Monocyte Adhesion to Decidual Endothelial Cells Is Increased in Pregnancies Complicated by Type 1 Diabetes but not by Gestational Diabetes

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Type 1 diabetes complicates ~1 of every 200 pregnancies and gestational diabetes a further 2–3% of pregnancies (1). Systemic atherosomatous vascular disease can develop or accelerate during diabetic pregnancy. We and others (2) have observed similar vascular lesions in placental bed vessels (Fig. 1A) associated with impaired placental function and fetal growth.

Genesis of atheroma involves adherence of peripheral blood monocytes to endothelium (3–5). To determine whether a similar process underlies the placental bed vasculopathy of diabetes, we examined cell adhesion in an in vitro coculture system using decidual endothelial cells from normal pregnancies and monocytes from both normal and diabetic pregnancies.

RESEARCH DESIGN AND METHODS — This study was approved by the Human Research Ethics Committee of Royal North Shore Hospital. All subjects gave written informed consent.

Blood for monocyte isolation was collected from three groups of women, i.e., normal (control), type 1 diabetes (confirmed prepregnancy by a history of diabetic ketoacidosis and/or presence of anti-GAD antibodies), and gestational diabetes (n = 6 in each group) groups, in the third trimester of pregnancy.

Endothelial cells were isolated from decidual biopsies collected from a separate group of nine healthy pregnant women at elective lower-segment caesarean section (6). Biopsies were diced, and after enzyme digestion, cells were purified by positive selection with lectin ulex europeus 1 (UEA1; Sigma, St. Louis, MO), giving a population of endothelial cells of >95% purity. Cells were pooled at passage three, seeded (1.2 × 10^5 cells/well) onto collagen type IV–coated coverslips (Sigma; 10% vol/vol), and allowed to adhere for 5 h. Monocytes were isolated from venous blood from the three study groups using density gradient centrifugation (7), which avoids artifactual activation encountered in monocytes isolated by methods of adhesion (8). As assessed by CD68 positivity and CD3 negativity, the purity of the monocyte preparations was >90% in all cases.

Monocyte adhesion to decidual endothelium

Monocytes from all subjects were seeded (2.5 × 10^5 cells/well) onto the decidual endothelial cell monolayer in 2% FCS in medium 199. After duplicate 1-h incubations, nonadherent cells were removed by gentle washing. Remaining adherent cells, fixed in 4% paraformaldehyde, were stored at 4°C until analysis. Monocytes were identified with a mouse anti-human CD68 monoclonal antibody (clone EBM11, Dako, Glostrup, Denmark) and the endothelial monolayer visualized by counter-staining with hematoxylin. Cells in five random sections on each coverslip were counted by two independent observers blinded to donor monocyte source.

Statistical methods

Data are presented as means ± SE. Between-group comparisons were made using unpaired Student’s t tests.

RESULTS — There was no statistically significant difference in gestational age among patient groups (data not shown) and no correlation between gestational age and level of monocyte adhesion in any group (control r^2 = 0.181, P = 0.431; type 1 diabetes r^2 = 0.208, P = 0.394; and gestational diabetes r^2 = 0.001, P = 0.956). All subjects had normal renal function and blood pressure. There was significant elevation of HbaA_1c levels in the type 1 diabetic group (7.0 ± 0.53%; P < 0.01) compared with the gestational diabetic group (4.7 ± 0.13%).

Adhesion of monocytes to decidual endothelium

Figure 1B shows representative immuno-cytochemical stains of monocytes and decidual endothelial cells in coculture and group data for the three groups. A significantly greater proportion of monocytes from women with type 1 diabetes adhered to the endothelial monolayer compared with that from normal pregnant women (P < 0.05). There was no significant difference from normal in the level of adhe-
sion of monocytes from women with gestational diabetes.

CONCLUSIONS — The placental vascular bed is essential for the supply of nutrients to the feto-placental unit. The function of this vascular bed is seriously impaired by development of the atheroma-like vascular lesions seen in diabetic pregnancy. These lesions have been well described in the literature and have been shown to be most severe in those with poor glycemic control (9). Representative biopsies from a normal and a diabetic woman, each delivered by caesarean section in our unit, are shown in Fig. 1 A. Since the majority of both normal and diabetic subjects in the current study delivered vaginally, placental bed biopsies were not able to be systematically collected.

Peripheral blood monocytes are preferentially recruited to the arterial intima early in the development of atheroma (3). They adhere to endothelium, migrate to the subendothelial space, and are transformed into lipid-laden foamy macrophages. They release proinflammatory cytokines, causing further endothelial activation and damage, further monocyte chemo-atraction, and acceleration of the vascular lesion (10).

We have described here, for the first time, increased adhesion to decidual endothelium of monocytes from pregnant women with type 1 but not gestational diabetes. Such an increase could be the initial step in the development of placental vascular disease. Differences were not explicable on the basis of gestational age or ambient blood glucose levels, which were within the normal range at the time of blood collection in both diabetic groups. They may nevertheless have been related to duration and adequacy of glycemic control, as HbA1c levels were higher in the type 1 diabetic group.

Using this novel model, future work can investigate mechanisms by which this increased adhesiveness to placental bed endothelium occurs in diabetic pregnancy and its significance for monocyte migration to subendothelial space, initiation of inflammation, and reduction of placental blood flow.

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References

Figure 1—A: Placental bed biopsy (H&E [hematoxylin-eosin], CD68-stained normal and diabetic patients). The atheromatous vessel in the diabetic subject is totally occluded, with marked perivascular infiltration by lipid-laden CD68+ macrophages. B: Adhesion of peripheral blood monocytes from normal, type 1 diabetic, and gestational diabetic pregnant women to a normal decidual endothelial cell monolayer. A representative section stained with CD68 (monocytes), counterstained with hematoxylin (endothelial cells), is shown with group data (mean ± SE, n = 6 in each group) below.