgE)-induced delayed hypersensitivity (Gregory et al., 2000; Wang et al., 1992). Previous researches (Santucci et al., 2001; Castagnoli et al., 1997; Chen et al., 2003) have indicated that HLA-DR and CD1a molecules have a high level expression in the local tissues within the keloid and hypertrophic scar, suggesting that the HLA-II molecule restricted and CD1a molecule restricted immune responses play a important role in the formation of keloid and hypertrophic scar.

In order to understand the expression and distribution of HLA-II molecules and CD1a molecules in the peripheral blood of patients with keloid and hypertrophic scar, we have determined in this study the amounts of HLA-DR, -DQ, -DP and CD1a molecules in the peripheral blood mononuclear cells in patients of keloid, hypertrophic scar, flat scar and normal people with the method of immunocytochemistry, and investigated the role of systemic immunogenetic factors in the pathogenesis of keloid and hypertrophic scar to provide a theoretical basis on the prevention and treatment of scars.
MATERIALS AND METHODS

Patients

Blood samples were obtained from the Research Center of Plastic surgery, Peking University Third Hospital. The keloid group included 10 cases (3 men and 7 women; 23–47 years old with a course of diseases for 4–20 years); the hypertrophic scar group had 10 cases (8 men and 2 women; 5–35 years old with a course of diseases for 0.5–6 years); the flat scar group had 10 cases (6 men and 4 women; 16–32 years old with a course of diseases for 0.5–16 years) and finally, the normal people group had 10 normal people (5 men and 5 women; 24–30 years old).

Blood collections were strictly done in accordance with the clinical diagnosis of keloid and hypertrophic scar, and all the blood sample donators had no autoimmune diseases and any other chronic diseases, had no blood transfusion history, had no history of recent local or general infection and had not had any drugs that might affect the blood and immune systems.

Preparation of smears for peripheral blood mononuclear cells (PBMC)

Fresh peripheral blood was taken and heparin was used for anticoagulation. The mononuclear cells were extracted from the sample by way of density gradient centrifugation after the sample blood was diluted with RPMI-1640 culture medium. Smears of 0.8 cm in diameter were prepared for use with a centrifugal smear maker and fixed with acetone.

Immunocytochemical staining

The method of streptavidin/peroxidase (SP) staining was used in displaying the HLA-DR, -DQ, -DP and CD1a molecules. The first antibodies, rat-anti-human HLA-DR, -DQ, -DP monoclonal antibodies and the Histostain™-SP kit were purchased from Beijing Zhongshan Biotechnology Co., Ltd., China, and the rat-anti-human CD1a antibody was bought from Tianjin Union Stem Cell Biotechnology Company. Diaminobenzidine (DAB) coloration was performed. Each kind of antigen and each sample was immunochemically stained for two pieces respectively; one piece was counter stained with hematoxylin to show the karyons and a cell counting analysis performed, while the other piece was used for image analysis without counter staining.

Cell counting

Under a 10×40 microscope, 200 mononuclear cells were counted in a selected region with the cells uniformly distributed and positive percentage of each group was analyzed. Cells with their surface stained brownish were defined as positive cells and otherwise the negative cells.

Image analysis

Images were acquired with a multifunctional CMIAS2001B color image analyzer under a multiple of 10×20. Five visions were selected for each piece of smear and the integrated optical density (IOD) of HLA-DR, -DQ, -DP and CD1a-positive cells in the peripheral blood of the patients in keloid, hypertrophic scar, flat scar and normal people groups was determined. Integral optical density is the summation of density distribution of the solely detected image points that is the integral optical density is given as: area of the detected object × average optical density. The protein content of HLA-DR, -DQ, -DP and CD1a molecules was expressed with integral optical density.

Statistical analysis

The experimental data were expressed as mean ± standard deviation (S.D) and SPSS for Windows 17.0 statistical program was used for statistical analysis. The acquired computation data were compared among multi-groups and a single factor variance analysis was performed. The least significant difference (LSD) method was used for paired comparisons. P<0.05 was defined as the standard of statistical significance.

RESULTS

Wright-Giemsa staining

The centrifuged smears were observed under an optical microscope, and mononuclear cells, including the lymphocytes were seen in a round shape with large karyons and with notches on the side of them and the karyons were in bluish purple. And there was less cytoplast, which was in azure. The mononuclear cells were larger and the karyons were in an elliptic or kidney shape in lilac, and the cytoplast was in grayish blue. Cells such as neutrophilic granulocytes, eosinophil granulocytes and basophilic granulocytes were seldom seen.

Counting and quantitative analysis of HLA-DR+, -DQ+ and -DP+ cells in the peripheral blood of the keloid, hypertrophic scar, flat scar patients and normal people

Cell counting

Expression of HLA-DR, -DQ, -DP molecules were found in the karyons and cytoplasm of the mononuclear cells and lymphocytes in patients from the different groups. HLA-DR, -DQ, -DP-positive cells were counted and analyzed and the results showed no significant difference in the number of HLA-DR, -DQ, -DP-positive cells among the different groups of keloid, hypertrophic scar, flat scar and normal people (P>0.05) (Table 1).

Quantitative analysis

The IOD of the HLA-DR+, -DQ+, -DP+ cells from the keloid and hypertrophic scar groups were both higher than those of the flat scar and normal people groups (P<0.05). IOD of the HLA-DR+, -DQ+, and -DP+ cells between group keloid and group hypertrophic scar and that between group flat scar and group normal people had no significant difference (P>0.05) (Table 2).
Table 1. Analysis on the numbers (%) of HLA-DR⁺, DQ⁺, DP⁺ cells in the peripheral blood of patients with keloid, hypertrophic scars, flat scars and of normal people (mean ± S.D).  

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>HLA-DR</th>
<th>HLA-DQ</th>
<th>HLA-DP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>10</td>
<td>61.05 ± 16.37</td>
<td>21.10 ± 8.16</td>
<td>23.80 ± 5.32</td>
</tr>
<tr>
<td>Flat scar</td>
<td>10</td>
<td>59.85 ± 13.75</td>
<td>29.20 ± 8.85</td>
<td>26.60 ± 15.48</td>
</tr>
<tr>
<td>Hypertrophic scar</td>
<td>10</td>
<td>54.80 ± 16.07</td>
<td>28.50 ± 10.12</td>
<td>19.10 ± 7.91</td>
</tr>
<tr>
<td>Keloid</td>
<td>10</td>
<td>62.82 ± 13.07</td>
<td>21.90 ± 8.86</td>
<td>19.05 ± 7.04</td>
</tr>
</tbody>
</table>

F-value: 0.535  
P-value: >0.05

No significant differences existed in the paired comparisons among the keloid, hypertrophic scars, flat scars and normal people control groups (P>0.05).

Table 2. Analysis on the integral optical density of HLA-DR⁺, DQ⁺, DP⁺ cells in the peripheral blood of patients with keloid, hypertrophic scars, flat scars and of normal people (Mean ± S.D).  

<table>
<thead>
<tr>
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<th>HLA-DR</th>
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<th>HLA-DP</th>
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<tr>
<td>Normal control</td>
<td>10</td>
<td>597.88 ± 166.36</td>
<td>308.57 ± 44.05</td>
<td>159.01 ± 31.99</td>
</tr>
<tr>
<td>Flat scar</td>
<td>10</td>
<td>636.22 ± 133.95</td>
<td>302.77 ± 89.77</td>
<td>192.95 ± 51.32</td>
</tr>
<tr>
<td>Hypertrophic scar</td>
<td>10</td>
<td>883.11 ± 146.71***</td>
<td>439.24 ± 146.65***</td>
<td>271.93 ± 70.38***</td>
</tr>
<tr>
<td>Keloid</td>
<td>10</td>
<td>813.16 ± 62.77***</td>
<td>420.12 ± 94.25***</td>
<td>259.40 ± 57.43***</td>
</tr>
</tbody>
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F-value: 10.613  
P-value: <0.01

Table 3. Analysis on the numbers (%) and the integral optical density of CD1a⁺ cells in the peripheral blood of patients with keloid, hypertrophic scars, flat scars and of normal people (mean ± SD).  

<table>
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<tr>
<th>Groups</th>
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<th>No. of CD1a⁺ cells (%)</th>
<th>IOD of CD1a⁺ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>10</td>
<td>29.70 ± 7.87</td>
<td>368.67 ± 117.18</td>
</tr>
<tr>
<td>Flat scar</td>
<td>10</td>
<td>35.60 ± 11.59</td>
<td>423.53 ± 142.36</td>
</tr>
<tr>
<td>Hypertrophic scar</td>
<td>10</td>
<td>32.65 ± 6.35</td>
<td>648.94 ± 185.46***</td>
</tr>
<tr>
<td>Keloid</td>
<td>10</td>
<td>31.25 ± 8.21</td>
<td>580.15 ± 108.48***</td>
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F value: 0.828  
P value: <0.05

Table 2. Analysis on the integral optical density of HLA-DR⁺, DQ⁺, DP⁺ cells in the peripheral blood of patients with keloid, hypertrophic scars, flat scars, and of normal people (Mean ± S.D).  

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F-value: 10.613  
P-value: <0.01

*P<0.05 in comparison with the normal people control group; ** P<0.05 in comparison with the flat scars group.

Table 3. Analysis on the numbers (%) and the integral optical density of CD1a⁺ cells in the peripheral blood of patients with keloid, hypertrophic scars, flat scars, and of normal people (Mean ± SD).  

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* P<0.05 in comparison with the normal control group; ** P<0.05 in comparison with the flat scars group.

**Counting and quantitative analysis of CD1a⁺ cells in the peripheral blood in patients from the keloid, hypertrophic scar, flat scar and normal people groups**

Expression of CD1a molecules was found in the karyons and cytoplasm of the mononuclear cells and lymphocytes in patients from the different groups, in consistency with the report from Gregory (Berman and Bieley, 1995). There was no significant difference in the number of CD1a⁺ cells in the patients of the keloid, hypertrophic scar, flat scar and normal people groups (P>0.05). The IOD of the CD1a⁺ cells from the groups keloid and hypertrophic scar were both higher than those of the flat scar and normal people groups (P<0.05); IOD of the CD1a⁺ cells between group keloid and group hypertrophic scar and that between group flat scar and group normal people had no significant difference (P>0.05) (Table 3).

**DISCUSSION**

Over-hyperplasia of scars may be associated with the increase in the content of HLA-DR, -DQ and -DP molecules of the body

The human major histocompatibility complex (MHC) is represented by the human leukocyte antigen (HLA), which is the earliest found disease-associated genetic system. A complex and the most polymorphic genetic...
system consists of a series of closely linked gene loci. Overall, 39.8% gene products of the 128 functional genes have immune function and immune response genes (Ir) are often HLA-II genes. Therefore, it is believed that HLA genes participate directly or indirectly in the pathogenesis of diseases (Chen, 2001; Gong, 1998; Gebe et al., 2002).

HLA-II molecules are the expressed product of HLA-II genes, including the HLA-DR, HLA-DQ and HLA-DP molecules, which are distributed mainly on the surface of B cells, mononuclear cells, macrophages, dendritic cells, endothelial cells and activated T cells. They are responsible for the exogenous antigen processing and presentation, and have some restricted effects on CD4+ T cells as well as participate in the immune response through a triple molecular compound of antigenic peptide-MHC-T cell (Van den Elsen et al., 2004). According to the literature (Berman, 1995), HLA-B14, -B21, -Bw16, -Bw35, -DR5, -DQw3 and blood type A are rather closely related to keloid and hypertrophic scar on a genetic basis. Simultaneous increase of CD4+ and HLA-DR+ T cells and HLA-DR2+ dendritic cells in the abnormal scar tissue shows that HLA-II molecules are playing an irreplaceable role in local immunoreactions (Santucci et al., 2001; Castagnoli et al., 1997).

Results of this experiment show that mononuclear cells in the peripheral blood in keloid, hypertrophic scar, flat scar and normal people groups can all express HLA-DR, -DQ, -DP, though there exists no significant difference in the numbers of HLA-DR, -DQ, -DP-positive cells among the groups, there is a difference in their content. The content of HLA-DR, -DQ, -DP in the patients of groups keloid and hypertrophic scar is higher than that of the patients with flat scars and those of the normal people. This indicates a increase in the amount of expression of HLA-DR, -DQ, -DP molecules in the mononuclear cells in the peripheral blood of patients with keloid and hypertrophic scar. Increased expression of HLA-II molecules of HLA-DR, -DQ and -DP indicates that the over-hyperplasia of scars may be associated with immunogenetic factors.

It is often explained as “cicatricial diathesis” in clinical practice — for the hurts in the same part and under the same actions of hurting factors, most people can come through a normal healing process, while a small number of people may form keloid and hypertrophic scar. According to the analysis on the essence of this phenomenon with the results of this study, it may be caused by HLA controlled immunogenetic factors. Individuals with different genetic factors may react differently to self or exogenous substances. Individuals in a high response state are easy to initiate an immune response and vice versa, when a human body receives stimulus from the same molecule (Chen et al., 2003). Hence, we concluded that immunogenetic characters of HLA might be in nature the basis for the formation of keloid and hypertrophic scar, and local wounds are only a inducing factor. It can be observed from Tables 1 and 2 that HLA-DR molecules in each group are at the highest level of expression, indicating that HLA-DR may play a dominant role in the process of antigen presentation. Meanwhile, the actions of HLA-DQ and HLA-DP molecules in the formation of scars await further investigations.

Over-hyperplasia of scars may be associated with the increase in the amount of CD1a molecules of the body

CD1a molecules belong to CD1 family, non-HLA gene coding, and without polymorphism. CD1a gene coding heavy chain is homologous with MHC-I molecules and is also related with β2 micro-globulin. CD1a are mainly distributed in T and B lymphocyte, mononuclear cells and dendritic cells. That CD1a is similar in structure to that of HLA-I molecules and close in distribution to that of HLA-II molecules make CD1a to have a function of antigen presenting. However, CD1a is different from HLA that participates in the presentation of peptide antigens, as it mainly presents lipid and glycolipid antigens. Expression of CD1a is related to the body’s activation state, it acts as a co-receptor in the superantigen-induced T cell activation and transfers activation signals through cell membranes (Gregory et al., 2000; Jayawardena-Wolf et al., 2001; Calabi et al., 2000). The high expression of CD1a molecules in the peripheral blood of the patients from the keloid and hypertrophic scar groups indicates that CD1a molecules and HLA-II molecules are presenting their respective effects on immunoregulation in two different ways, contributing in making the body in a state of high immune response. It was reported (Spada et al., 2000) that antigen specific hyperplasia of some of the lymphocytes in the body is the key to acquiring immunity and that although some special lymphocyte subpopulations are as a result of lack of obvious stimulation from exogenous antigens in their adult stage, they represent obvious hyperplasia and potential or obvious autoimmune response to autoantigens. These lymphocytes account for 10 - 50% of the adult lymphocytes in various tissues such as the abdominal cavity, gastrointestinal tract, liver, spleen, bone marrow and the blood. Therefore, they are play an important role in initiating immune response before the T and B lymphocytes in the infected area are activated.

Generally, CD1 molecules have the functions in restricting congenital immune responses of cells, acting as a bridge in between the congenital and acquired immunities. Therefore, the high expression of CD1a molecules in the peripheral blood of the patients from the keloid and hypertrophic scar groups indicates that patients with scars have autoantigens, which further explains that over-hyperplasia of the scars may be associated with autoimmune responses.
REFERENCES

