Isolation and Characterization of Endometrial Leukocyte Populations by Flow Cytometry

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UDC 618.145-008.853-076.5

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 1, pp. 68-71, January, 1994
Original article submitted October 28, 1993

Potentialities of the flow cytometry method in studies of the qualitative and quantitative composition of endometrial immunocompetent cells are demonstrated. The studied leukocyte populations are found to occur in negligible quantities in the endometrium.

Key Words: *flow cytometry; endometrial leukocyte populations*

Local immune response parameters have attracted increasing attention on the part of scientists investigating reproduction immunology in the last 5 years due to progress in fundamental immunology. Of paramount importance among these parameters is the qualitative and quantitative composition of endometrial immunocompetent cells. Previously the possibility of using flow cytometry to study lymphocyte populations in the decidual tissue of the first trimester of pregnancy was demonstrated [5,8,12]. Enzymatic disaggregation, for example [5], leads to loss of a number of surface markers and changes the ratio of lymphoid cell populations. Mechanical disaggregation of cells followed by isolation of mononuclears by gradient centrifugation is a more sparing procedure, although it may yield a reduced concentration of macrophages in comparison with the immunohistochemical examination [2]. However, immunohistochemical analysis of the endometrium or decidual tissue is rather difficult and does not permit the investigator to obtain quantitative characteristics [13].

The aim of this research was to develop a flow cytometry technique for analysis of endometrial leukocyte populations during various phases of the menstrual cycle in health and disease (endometritis, endometriosis).

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MATERIALS AND METHODS

Preparation of a cell suspension from the endometrium. Endometrium was obtained from 8 women with normal reproductive function (3 of these in the first and 5 in the second phase of the cycle), 25 women with disseminated endometriosis, and 16 with endometritis. For control, decidual tissue from a healthy woman in the first trimester of pregnancy was examined. The leukocytic fraction of endometrial cells was obtained after mechanical homogenization of tissue fragments weighing 0.2 to 1.0 g followed by centrifugation in a Ficoll-Paque gradient at 1700 g for 40 min. Mononuclear cells were collected in interphases and after double washing in PBS were used for phenotypic marking. The cells were counted in Goryaev's chamber in a mixture with trypan blue (0.1%) and 3% acetic acid used for red cell lysis.

Analysis of cell phenotypes by flow cytometry. The following FITC-labeled monoclonal antibodies were used: anti-Leu-12 (CD19), anti-Leu-9 (CD7), anti-Leu-4 (CD3), and anti-Leu-11c (CD16), anti-Leu-M3 (CD14), anti-HLA-DR (Becton Dickinson, USA).

0007-4888/94/0001-0071$12.50

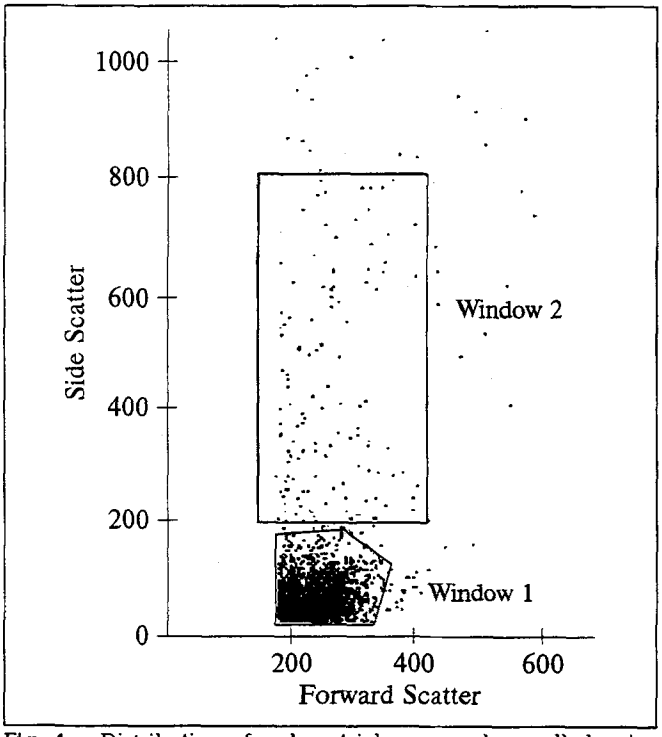

Fig. 1. Distribution of endometrial mononuclear cells by size and light dispersion: window 1: cells corresponding by parameters to peripheral blood lymphocytes; window 2: "large" cells.

Cell samples (50×10^3 per sample) with monoclonal antibodies were incubated at 4°C for 20 min and then washed twice in PBS. The samples were analyzed in a FACScan flow cytometer (Becton Dickinson), each sample containing not less than 5×10^3 cells. Cell viability was assessed by staining with propidium iodide. Native murine IgG of a relevant isotype was used for control of nonspecific binding of immunoglobulins.

RESULTS

Mechanical disaggregation of endometrial cells followed by isolation of the mononuclear fraction in a Ficoll-Paque gradient helped obtain a cell suspension with at least 90% viability by the trypan blue exclusion technique. Phenotypic marking of cell samples reduced cell viability assessed by staining with propidium iodide, but not lower than 75%. In the present research we analyzed the expression of surface markers only on live cells.

The total leukocyte count estimated per gram of endometrial tissue was virtually the same in healthy women (both in the first and second phases of the cycle) and in those with endometriosis or endometritis in the second phase of the cycle: $1.5\pm0.8\times10^6$ cells (Tables 1, 2). This parameter was markedly increased only in endometritis patients examined during the first phase of the cycle (Table 1), which may be indicative of a local inflammatory process. The total leukocyte count per gram of decidual tissue in healthy women during the first trimester of pregnancy was 1.0×10^6 cells in our study, this corresponding to the results of other workers, $0.7\text{-}2.5\times10^6$ cells [3]. Hence, transformation of the endometrium into decidual tissue during pregnancy evidently does not lead to an increase in the count of mononuclears.

Phenotypic analysis of the isolated leukocytes was carried out separately for cells corresponding in size to peripheral blood lymphocytes (window 1) and for large cells falling into window 2 (Fig. 1). As one can see from the results presented in Table 1, the overwhelming majority of mononuclears in the endometrium of women with normal reproductive function, at least in the second phase of the cycle, do not carry the markers used: CD19, CD7, CD16, and CD14.

Hence, B, T, and NK cells and macrophages are present only in negligible numbers in the endometrium of nonpregnant women, and, although just a few healthy women were examined, we may assume that this corresponds to the actual distribution of leukocyte populations in the nonpregnant endometrium, because similar results were obtained in 25 patients with endometriosis (Table 2). Examinations of normal decidual tissue confirmed once again that these data did not result from cell loss in the course of their isolation and phenotypic characterization. For example, in the total mononuclear count in decidual tissue of 1.0×10^6/g, T lymphocytes constituted more than 50%.

Decidual tissue leukocyte populations were represented as follows: window 1: 2×10^3 CD19+, 455×10^3 CD7+, and 28×10^3 HLA-DR+ cells; window 2: 4×10^3 CD19+, 23×10^3 CD7+, and 6×10^3 HLA-DR+ cells. Other authors report similar results of studies of decidual tissue from women in the first trimester of pregnancy [9]. Three main leukocyte populations were identified: macrophages, large granular lymphocytes with an uncommon phenotype (CD2+, CD7+, CD38+, CD56+, CD3-, CD16-, CD57-, CD5-, CD4-, CD8-, CD25-), and a few T lymphocytes. Decidual macrophages express CD14, CD11c, and HLA-DR and participate in phagocytosis [1], in antigen presentation [11], and in the production of various lymphokines [6]. Large granular lymphocytes 15 to 25 μ in size were first described in 1954 [10], but the function of these cells is still unclear. They are known to be capable of lysing K-562 targets and trophoblast cells after IL-2 treatment [7] and to secrete TNF and γ-IF [4].

TABLE 1. Leukocyte Populations in the Endometrium of Women with Normal Reproductive Function and in Endometritis Patients in Various Phases of the Cycle

Group	Cells, $\times 10^6$/g tissue	Window	CD19+	CD7+	CD16+	CD14+
			$\times 10^3$/g tissue			
Healthy women						
Phase 1 ($n=3$)	1.2±1.0	window 1	47±30	146±42	73±17	15±7
		window 2	1±1	1±1	14±5	3±2
Phase 2 ($n=5$)	1.6±0.8	window 1	12±2	73±28	44±15	18±8
		window 2	1±1	3±2	4±3	1±1
Endometritis patients						
Phase 1 ($n=7$)	5.2±2.1*	window 1	59±16	1222±485*	247±97*	35±10
		window 2	11±2*	113±55*	110±51*	56±5*
Phase 2 ($n=9$)	1.8±0.5	window 1	5±4	226±83*	44±14	28±14
		window 2	2±2	31±13*	39±15*	21±15*

Note. Asterisk shows a statistically reliable difference in comparison with the relevant control ($p<0.05$).

Hence, transformation of the endometrium into decidual tissue leads to a noticeable increase of T cells without changing the total count of mononuclears.

Analysis of the endometrium in women with endometritis in the first phase of the cycle indicated development of inflammatory cell reactions (Table 1) manifested by increases in the leukocytic infiltration of the endometrium, the T lymphocyte count, and the number of "large" cells (window 2) expressing the following markers: CD19 (plasma cells), CD7 (T lymphocyte blast forms), CD16 (natural killers), and CD14 (macrophages) in comparison with the group of women without endometritis.

The population composition of endometrial leukocytes in endometriosis did not differ much from that in healthy women (Table 2). Statistically reliable differences were revealed only between "large" cells (window 2): CD7+CD3- (large granular lymphocytes), CD16+ and CD14+ ($p<0.05$) manifested in an increased number of the said phenotypes. Investigation of endometrial cells in endometriosis patients revealed a peculiar feature in leukocyte populations depending on the presence of endometrioid cysts of the ovaries. The presence of ovarian cysts leads to an increase in the number of T lymphocytes, particularly natural killers. Moreover, an increase in the count of "large" CD16+ cells was most characteristic of such patients. The detected difference may be related to a changed concentration of ovarian hormones in the presence of cysts, for it has been shown that at least the large granular lymphocytes may be a good criterion of hormonal differentiation of the endometrium due to their progesterone sensitivity [9,10].

Flow cytometric analysis permitted identification of the following populations of endometrial leukocytes in nonpregnant women: T and B lymphocytes, large granular lymphocytes, NK cells, and macrophages. T cells were the most numerous of these cells expressing CD7 on their surface, a phenomenon which has been observed previously in immunohistochemical analysis [13].

The function of endometrial T lymphocytes in nonpregnant women is known even less than that of decidual tissue in the first trimester of pregnancy. Some workers have proposed that this population of immunocompetent cells is functionally heterogeneous and represented by cytotoxic T lymphocytes controlled by T suppressors [10], and that large granular lymphocytes act as such suppressors. The supposition afout the functional heterogeneity of endometrial T lymphocytes seems very attrac-

TABLE 2. Differences in Population Composition of Endometrial Leukocytes of Women with Endometriosis with or without Endometrioid Cysts in the Second Phase of the Cycle

Group	Cells, $\times 10^6$/g tissue	Window	CD19+	CD3+	CD7+	CD16+	CD14+
			$\times 10^3$/g tissue				
Without cysts ($n=15$)	1.6±0.3	window 1	6±3	45±15	99±25	23±6	6±2
		window 2	1±0.4	8±6	17±3	19±5	10±2
With cysts ($n=10$)	1.4±0.2	window 1	26±20	58±16	227±84*	53±16*	15±15
		window 2	5±2	14±10	31±10	73±15*	8±2

Note. Asterisk shows statistically reliable differences between studied parameters in groups of patients ($p<0.05$).

tive from the viewpoint of the hypothesis on the possibility of extrathymic differentiation of endometrial lymphocytes. For the first time we revealed in the endometrium numerous cells expressing the CD16 marker. It is very important to find out whether these cells are functionally natural killers.

Hence, our studies of the qualitative and quantitative characteristics of endometrial mononuclears in nonpregnant women using the flow cytometry technique helped detect immunological specificities of transformation of endometrial tissue into decidual tissue and demonstrate the diagnostic significance of analyzing endometrial leukocyte populations in patients with endometritis and endometriosis.

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