EXPRESSION OF NATURAL CYTOTOXICITY RECEPTORS IN PERIPHERAL BLOOD NK CELL SUBSETS OF WOMEN WITH RECURRENT SPONTANEOUS ABORTIONS OR IMPLANTATION FAILURES

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INTRODUCTION

NK cells are composed of about 5–10 % of peripheral blood lymphocytes (PBL) and can be distinguished from other cell types by the expression of NK specific surface markers, i.e. CD56. Human NK cell subsets can be distinguished by their CD26 expression to CD56^{dim} NK cells and CD56{bright} NK cells. In peripheral blood, the main population of NK cells is CD56{dim}, whereas in the uterus the main population is CD56^{bright} NK cells. NK cells play an important role in human pregnancy. Studies showed that regulating peripheral blood NK cell activity contributed to reproductive success.

Natural cytotoxicity receptors (NCRs), which include NKp30, NKp40 and NKp46 appear to be expressed exclusively on NK cells. The NKp30 and NKp46 receptors are expressed on the surface of activated and non-activated NK cells. However, the NKp44 receptor is expressed on the surface of activated NK cells only. In addition, the NKp30 and NKp46 receptors function not only to the cytotoxic activity of NK cells but also to NK cell cytokine production.

MATERIALS AND METHODS

Study subjects

Peripheral blood was obtained from women with RSA (n=14), IVF-ET failures (n=16) and non-pregnant healthy controls (n=9). All study subjects had signed informed consents prior to entering the study, according to local IRB protocol. Blood was drawn from study groups prior to any treatment. Consilium of time the blood was drawn with the menstrual cycle of the subjects was not considered in this study.

Flow cytometric analysis of the peripheral blood leukocytes

The following monoclonal antibodies (mAbs) were used to analyze the surface antigens of peripheral blood leukocytes: anti-CD3 (eBioscience, San Jose, CA USA), anti-CD56 FITC (BD Bioscience, San Jose, CA USA), anti-CD26 PE (BD Bioscience, San Jose, CA USA), anti-CD33 (BD Bioscience, San Jose, CA USA) and anti-CD165 (BD Bioscience, San Jose, CA USA). Peripheral blood leukocytes with this panel of mAbs was performed using standard techniques. Brieﬂy, 100 µl of whole blood was incubated with mAbs for 20 min at 4 °C, and cells were lysed and fixed, following with wash in phosphate-buffered saline (PBS). Flow cytometric analysis was performed using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) with computer interfacing to BD CellQuest for full-lis-mode data storage, recovery, and analysis. The gate was set on the lymphocyte region. For each sample, 10{sup 5} peripheral blood lymphocytes were analyzed.

Statistical analysis

We classified patients by their histories as follows: the RSA group, women with a history of more than three spontaneous abortions; the implantation failure group, women with a history of more than two IVF-ET failures; controls, women with normal female fertility. Results were analyzed by Fisher’s exact probability test of frequency distribution or by paired t test. Differences were considered significant for probability < 0.05.

RESULTS

Patient characteristics are shown in Table 1. There was significant difference in age, number of pregnancy, number of delivery and number of abortion among three groups.

<table>
<thead>
<tr>
<th>No. of Pregnancy</th>
<th>Age (y)</th>
<th>RSA (n = 14)</th>
<th>Implantation Failures (n = 16)</th>
<th>Control (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>25.6 ± 2.8</td>
<td>33.4 ± 4.0</td>
<td>33.7 ± 4.1</td>
<td>33.4 ± 4.0</td>
</tr>
<tr>
<td>1</td>
<td>3.8 ± 1.7 *</td>
<td>0.5 ± 0.7</td>
<td>0.5 ± 0.7</td>
<td>0.5 ± 0.7</td>
</tr>
<tr>
<td>2</td>
<td>4.1 ± 1.8 †</td>
<td>0.5 ± 0.7</td>
<td>0.5 ± 0.7</td>
<td>0.5 ± 0.7</td>
</tr>
<tr>
<td>3</td>
<td>1.2 ± 1.1 ‡</td>
<td>0.5 ± 0.7</td>
<td>0.5 ± 0.7</td>
<td>0.5 ± 0.7</td>
</tr>
</tbody>
</table>

Flow cytometric analysis of NCRs and a2V-ATPase expression in peripheral blood NK cell subsets in women with RSA, implantation failures and normal healthy controls are shown in Figure 1. For the expression of NKp46, NKp44 and a2V-ATPase on NK cells (CD56{bright}, CD56{dim} and NKT cells), there were no significant differences between RSA, implantation failures and normal healthy control. For the expression of NKp44, the percentage of CD56{bright} NK cells was significantly lower than that in control (p<0.05). However, for another expression of NKp44 on NK cells and NKT cells, there were no significant differences between RSA, implantation failures and normal healthy controls.

CONCLUSIONS

The differential expression of NCRs and a2V-ATPase in CD56{bright} and CD56{dim} NK cell subsets of women with RSA and IVF-ET failures suggested different function of NK cell subsets such as cytotoxicity and cytokine production.

Women with RSA demonstrated significantly lower NKp44 expression on CD56{dim} NK cell subsets as compared to those of CD56{bright} NK cells were present in women with RSA or IVF-ET failures but not in normal controls.

Analysis of NCRs and a2V-ATPase expression in peripheral blood NK cell subsets may contribute to a better understanding of the biology of NK cells in women with RSA or IVF-ET failures.