Comparison of Insulin-Binding Activity of Blood Cells in Diabetes Mellitus in Pregnant Women

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Insulin-binding activity of blood cells in pregnant women is shown to vary considerably in health and in diabetes mellitus with different forms and stages of compensation. More stable changes were observed just in erythrocytes.

Key Words: *insulin sensitivity of cells; diabetes mellitus*

Insulin-dependent diabetes mellitus (IDDM), primarily caused by absolute insulin deficiency, is characterized by the development of secondary insulin resistance which depends on the state of the insulin receptors [2,9,13]. Insulin receptors (IR) are the primary target of insulin action on the cell. Alteration in the state of IR affects the sensitivity of tissues to insulin, i.e., may leads to insulin resistance and to disturbances of insulin-dependent metabolic pathways, primarily, carbohydrate metabolism.

Published data point to a great variability of both the nature of insulin-receptor interaction and the insulin sensitivity of cells in patients with IDDM. For instance, the level of insulin binding by erythrocyte (Er) and monocyte receptors in patients with initially detected untreated IDDM varied from low to high, while in long-term and well-compensated diabetes mellitus (DM) it did not differ from that in healthy subjects [3,5,14]. A comparative study of the insulin-receptor interaction on Er, monocytes, and target cells for insulin revealed that, despite normal insulin binding by erythrocyte and monocyte receptors, it was lowered in living cells. This phenomenon was attributed to enhanced insulinization of peripheral tissues, in particular adipose tissue due to high level of exogenous insulin and its predominant effect on IR [3,14]. However, blood cells may be adapted to higher concentrations of the hormone, and therefore the steady-state level of hormone binding to blood cells may not change perceptibly.

The insulin-binding activity (IBA) of blood cells in pregnant women has been little studied, and the results which have been reported are contradictory and do not allow one to explain the changes in insulin-receptor interaction in blood cells by the action of insulin alone. In pregnant women with DM the IR may be affected not only by exogenous insulin but also by stress-induced metabolic shifts caused by insulintherapy [2].

In view of all this, we aimed to investigate IBA in different blood cells in various forms and stages of compensation of DM in women in the third trimester of pregnancy.

MATERIALS AND METHODS

A comparative characteristics of IBA in blood cells was carried out in 77 women with IDDM in the third trimester of pregnancy. The age of the patients ranged from 18 to 40, the mean age was

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TABLE 1. IBA of Blood Cells and Expression of Fc_γ Receptors in Pregnant Women with DM ($M \pm m$)

Parameter		Examined group			
		Control (physiological course of pregnancy)	Pregnant women with compensated and subcompensated IDDM	Pregnant women with decompensated IDDM	Pregnant women with GDM
Number of leukocytes, μl		8800±500	7330±700	6660±600*	7500±740
Number of lymphocytes:	%	24.5±2.34	19.14±3.14	24.2±1.8	21.0±2.4
	abs.	2026±166	1353±223*	1479±171*	1572±227
Number of Fc_γ,	%	30.2±1.96	31.31±2.13	34.5±1.2	31.1±1.69
	abs.	575.5±53	396.5±43*	478.9±71.1	393.3±72
Number of T_γ,	%	29.4±1.61	26.25±2.8	26.2±2.3	38.2±3.1*
	abs.	392.8±32.8	325±68.4	256.7±38.6*	293±62
Number of T_μ,	%	35.4±1.8	39±0.7	29.5±2.81	28.75±7.1*
	abs	285.6±27.2	505±60.45*	263.5±60	284.4±58.9
% of specific binding:	on MNC	29.75±2.42	35.47±1.94*	21.67±0.9*	35.55±1.74*
	on T_γ	18.1±2.6	14.9±1.98*	8.4±2.3	7.3±1.3*
	on T_μ	20.1±1.9	17.81±1.01*	24.85±2.07*	16.6±2.04*
	on Er	11.2±0.7	10.88±1.11	9.3±0.98*	9.2±1.3*
Glucose, mM		4.8±0.38	8.14±0.67*	11.77±0.89*	7.96±0.63*
Insulin resistance, μIU/ml		9.67±1.0	8.87±0.9	7.1±0.3*	–

Note. Here and in Table 2: * – $p<0.05$.

26±5.4 years; the history of the disease varied from 5 to 16 years. We studied insulin-receptor interactions in Er, mononuclear cells (MNC), and lymphocytes and their subfractions in pregnant women with IDDM who had a pronounced insulin resistance (daily dose of insulin more than 40 IU, 72 IU on average). At the time of examination diabetic shifts were compensated in 28 patients (group I), which manifested itself in normoglycemia after fasting and over 24 h and in the absence of glucosuria and ketoacidosis. Group II comprised 11 pregnant women with decompensated IDDM, and group III included 18 pregnant women with gestation diabetes mellitus (GDM). The control group consisted of 20 women in the third trimester of pregnancy without endocrine pathology.

The serum concentration of insulin was determined with radioimmunoassay kits; the methods of separation of the blood cells and determination of the number of IR in the plasma membranes of Er and MNC we described earlier [2]. Lymphocytes were isolated and the subpopulation of T lymphocytes was quantitatively characterized using the method of rosette formation [6]. Binding of ^{125}I-insulin to receptors on Er, MNC, lymphocytes, and their subfractions was assessed [15] using elimination of ^{125}I-insulin from hormone-receptor complex by increasing concentrations of unlabeled insulin under equilibrium conditions [9]. The total number of insulin-binding sites and their affinity were measured [12,16]. The sensitivity of the cells to insulin was evaluated by the amount of utilized glucose.

RESULTS

The comparison of the maximal specific binding of ^{125}I-insulin to lymphocytes and their subfractions, and to Er in pregnant women with compensated and decompensated IDDM and with GDM revealed reliable differences. As is seen from Table 1, IBA depends not only on the stage of compensation and on the form of diabetes, but also on the type of cells. For instance, in MNC binding of ^{125}I-insulin was increased in compensated DM and GDM, and decreased in decompensated DM ($p<0.05$). In Er specific binding of ^{125}I-insulin was reliably decreased in decompensated DM and GDM, while in compensated IDDM it differed little from that in the control group at the same stage of gestation.

These data suggest that the shifts of IBA in the same pathological states are not identical in different blood cells. This can probably be attributed to other causes. In particular, unlike other cells, Er have no nucleus and therefore they are unable to synthesize proteins, including the receptor protein. Er persist in the circulation for a longer time (120 days) than other blood cells. This probably explains the absence of complete identity in insulin binding by receptors on monocytes and erythrocytes under fast changing condi-

tions. Er are more "rigid" cells for manifestations of changes in insulin receptor activity and therefore they are mor suitable for studies of the receptors in more prolonged pathological and physiological states, in particular, in pregnancy. The receptor parameters are known to vary even within one cell population on different stages of differentiation (erythroblasts, reticulocytes, young and mature erythrocytes), a higher binding capacity being characteristic for less differentiated cells [4,8]. Therefore, the data on insulin receptors obtained on certain cells cannot be extrapolated to others, as is confirmed by our results presented in Table 1.

Lymphocytes represent a convenient model for study of insulin resistance, since they are target cells for insulin [11] and, like other blood cells, they lend themselves readily to clinical study. At the same time, the binding of insulin with receptors in lymphocytes reflects the immunoreactivity of the latter [7], which is of interest in immunodeficiency states. From this point of view, the study of IBA of T lymphocytes in pregnant women with IDDM merits special attention. Insulin receptors on T cells are thought to appear only in response to stimulation with mitogens, allogenic cells, etc. [1,10]. Intact T lymphocytes isolated from healthy subjects and from patients with DM are virtually unable to bind insulin. This, however, does not exclude the existence of certain forms of DM characterized by the functioning of activated T lymphocytes. It is possible that such forms can be detected within the T lymphocyte subpopulation, or that specific activation of T lymphocytes occurs at a certain stage of the disease, during a certain period, and in a certain physiological state of the organism. In view of all this, insulin-receptor interaction in T lymphocytes (as in all other cells) was studied in pregnant women in the third trimester both in health and in DM. It was found that in the third trimester lymphocytes possess a rather high IBA in both health and disease. The absolute number of lymphocytes was reduced only in IDDM ($p<0.05$), whereas the level of insulin binding in T lymphocytes was lowered in all stages and forms of DM and did not correlated with the degree of insulin resistance ($r=0.31$, $p>0.05$) and blood sugar ($r=0.24$), suggesting an immunologically active process.

Taking into account the cell heterogeneity and different insulin-binding capacity of MNC, in our next experiments we determined the activity of IR of T lymphocytes (Table 2). Of particular interest is the clear-cut distribution of the patients with IDDM into two opposite subgroups with high ($36.82\pm1.31\%$, $p<0.01$) and low ($9.86\pm1.44\%$, $p<0.001$) IBA of T lymphocytes. Pregnant women with low IBA of T lymphocytes were receiving insulin in high doses and this was probably responsible for the insulin resistance in decompensated patients and for the excess of insulin in compensated patients. This was primarily observed (in 8 cases of 9) in pregnant women with diabetic microangiopathy, regardless of the degree of compensation.

TABLE 2. IBA of MNC and T Lymphocytes in Blood of Pregnant Women with DM as a Function of the Degree of Compensation ($M\pm m$)

Group	IBA, %	
	MNC	T lymphocytes
Health ($n=11$)	34.02±3.94	24.50±2.53
IDDM ($n=25$)	23.50±2.61*	22.44±3.72
decompensated	26.64±2.90	24.85±4.77
compensated	20.28±4.09*	20.33±5.77
GDM	16.38±5.02*	16.63±3.44

The revealed differences in IBA of MNC and T lymphocytes are related to both the heterogeneity of the examined patients and the multifactor effect of hormonal and metabolic shifts caused by pregnancy, on the one hand, and DM on the other. As is seen from Table 1, the absolute number of T_γ lymphocytes in GDM drops from 392.8±32.8 to 293.2±61.6 cells ($p<0.05$). The number of T lymphocytes in decompensated IDDM decreases 2.5-fold. The content of T_μ cells rises just in compensated DM, while in other forms it does not differ from the norm. IBA of T_μ cells is reliably decreased in GDM and IDDM and reliably increased in decompensated DM in comparison with the control group. A close dependence is noted between IBA and the number of T_μ lymphocytes ($r=0.76$). The decreased IBA of T lymphocytes points to a defect on the receptor level, which may lead to a change of the immune response in DM. In compensated DM the number of T lymphocytes possessing Fc_γ-receptor decreased from 575.5±53 to 396.5±43 ($p<0.05$), while in other cases it did not differ from that in healthy subjects ($p<0.05$).

Thus, IBA of different lymphocyte populations differs in different forms and stages of DM; in decompensated IDDM and GDM a deficit of T suppressors against the background of a normal content of T helpers in peripheral blood of women in the third trimester of pregnancy. The imbalance was revealed during both the quantitative and functional (IBA) evaluation of T cells.

The comparison of IBA in different blood cells in health and in different forms of DM and in

different stages of compensation of IDDM revealed significant differences in the test parameters, and therefore the data obtained on one type of cells cannot be extrapolated to others. More stable changes were obtained on Er, and hence these cells are preferably used for the study of insulin-receptor interaction in DM in pregnancy. The study of insulin binding capacity in Er and lymphocytes is important not only for the monitoring of insulin therapy but also for the prevention of chronic overdose of the hormone in insulin resistance and for the identification of the "high risk group" among mothers and their children. Complete evaluation of quantitative parameters of peripheral lymphocytes, Fc_γ receptor expression, IBA of immunocompetent cells, and the interrelationship between these parameters will improve the diagnostics of the level and the degree of disturbances of T immunity in DM and broaden our knowledge of their pathogenetic role in various clinico-metabolic states.

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