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Lymphocyte subsets and cytokines in women with gestational diabetes mellitus and their newborn

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Abstract

This study aimed to identify potential immunological markers for predicting type 1 diabetes in patients with gestational diabetes mellitus (GDM) and any immunological impairment in their newborn. In 62 GDM patients and 74 women with normal glucose tolerance (NGT), and their babies, we assessed total lymphocytes, T lymphocyte subsets CD3 and CD8 expressing T cell receptor (TCR) alpha/beta or gamma/delta, CD16 and CD19, pancreatic autoantibodies and cytokines (IL-5, IL-2, soluble receptor IL-2). At delivery, umbilical cord blood samples were taken for lymphocyte subpopulations and cytokine measurements. GDM mothers had higher levels of total lymphocytes, CD8 expressing TCR gamma/delta, and lower levels of CD3 expressing TCR alpha/beta than NGT controls. Insulin-treated GDM mothers had lower CD4 and CD4/CD8 ratios, and higher CD8 and IL-5 than diet-treated GDM or controls. Five women were positive for pancreatic autoantibodies, with lower CD4 ($p < 0.01$) and CD4/CD8 ratios ($p < 0.05$), and higher CD8 ($p < 0.03$) and CD19 than GDM and control mothers negative for autoantibodies. GDM newborn had higher CD8 gamma/delta and lower CD16 than NGT babies. There were no significant differences in TNF-alpha concentrations in the cord blood obtained from the GDM and NGT newborn. In conclusion, GDM women and their newborn have lymphocyte subset impairments, which are more important in patients positive for autoantibodies and/or treated with insulin.

Keywords: Pregnancy; Diabetes; Lymphocyte subsets; Immunological impairment

1. Introduction

Gestational diabetes mellitus (GDM), defined as a carbohydrate intolerance of varying severity developing or first recognized during pregnancy, is the most frequent metabolic disorder of pregnancy, occurring in 1–10% of all pregnancies [1]. GDM is considered a heterogeneous disease, the pathogenesis of which has yet to be completely clarified [2–4]. It is also characterized by a high incidence of diabetes developing after pregnancy, with an incidence ranging from 6 to 62%, depending on the population examined and the length of the follow-up considered [5–8].

Abbreviations: BMI, body mass index; CV, coefficient of variation; FPG, fasting plasma glucose; FITC, fluorescein isothiocyanate; GDM, gestational diabetes mellitus; GLM, general linear model; GCT, glucose challenge test; GADA Abs, glutamic acid decarboxylase autoantibodies; gw, gestational weeks; HPLC, high-pressure liquid chromatography; HbA1c, glycated hemoglobin; IA_2 Abs, tyrosine-phosphatase autoantibodies; ICA, islet cell autoantibodies; IL, interleukins; LADA, latent autoimmune diabetes in adults; LGA, large for gestational age; NK, natural killer; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PPPG, post-prandial plasma glucose; PE, phycoerythrin; RIA, radioimmunoassay; TCR, T cell receptor; TNF-alpha, tumor necrosis factor.

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The thymus-dependent immune system is involved in the disease processes underlying type 1 diabetes. Several studies have shown that the distribution of peripheral T lymphocyte subsets is impaired in diabetic patients, both at diagnosis and during the course of the disease [9,10].

In this context, studies have demonstrated an increase in T lymphocytes expressing T cell receptor gamma/delta in the pancreatic tissues and peripheral blood of type 1 diabetic subjects, suggesting their involvement in the insulitis process [11].

Activated T lymphocytes release several lymphokines, which facilitate their proliferation and differentiation into effector cells and enhance their activity [12–14]. IL-2 is required for T cell growth and the activation of natural killer cells. IL-5 is expressed by several cells, including eosinophils, NK cells, TC2 CD8 T cells, gamma/delta T cells and IL-1b-activated endothelial cells. In B-cells, IL-5 induces the differentiation of activated cells into Ig-secreting cells; in T cells, it triggers the differentiation of thymocytes into cytotoxic lymphocytes.

In a previous study, we showed an increase in T cell receptor gamma/delta in GDM patients, suggesting its involvement in the subsequent occurrence of diabetes in GDM, but the study involved only a small number of women [15].

Hence our decision was to evaluate the pattern of lymphocyte subpopulations in a larger number of GDM mothers and their newborn in order to confirm a TCR rearrangement and ascertain whether it increases the risk of type 1 diabetes after pregnancy. We also aimed to establish whether TCR rearrangement expresses an immunologically impaired pattern in fetal life. Given the potential role of interleukins in the onset of diabetes and their possible effects on the fetus, we evaluated IL-2, IL-2 soluble receptor, and IL-5 in both mothers and newborn. Finally, since TNF-alpha is a potent NK cell modulator, we evaluated its levels in the newborn [16,17].

2. Materials and methods

2.1. Subjects

We evaluated 136 pregnant Caucasian women, 62 with GDM and 74 with a normal glucose tolerance (NGT).

The GDM women were selected by a screening and diagnostic program, described elsewhere [18]. Briefly, GDM was diagnosed according to Carpenter and Coustan's criteria, after screening with the Glucose Challenge Test (GCT) at 24–28 weeks of pregnancy.

GDM patients were put on a diet and their fasting (FPG) and 1 h post-prandial plasma glucose (PPPG) levels were monitored. Insulin treatment was started if FPG and/or PPPG were higher than 95 and 140 mg/dl, respectively. Metabolic and obstetric monitoring was continued on all patients until delivery. The women generally delivered at term unless obstetric complications set in.

The maternal parameters evaluated were: family history of diabetes (first-degree relatives), age, pre-pregnancy BMI, blood pressure, timing and mode of delivery and morbidity (pre-eclampsia). In the third trimester of pregnancy (28–34 gestational weeks [gw]), several metabolic parameters (FPG, HbA1c), fasting insulin, autoantibodies (ICA, GAD Abs, IA2 Abs) and microalbuminuria and/or proteinuria (24-h urine collection) were evaluated in all subjects. At the same time, lymphocyte subpopulations (CD3, CD4, CD8 as total and percentage expressing TCR alpha/beta or gamma/delta, CD16, CD19) and interleukins (IL-2, IL-5, soluble receptor RsIL-2) were assessed in all GDM patients and in 36 randomly selected NGT women.

Fetal parameters – length, weight, Apgar score and morbidity (hypoglycemia, fetal distress, neonatal asphyxia, hypocalcemia, hyperbilirubinemia, polycythemia) – were recorded. The newborn were considered large for gestational age (LGA) when their birth weight was $>90^{\circ}$ percentile, on the basis of standard growth and development parameters for our population (provided by the Department of Pediatrics, University of Padova). In addition, a blood sample was taken from the umbilical cord at delivery to evaluate lymphocyte subpopulations, interleukins and TNF-alpha.

The NGT pregnant women had no GDM risk factors (no family history of diabetes in first-degree relatives; pre-pregnancy BMI below 28; age under 35 years; no previous impaired glucose tolerance or gestational diabetes; no previous macrosomia or LGA babies, unexplained stillbirths, persistent glycosuria or asymmetric fetal growth in the current pregnancy) and they all had normal screening results at the GCT followed by normal OGTT results.

None of the women had any infections, drug use, or other factors capable of influencing the immune system.

The study was approved by the local Ethics Committee and conducted in accordance with the ethical standards of the Helsinki Declaration. All patients gave their informed consent.

2.2. Laboratory methods

2.2.1. Metabolic parameters

Plasma glucose was measured using a glucose-oxidase method [19]; inter- and intra-assay coefficients of variation (CV) were 1.10% and 0.5%, respectively.

HbA1c was assayed by HPLC [20]; inter- and intra-assay CVs were 1.11% and 0.42%, respectively.