EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Insulin-Binding Activity of Lymphocytes Carrying Fcγ Receptors in Pregnant Women with Diabetes Mellitus

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Differences in insulin-binding activity of mononuclear cells and T lymphocytes due to heterogeneity of insulin receptors and effects of the hormonal and metabolic shifts are detected in pregnant women with insulin-dependent diabetes mellitus. In healthy women high insulin-binding activity of mononuclear cells during the third trimester correlates with the content of Fcγ-R$^+$ cells, but does not depend on the number of Tγ lymphocyte, despite an increase in their blood count. No correlation between insulin-binding activity of T fraction and the content of Fcγ lymphocytes is detected. The relationship between insulin-binding activity of T lymphocytes and the number of Tγ cells in pregnant diabetics differs from that in healthy women and is different in high and low activity.

Key Words: *insulin-binding activity; mononuclear cells; insulin-dependent diabetes mellitus; heterogeneity*

Hormonal and metabolic shifts associated with normal pregnancy can act as a diabetogenic factor. On the other hand, diabetes mellitus (DM) complicates the course of pregnancy and affects fetal development [1].

Increased activities of the suppressor components of the immune system [6,7] and insulin interaction with insulin receptors in immunocompetent cells [2] are justified in normal pregnancy. These shifts can underlie decompensation of insulin-dependent DM (IDDM). Generation of suppressor T lymphocytes is associated with the expression of membrane receptors to Fcγ fragment and IgG (Fcγ-R), which is characteristic of the early stage of cells activation. Insulin receptors are late activation markers of T lymphocytes undetectable on resting cells [11]. Therefore, the relationship between insulin-binding activity (IBA) of insulin receptors and the number of lymphocytes carrying Fcγ receptors is of practicular interest.

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We investigated the relationship between the IBA of immunocompetent cell and the number of Fcγ-R cells in the peripheral blood of pregnant women with DM.

MATERIALS AND METHODS

Peripheral blood mononuclear cells (MNC) and T lymphocytes of 31 pregnant women during the third trimester (30-36 weeks) aged 19-36 years were examined. Twenty-one women had IDDM (group 1) and 10 had gestation DM which developed at 20-33 weeks gestation (group 2).

Blood sugar level in pregnant patients with decompensated IDDM (n=11) was 10.4-20.0 mmol/liter and the mean daily dose of insulin was 54.3±7.45 U (22-82 U). In 10 patients with clinically and metabolically compensated IDDM glycemia varied from 5 to 10 mmol/liter and daily insulin dose was 41.3±4.4 U (35-50 U). IDDM ran a severe course in the majori-

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ty of patients; the history of diabetes was 3-17 years. In eight patients diabetes was complicated by angiopathy and three patients developed concomitant Hashimoto's thyroiditis (1), diffuse goiter (1), and chronic pyelonephritis (1). Three patients developed gestosis.

Half of group 2 patients suffered from obesity of the first-second degree.

The control group consisted of 17 women with normal pregnancy at the same terms.

Peripheral blood MNC were isolated by centrifugation in the Ficoll-Verograffin density gradient [4]. T cell-enriched fraction was isolated by E-rosette formation with neuraminidase-treated sheep red blood cells followed by re-centrifugation in the Ficoll-Verograffin gradient [3].

Insulin receptors in MNC and T lymphocytes were determined by radioimmunoassay [10]. IBA was calculated by the formula: IBA=$(A-B)/A\times100$, where A and B are total and nonspecific (in the presence of unlabeled hormone) binding (cpm/cell).

The number of Fcγ-R-positive cells (Tγ) was assessed by EA-rosette formation with bovine erythrocytes sensitized by rabbit specific IgG [12].

The data were processed by the method of variation statistics using Student's t test.

RESULTS

The IBA of MNC from healthy pregnant women varied from 25 to 42% (average 34.02±3.93%) (Fig. 1, *a*). Insulin receptor binding of MNC of pregnant diabetics in both groups was decreased in comparison with donors (Table 1). In healthy pregnant women the IBA values were within M±σ, while in IDDM they did not surpass the mean value in healthy women and in half patients were lower than M±σ. Individual values of IBA in women with gestation DM were lower than the least values in healthy pregnant women, particularly in obese women, and in general there was a statistically significant decrease in comparison with healthy pregnant women and IDDM patients ($p<0.005$). No relationship between IBA and the clinical and metabolic status of pregnant patients with IDDM was detected, but the lowest values were observed in women with subcompensated diabetes.

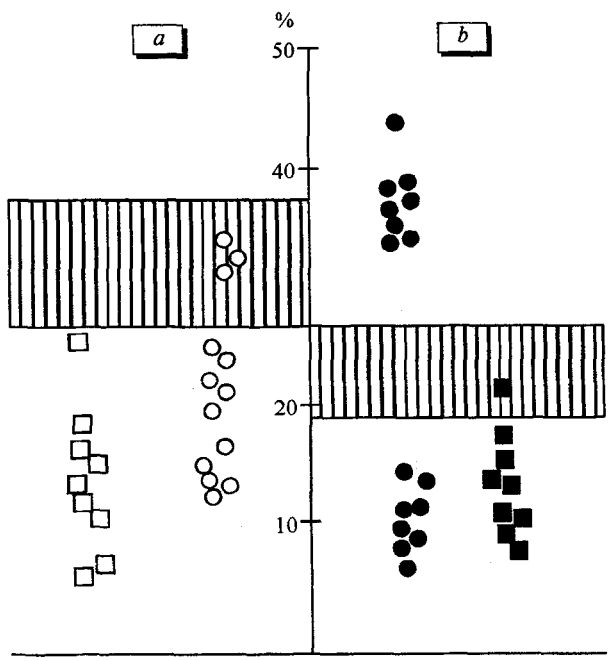

Fig. 1. Individual values of insulin-binding activity of mononuclear cells (*a*) and T lymphocytes (*b*) in pregnant women with insulin-dependent (squares) and gestation (circles) diabetes mellitus. Cross-hatched zone: mean values.

Since the MNC suspension was heterogeneous, we evaluated insulin receptors activity of the T lymphocyte fraction. In healthy pregnant women the IBA of T cell varied within 15-32% (average 24.50±2.53%) (Table 1) indicating the presence of activated T lymphocytes in the circulation, which is in line with the data on physiologically justified activation of the T cell immunity during pregnancy [7]. Patients with IDDM can be divided into two subgroups by the ^{125}I insulin-binding capacity of T lymphocytes: T lymphocytes with high IBA (32.5-43.4%, average 36.85± 1.31%, $p<0.01$ in comparison with healthy pregnant women) and low IBA (3.3-13.0%, average 9.86± 1.44%, $p<0.01$ in comparison with healthy pregnant women). This can be explained by insulin resistance in decompensated patients or insulin excess in compensated patients. Low IBA was observed in eight patients with diabetic microangiopathies irrespective of compensation. Comparison of IBA of MNC and T lymphocytes showed that the T-cell IBA in healthy pregnant women was lower than the MNC IBA in almost all examines.

A similar relationship was revealed in IDDM patients with diabetic microangiopathy and low IBA of both T lymphocytes and MNC, but we did not observe such tendencies in patients with gestation DM; although IBA of both cell suspensions was low in obese patients, IBA of the T fraction was higher.

In none of the groups IBA of T cells correlated with the number of these cells. In healthy pregnant women the IBA of MNC strongly correlated with the number of Fcγ-R^+ cells ($r=0.89$) due to non-T Fcγ-R^+ cells ($r=0.77$), while in IDDM this correlation became weaker. Moreover, in healthy pregnant women IBA inversely depended on the number of T lymphocytes ($r=-0.62$). It can be hypothesized that in normal pregnancy the IBA of MNC is to a greater extent associated with monocytes and B lymphocytes [8] than in DM.

The divergence between IBA of T lymphocytes and the number of these cells in the blood of healthy

TABLE 1. IBA of MNC and T Lymphocytes of Pregnant Patients with DM with Different Degree of Compensation ($M \pm \sigma$)

Group of pregnant women	Insulin-binding activity, %	
	MNC	T lymphocytes
Healthy (n=17)	34.02±3.93	24.50±2.53
IDDM (n=21)	23.50±2.61*	22.44±3.21
decompensated (n=11)	26.64±2.90	24.85±4.13
compensated (n=10)	20.28±4.09*	20.33±5.1
Gestation DM (n=10)	16.1±5.02*	16.6±3.44

Note. *p<0.05 *vs.* healthy pregnant women.

pregnant women and in diabetics prompted us to study the relationship between these parameters. In normal pregnant women, despite high IBA of the T fraction and an increased number of Tγ cells (29.42± 1.61%, 392.8±32.8 cells/ml), these parameters did not correlate (r=-0.14).

In IDDM patients with high IBA we detected a medium inverse correlation between IBA of T lymphocytes and the relative content of Tγ cells (r=-0.38). By contrast, in diabetics with low IBA and decreased count of Tγ lymphocytes (24.1±1.5%) these parameters were in strong inverse correlation in both IDDM (r=-0.72) and gestation DM (r=-0.73). The positive correlation between IBA of T lymphocytes and the number of non-Tγ cells (r=0.72 in group 1 and r=0.73 in group 2) confirms the hypothesis on the expression of insulin receptors on helper and inductor lymphocytes [5], although reduced avidity and affinity of insulin receptors on Tγ cell cannot be ruled out. Medium inverse correlation between the parameters in the subgroup of pregnant IDDM patients with high IBA can be due to increased number of cells with insulin receptors and to increased number avidity and affinity of receptors expressed on $CD8^+$ [8] and $CD4^+$ lymphocytes [5].

Analysis of correlations showed that in healthy women during the third trimester of gestation high IBA of MNC correlated with the number of $Fc\gamma\text{-}R^+$ cells and did not depend on the number of Tγ lymphocytes, despite their increased blood count. No correlation between the IBA of the T fraction and number of Tγ lymphocytes was found.

In diabetics the relationship between IBA of T lymphocyte and number of Tγ cells was different, and its pattern varied in high and low IBA.

Evaluation of IBA of immunocompetent cell is important for adequate insulin therapy and prevention of chronic insulin overdose in insulin resistance, and for detection of high-risk mothers and their children.

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