Journal of Reproductive Immunology 95 (2012) 87–92

Contents lists available at SciVerse ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jreprimm

Enhancement of peripheral blood CD56^{dim} cell and NK cell cytotoxicity in women with recurrent spontaneous abortion or in vitro fertilization failure

Najmeh Karami[a], Mehri G. Boroujerdnia[a,d], Roshan Nikbakht[c,d], Ali Khodadadi[a,b,*]

[a] Department of Immunology, Faculty of Medicine, Ahwaz Jundishapur University of Medical Sciences, Ahwaz, Iran
[b] Cancer Research Center, Ahwaz Jundishapur University of Medical Sciences, Ahwaz, Iran
[c] Department of Obstetrics and Gynecology, Ahwaz Jundishapur University of Medical Sciences, Ahwaz, Iran
[d] Fertility and Infertility Research Center Imam Khomeini Hospital, Ahwaz Jundishapur University of Medical Sciences, Ahwaz, Iran

ARTICLE INFO

Article history:
Received 4 May 2012
Received in revised form 22 June 2012
Accepted 27 June 2012

Keywords:
NK cells
Cytotoxicity
LDH
CD56
RSA
IVF failure

ABSTRACT

Recent studies support the concept that NK cells play an important role in the success or failure of embryo implantation. Recurrent spontaneous abortion (RSA) is the most common complication of pregnancy. Some couples suffer from infertility of unknown cause. In vitro fertilization (IVF) is one of the useful treatment methods used for treatment of this type for infertility with variable outcomes. The aim of this study was to compare the percentage of peripheral blood CD56^{+} (CD56^{dim} and CD56^{bright}) cells and the level of NK cell cytotoxicity in patients with RSA and patients with IVF failure with those of healthy multiparous and successful IVF control women. In this case–control study peripheral blood samples from 43 patients, which included 23 women with RSA and 20 with IVF failure, plus 43 healthy control women comprising 36 normal multiparous women and 7 women with successful IVF, were collected. The percentage of peripheral blood NK cells (CD56^{+}) was identified by flow cytometry, then peripheral blood mononuclear cells (PBMC) were isolated by density centrifugation (Ficol-Hypaque) and incubated with NK-sensitive K562 cells. The NK cell cytotoxicity level was determined by lactate dehydrogenase (LDH) release assay. The percentage of CD56^{dim} cells and the level of peripheral blood NK cell cytotoxicity in RSA patients and women with IVF failure were significantly higher than in both the healthy multiparous and successful IVF control groups ($P < 0.001$). The findings of the present study suggest that increases in the percentage of CD56^{dim} cells and NK cytotoxicity in peripheral blood may be important contributing factors for both RSA and IVF failure.
© 2012 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Natural killer (NK) cells are the most abundant immune cells infiltrating the uterine implantation site (Moffett-King, 2002). These cells represent the first cellular immune defense mechanism and have close contact with the conceptus and placenta. NK cells comprise 5–12% of all lymphocytes (Thum et al., 2004) and are classified into CD16^{+} CD56^{dim} NK cells and CD16^{-} CD56^{bright} NK cells by their surface markers (Saito et al., 2008). In the peripheral blood, the main population of NK cells consists of CD16^{+} CD56^{dim} NK cells whereas CD16^{-} CD56^{bright} NK cells are the main population in the endometrium (Moffett-King, 2002; Dosiou and Giudice, 2005).

About 0.5–1% of couples who are trying to conceive will suffer subsequent spontaneous abortion (Emmer et al., 2000). Approximately 80% of recurrent spontaneous

* Corresponding author at: Department of Immunology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Golestan Avenue, P.O. Box 61355-45, Ahvaz, Iran. Tel.: +98 611 3330074; fax: +98 611 3332036; mobile: +98 9161183357.
E-mail addresses: akhodadadi2@gmail.com, akhodadadi@ajums.ac.ir (A. Khodadadi).

0165-0378/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jri.2012.06.005

miscarriages cannot be accounted for by hormonal disorders, chromosomal deficiencies or uterine abnormalities. Evidence is accumulating that these unexplained miscarriages could have an immunological background (Van Wijk et al., 1996).

The results of the evaluation of different lymphocyte subpopulations in infertility or recurrent miscarriages in the peripheral blood and in endometrial tissues are controversial (Beer et al., 1996; Furuya et al., 2003). Additionally there is no clear cause of at least of 20% of infertility cases, and this reproductive failure is also liked with immunological mechanisms (Baczkowski and Kurzawa, 2007).

A previous study showed that an elevated percentage of peripheral blood NK cells is associated with recurrent failed IVF treatment cycles (Beer et al., 1996). A subsequent study showed that increased peripheral blood NK cell cytotoxicity level is also associated with an increased rate of recurrent failed implantation after IVF treatment (Thum et al., 2004).

NK cells play an important role in the cellular recognition and killing of virus-infected and tumor cells (Imai et al., 2000). This process is accomplished through various immune effector mechanisms (Vivier et al., 2008). NK activity is accomplished by nonspecific lysing of infected targets through the use of NK receptors, or the FcγII (CD16) receptor, which recognizes IgG bound to specific antigens on the target cell surface (Imai et al., 2000). Cytotoxicity assays provide an in vitro evaluation of the lytic activity of NK and T cells against tumors or transformed target cells. The evaluation of lytic activity is therefore of great importance in monitoring the functional capability of these cells. CD56 is one of the natural cell adhesion molecule (NCAM) isoforms that are expressed in NK cells. A recent study has suggested that this molecule could be a mediator in the interaction between NK cells and their target cells (Vivier et al., 2008).

The aim of this study was to evaluate the percentage of peripheral blood NK cells ($CD56^{dim}$ and $CD56^{bright}$) by flow cytometry and then to compare the cytotoxicity activity within peripheral blood NK cells in patients with RSA and IVF, with those of normal multiparous and successful IVF women, using a lactate dehydrogenase (LDH) measurement method.

2. Materials and methods

2.1. Study population

A total of 86 women volunteered to participate in this case–control study. The patient groups comprised 43 women (23 with RSA and 20 with IVF failures). The grade of embryo in the IVF cycles was A or B, where the cleaving embryos were scored according to equality, size of the blastomers and proportion of anucleate fragments. Four categories were distinguished within this scoring system. Type A was defined as an equal sized embryo without anucleate fragments. Type B was defined as a non-equal-sized embryo and a maximum of 20% of the volume of the embryo was filled with anuclear fragments (Staessen et al., 1989).

The control groups included 43 nonpregnant healthy women, which consisted of 36 normal multiparous and 7 women successfully treated with IVF. Blood samples were taken from all women at the secretory phase of the menstrual cycle, which was defined by self-reporting. All persons provided a written informed consent before their participation in the study. Inclusion criteria for women with RSA were a history of at least two consecutive idiopathic miscarriages with the same partner and a desire for pregnancy. A miscarriage was defined as a spontaneous pregnancy loss before 22 weeks of gestation.

The inclusion criteria for IVF failure group were: women who were diagnosed by a gynecologist as having primary or secondary infertility of unknown cause with at least two episodes of IVF failure, no male factor, and with a normal gynecological, hormonal, and anatomical state. While the exclusion criteria included chromosomal rearrangement in either partner, anatomical–hormonal or infectious causes of RSA, immunological disease (presence of antiphospholipid antibodies, lupus anticoagulant, anticardiolipin antibodies), diabetes mellitus, polycystic ovarian syndrome, and thyroid dysfunction, uterine abnormality (fibroid, uterine polyp, uterine septum), normal gynecological, hormonal, and anatomical state. In addition, a history of previous spontaneous abortions, PID, curettage, hydrosalpinx, endometriosis, endocrinological, and metabolical diseases, gynecological interventions (leiomyomas, endometrial polyps, and pelvic adhesion removal) were other exclusion criteria. A gynecologist, who is a specialist in infertility, carried out the patient selection carefully. All of the patients selected had a normal karyotype.

The inclusion criteria for control groups were age less than 45 years; normal gynecological, hormonal, and anatomical state; a minimum of one delivery; no history of miscarriage or complications during previous pregnancies. The characteristics of the study population are shown in Table 1.

2.2. Flow cytometric assay

Ten milliliters of peripheral blood was collected in heparinized tubes. The flow cytometry examination was done on the fresh blood. Phycoerythrin (PE)-conjugated antihuman-CD56 monoclonal antibodies (BD Biosciences, USA) were used for NK cell detection. Erythrocytes were lysed using lysing solution (DAKO cytomation, Germany) and washed twice with phosphate buffer saline (PBS). A total of 10,000 events were acquired. $CD56^{+}$ cell analysis was performed within the lymphocyte cells range. Flow cytometric data were analyzed by WINMDI software.

2.3. Cytotoxicity assay

For the cytotoxicity assay, mononuclear cells (PBMC) were isolated by density gradient centrifugation (Ficol-Hypaque), washed three times and then resuspended in a freezing medium that included complete medium containing 10% dimethyl sulfoxide (DMSO) and stored in liquid nitrogen until use. The K562 tumor cell line (National Cell Bank of Iran, Pasteur Institute, Tehran) was maintained in a continuous suspension culture in RPMI 1640 + 10% FCS supplemented with 3 mmol/L L-glutamine, 100 μg/mL streptomycin, and 100 U/mL penicillin at 37 °C (Jurisic et al.,

Download English Version:

https://daneshyari.com/en/article/3964398

Download Persian Version:

https://daneshyari.com/article/3964398

Daneshyari.com