Expression of Activation Markers on Decidual Lymphocytes during the First Trimester of Pregnancy

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Abundant evidence of an important role of maternal immunoregulatory factors in normal fetal development has been obtained of late. Active regulation of a potentially dangerous maternal immune response to fetal antigens occurs primarily at the placental level [6]. Three principal leukocyte subpopulations are present in the decidual membrane (maternal portion of the placenta) in the first trimester of pregnancy: macrophages, large granular lymphocytes with the uncommon phenotypes $CD56^+$, $CD3^+$, $CD7^+$, $CD16^-$, $CD57^-$, $CD3^-$; and T lymphocytes [4,7]. Previously we developed ways to analyze the phenotype of decidual lymphocytes obtained by mechanical disintegration of the decidual membrane using flow cytofluorometry and characterized in detail decidual $CD7^+$, $CD3^+$, and $CD8^+$ lymphocytes [7].

The aim of the present study was to elucidate the activation status of various subpopulations of decidual lymphocytes by analyzing the expression of activation markers CD69 and HLA-DR, receptors for interleukin-2 (IL-2) and transferrin.

MATERIALS AND METHODS

Decidual tissue was obtained from women with normal pregnancies after induced abortions, thoroughly washed free of blood in phosphate buffered normal saline, minced with scissors, pressed through a steel grid (100 μ) with the plunger of a glass syringe, and filtered 8 through layers of gauze. Mononuclear cells were isolated in a Ficoll-Hypaque (Pharmacia) density gradient. Simultaneously peripheral blood was collected, followed by isolation of mononuclears in the density gradient.

Monoclonal antibodies conjugated with fluorescein isothiocyanate or phycoerythrin (Becton Dickinson): CD3 (Leu-4), CD56 (Leu-19, CD69 (Leu-23), CD25 (IL-2 receptor α; IL-2Rα), CD71 (transferrin receptor), and HLA-DR were used to label the cells. Cytofluorometric analysis was carried out using a FACScan flow cytofluorometer (Becton Dickinson),placing the discrimination window on $CD3^+$ or $CD56^+$ lymphocytes. Simultest Control (mouse IgG1FITC+IgG2aPE, Becton Dickinson) was used for nonspecific staining analysis. Nonspecific binding of murine immunoglobulins to decidual cells was blocked by aggregated rabbit immunoglobulins.

RESULTS

Use of two-colored flow cytofluorometry allowed us to detect differences in the expression of activation markers on decidual membrane (DM) and peripheral blood (PB) lymphocytes (Table 1). A high level of expression of T and natural killer (NK) lymphocyte early activation marker CD69 was cha-

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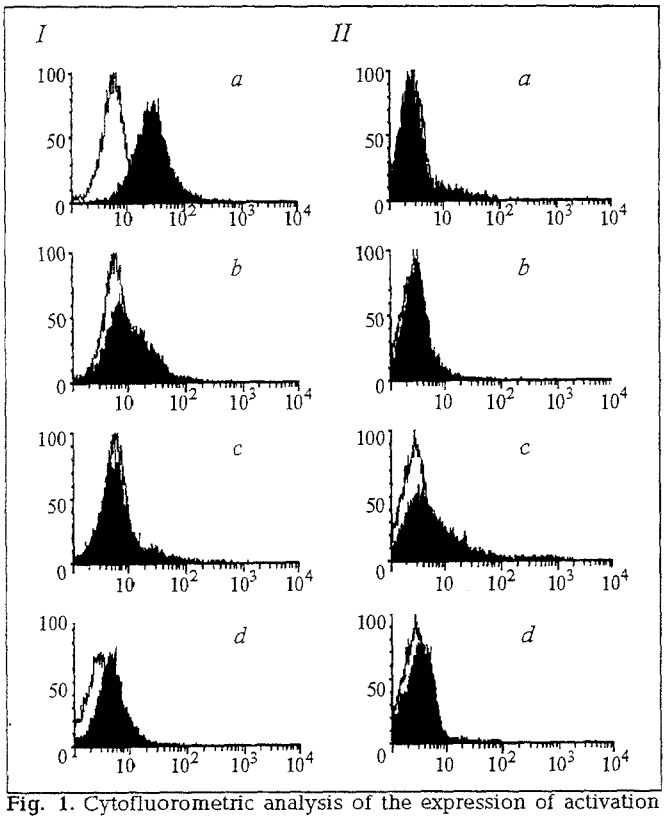

Fig. 1. Cytofluorometric analysis of the expression of activation markers CD69 (*a*), IL−2Rα (*b*), HLA−DR antigen (*c*), and transferrin receptor (*d*) on decidual (*I*) and peripheral blood (*II*) $CD3^+$ lymphocytes. White histogram: control; black histogram: cells labeled with relevant antibodies.

racteristic of DM T lymphocytes ($CD3^+$) and NK ($CD56^+$). In contrast to PB lymphocytes, a much higher fraction of decidual $CD56^+$ lymphocytes expressed IL-2Rα and a much lower fraction expressed HLA-DR antigen. At the same time a high level of HLA-DR antigen expression on decidual T lymphocytes was revealed, paralleled by a low level of IL-2Rα expression.

The differences in transferrin receptor expression on DM and PB lymphocytes were less marked, but the fraction of decidual T and NK lymphocytes expressing this receptor was markedly higher than that of analogous subpopulations of PB lymphocytes. The results of cytofluorometric analysis of activation marker expression on DM and PB lymphocytes are presented in Figs. 1 and 2.

Earlier *in vitro* studies demonstrated CD56 marker expression as soon as 2 h after lymphocyte activation [11], and after 24 h lymphocytes were found to express IL-2Rα [5]. IL-2 binding with the cell initiated a number of processes, including transferrin receptor expression, this resulting in lymphocyte proliferation [12]. HLA-DR antigens are expressed on days 3-4 after activation [8]. Unlike PB lymphocytes, decidual T lymphocytes are activated, as is indicated by a high level of CD69 and HLA-DR antigen expression. At the same time, these lymphocytes are characterized by a low level of IL-2Rα and transferrin receptor expression. Specific features of the expression of activation markers on decidual lymphocytes may be indicative of specific mechanisms regulating the activation processes in the placenta. Trophoblasts are known to synthesize IL-1 and IL-2 [3,9], and placental macrophages are capable of synthesizing colony-stimulating growth factors TNF-α and TGF-β [9]. These factors may have variously directed effects on T lymphocyte activation, and therefore peculiarities of activation marker expression on decidual T lymphocytes are due to the release of a specific group of cytokines in the placenta. Evidently, the specificities of activation marker expression on the decidual membrane provide the optimal conditions for maintaining a normal pregnancy. Biochemical analysis has demonstrated that the CD69 molecule contributes to the transfer of the signal initiating T lymphocyte proliferation; but the signals mediated via the CD69 molecule are not capable of inducing $CD69^+$-activated T lymphocyte cytotoxic activity [14]. Hence, the absence of α-receptors to IL-2 and, at the same time, CD69 expression on decidual T cells

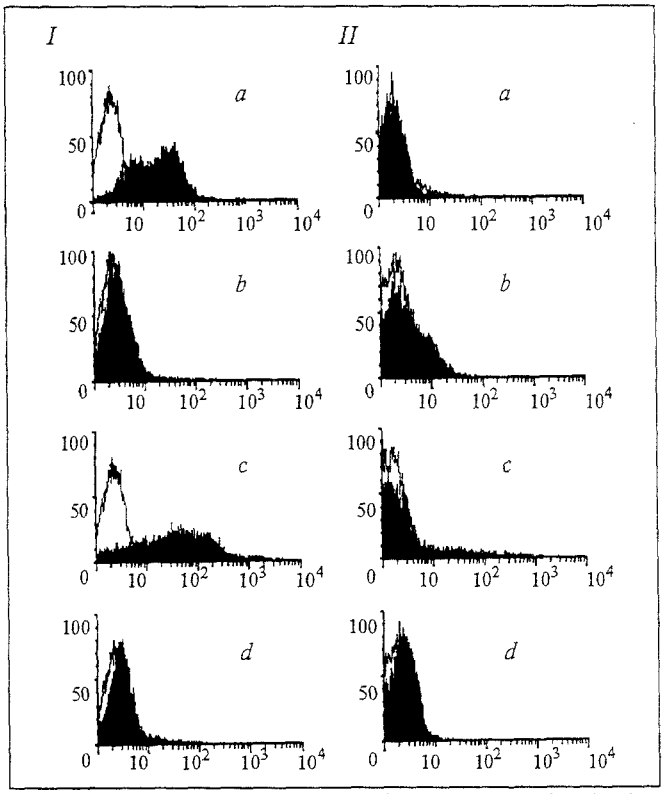

Fig. 2. Cytofluorometric analysis of the expression of activation markers CD69 (*a*), IL−2Rα (*b*), HLA−DR antigen (*c*), and transferrin receptor (*d*) on decidual (*I*) and peripheral blood (*II*) $CD56^+$ lymphocytes. White histogram: control; black histogram: cells labeled with relevant antibodies.

TABLE 1. Expression of Activation Markers (in % on Placental Decidual Membrane and Peripheral Blood Natural Killers ($CD56^+$) and T Lymphocytes ($CD3^+$) (Mean±SEM)

Activation marker	$CD56^+$ lymphocytes		$CD3^+$ lymphocytes	
	DM	PB	DM	PB
CD 69	62.3±4.0**	11.9±2.0	57.3±3.7**	1.5±0.7
IL−2R	17.5±4.5*	2.6±1.6	5.5±0.9*	12.4±2.2
Transferrin receptors	6.1±1.2*	2.2±0.4	8.2±2.3*	1.3±0.4
HLA−DR	8.1±1.2*	18.2±3.2	56.5±3.0**	9.3±2.4

Note. DM: decidual membrane; PB: peripheral blood; one asterisk: $p<0.01$ vis−a−vis peripheral blood lymphocytes; two asterisks: $p<0.001$ for $n=12$.

may be indicative of the inability of these cells to generate cytotoxic activity targeted at fetal antigen-carrying cells.

On the other hand, an activated state of decidual T lymphocytes indicates their capacity for synthesizing cytokines, whereas resting T cells are incapable of this. According to Wegmann's hypothesis, a key role in pregnancy maintenance is played by immunostimulation processes, because soluble factors produced by T lymphocytes are capable of maintaining trophoblast growth [15]. Decidual NK cells may also be important in cytokine production, this being indicated by a high level of antigen CD69 expression on these cells, and stimulation of this antigen leads to activation of IFN-γ and IL-2 genes [14]. Colony-stimulating factors IL-2, IL-3, IL-4, IL-5, IL-6, TNF-α, and IFN-γ mRNA were detected in decidual $CD56^+$ lymphocytes [10].

Decidual membrane $CD56^+CD16^-$ lymphocytes are thought to be immature NK cells selectively accumulated in the decidual membrane; however, immature NK cells are characterized by a high level of HLA-DR antigen expression [2]. Our studies showed that HLA-DR antigen was expressed by only 8% of decidual $CD56^+$ lymphocytes, so that we cannot speak about immaturity of the decidual NK cells.

Peripheral blood NK cells were reported to express only IL-2 β-receptor but not IL-2 α-receptor [13]; our studies demonstrated a high level of IL-2 α- and IL-2 β-receptor expression on decidual $CD56^+$ lymphocytes; the simultaneous expression of IL-2 α- and β-receptors may be indicative of a high sensitivity of decidual NK cells to IL-2.

The negligible level of transferrin receptor expression on decidual lymphocytes appears to be explained by their low proliferative activity. A low level of proliferating lymphocytes in the decidual membrane was also demonstrated using the Ki67 marker [4] and cell cycle analysis [1].

Hence, our data indicate a local activation of decidual lymphocytes which are therefore capable of secreting lymphokines necessary from placental growth and development. The specificities of activation marker expression on decidual lymphocytes appear to be related to the need for providing the immunologically optimal conditions for pregnancy development.

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