Preconceptional natural-killer subsets in the peripheral blood as a predictor for prognosis of pregnancy

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Introduction

Spontaneous abortion is the most common complication of pregnancy. Estimates show that 2% to 5% of couples are childless because of repeated spontaneous abortion. (1) Although genetic, anatomic, hormonal factors, and autoimmune disorders have been implicated, a substantial proportion of cases remain unexplained. An immunologic cause has been suggested for many such affected couples. Because mammalian pregnancy is associated with the induction and adaptation of maternal allogenic reactions, inappropriate allogenic responses have been suggested as a cause of recurrent pregnancy loss in many couples. The sharing of HLA antigens between husband and wife has thus far been considered as an immunological indicator of habitual abortion due to inappropriate allogenic response. However, recently, the reliability of this indicator has been thrown into doubt. (2)

Therefore, immunological parameters that could be used to diagnose immunological abortion must be established as soon as possible. In the 8th annual meeting held in Nara, December, 1993, we reported that preconceptional NK activity might be a useful parameter for prediction of outcome of next pregnancy in the groups of the recurrent spontaneous aborters. (3) In this study, we assessed the NK cell subsets in the peripheral blood lymphocytes of the patients with history of recurrent spontaneous abortion and checked the possible correlations with the outcome of the next pregnancy.

Material and method

(Subjects)
A total of 42 women who had histories of two consecutive first-trimester abortions and no live births after conception with the same partner were evaluated in our department. No patient had any identifiable cause of recurrent miscarriages, such as uterine anomalies, endocrine abnormalities including luteal-phase defects, genetic abnormalities in either partner, or autoimmune abnormalities including evidence of antiphospholipid antibodies.

(Flow cytometry analysis)
Three ml of heparinized blood was drawn from each woman after informed consents had been obtained. The subsets of lymphocyte expressing CD16 and/or CD56 were identified by two-color flow cytometry and expressed as a percentage of lymphocytes present. Peripheral blood was mixed with FITC (fluorescein isothiocyanate) monoclonal antibody to CD16 and PE (phycoerythrin) monoclonal antibody to CD56. Immunofluorescence analysis of the lymphocyte population was performed on a Cytron Absolute Flow Cytometer (Ortho Diagnostics, Raritan, NJ).

(Statistical analysis)
Statistical analysis of data was carried out by Student's t test. The results were considered significantly different when p<0.05.

Results

1. In the study group, the percentage of CD56(+)16(+) cell was 7.9 ± 4.5%; CD56(+)16(-) 3.0 ± 2.0%; CD56(-)16(+) 6.6 ± 3.7%.

2. A total of 42 patients received no treatment in the next pregnancy. 24 of 42 patients maintained the next pregnancy normally (NP), 18 aborted in the first trimester of the next pregnancy (SA).

   The percentage of CD56(+)16(+) in NP; 6.1 ± 4.1% was significant lower compared with that of SA; 10.2 ± 4.2%.

   (p<0.05) (Fig. 1)

   In other subsets; CD56(+)16(+) and CD56(-)16(+), there were no significant differences between the two groups.

3. The percentage of CD56-positive cells was 8.7 ± 4.3% in NP, 13.7 ± 5.3% in SA; significant difference was found out between the two groups. (Fig.2)

4. Frequency of CD56-positive cells ≥12.5% and <12.5% between viable and nonviable pregnancy was shown in table 1. The sensitivity was 72.4%, specificity 76.9%. Positive predictive value 87.5%.
Discussion

The association between pregnancy outcome and NK cell subsets was revealed. Especially percentage of circulating CD56-positive cells in peripheral blood lymphocytes can predict the outcome of next pregnancy in women with obstetrical history of recurrent abortion. When the percentage of CD56-positive cells is less than 12.5%, the possibility of maintenance of pregnancy is considered to be high without specific treatment. On the other hand, when the percentage of these cells is more than 12.5%, the chance of maintaining pregnancy is lower.

Human decidual tissue appears to play an important role not only in nurturing the implanted embryo, but also in preventing its rejection by the maternal immune system. Compared with the uterine endometrium, early decidual tissue has been shown to contain a markedly increased number of various kinds of lymphocytes and macrophages, which affect local immunity. Among these decidual cells, the large granular lymphocytes (LGL) are especially abundant in the first trimester of pregnancy. Decidual LGL contain many cells expressing CD56, a surface marker of natural killer cells. (4) Moreover, decidual LGL have been revealed to have NK activity as measured by cytotoxicity against the NK-sensitive cell K-562. (5) Since additionally decidual LGL could kill human trophoblast cells after prior stimulation with interleukin-2, it is considered that decidual LGL play a pivotal role in the immunological maintenance of pregnancy. (6)

Considering the above results, it is considered as possible that circulating CD56-positive cells in the peripheral blood might be a useful parameter for immunological recurrent abortion.

Reference

(4) Starkey PM, Sargent IL, Redman CWG. Cell populations in the early human decidua: Characterization and isolation of large granular lymphocytes by flow cytometry. Immunology 1988; 65: 129-134
(6) King A, Loke YW. Human trophoblast and JEG choriocarcinoma cells are sensitive to lysis by IL-2 stimulated decidua NK cells. Cell Immunol 1990; 129: 435-438

Table 1

| C D 56(+) < 12.5% | 21 | 8 |
| C D 56(+) ≥ 12.5% | 3  | 10 |

Sensitivity: 72.4%
Specificity: 76.9%
Positive predictive value: 87.5%