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INTRODUCTION

Recurrent miscarriage (RM) is a common clinical reproductive problem occurring in approximately 1–2% of pregnancies [1,2] and is defined as three or more consecutive spontaneous miscarriages prior to 20 weeks' gestation.

Although genetic, anatomical and hormonal factors have been implicated in the etiology of recurrent miscarriage, more than 60% of cases remain unexplained.

Investigations are currently focused on mediators of immune activation and subsequent inflammatory and thrombotic tissue injuries at the maternal–fetal interface.

The fetus is considered a semi-allograft because of the genetic contribution from the father, resulting in a disturbance in alloimmune factors in the placental environment. Therefore, the immunological rejection of the fetus could be a consequence of a breakdown in the mechanisms that normally prevent the maternal immune system from becoming activated by the paternal antigens expressed in the developing fetus.

Thomas Wegmann, who proposed the original immunotrophic hypothesis in 1984, focused his attention on the role of cytokines in the feto-maternal relationship [6] and proposed that successful pregnancy may depend, at least in part, on the maternal immune response shifting away from cell-mediated inflammatory Th1 type responses toward a Th2 phenotype with humoral responses [7].

The hypothesis regarding the Th1 and Th2 cytokine responses in recurrent miscarriage is probably too simplistic and rigid. Therefore, it is important to investigate the overall role of cytokines at the maternal–fetal interface.

Cytokines are polypeptides involved in the control of the local and systemic events that make up the immune response: inflammatory reactions, healing, and hematopoiesis. Our group has already reported that in trophoblastic tissue from recurrent miscarriage patients, compared with tissue from women undergoing elective termination of a pregnancy, there is a reduction in the thrombomodulin expression levels [8], which is involved in the thrombotic aspects of recurrent miscarriage, and the Alpha Hemoglobin Stabilizing Protein [9], which is related to the hypothetical hypoxic aspects of recurrent miscarriage, is downregulated.

The objective of this study was to elucidate the role of inflammatory processes involved in alloimmune activation at the placental interface in the pathophysiology of recurrent miscarriage. In particular, we investigated the expression pattern of inflammatory cytokines and their receptors using cDNA microarray technology.

To confirm the microarray analysis results and to examine the cytokine expression levels more quantitatively, we also performed a real-time PCR assay, and we identified a number of differentially expressed cytokines that may be involved in the pathophysiology of unexplained recurrent miscarriage.

RESULTS

To explore whether tissue microarray data can be used to identify new molecules involved in the occurrence of recurrent miscarriage, we first compared the expression of 96 genes involved in the inflammatory response in the trophoblastic tissue from women who experienced recurrent miscarriages and in women who underwent elective termination of pregnancy. A commercially available filter array designed for exploring changes in the expression of inflammatory cytokines and their receptors was used.

Side-by-side hybridization with cDNA probes prepared from RNA isolated from trophoblastic tissues was evaluated, and the expression profiles of these genes were determined. Fig. 1 shows that the expression levels of 18 genes were significantly different between the two groups; specifically, 4 genes were upregulated and 14 were downregulated in the RM group compared with elective termination group. To investigate those differences, quantitative real-time PCR was performed. Differential gene expression measurements (RM vs. controls) revealed significant downregulation of TGF- β 3 and IL-25 (5-fold and 2.5-fold reductions, respectively), while the expression of CD-25 was significantly upregulated (7-fold increase). The relative gene expression levels (RM/controls) are depicted in Fig. 2. Table 1 shows the results expressed as the median and interquartile range of Δ Ct. The median Δ Ct of TGF- β 3 was 8.2 (interquartile range, 7.67–8.9) in the recurrent miscarriage group compared with 5.85 (interquartile range, 5.3–6.09) in the elective termination group; the median Δ Ct of IL-25 was 5.18 (interquartile range 4.46–5.76) in the recurrent miscarriage group compared with 3.85 (interquartile range 3.6–4.51) in the elective termination group; and the median of Δ Ct of CD-25 was 9.62 (interquartile range 7.81–12.42) in the recurrent miscarriage group compared with 12.44 (interquartile range 11.02–13.86) in the elective termination group.

TABLE 1

Differential gene expression measurements performed by quantitative real-time PCR.

	TGF- β 3	IL-25	CD-25
RM	8.2	5.18	9.62
1°–3° quartile	7.67–8.9	4.46–5.76	7.81–12.42
Controls	5.85	3.85	12.44
1°–3° quartile	5.3–6.09	3.6–4.51	11.02–13.86
Mann–Whitney test p value	0.0001	0.0001	0.036

All values are expressed as median Δ Ct, and first and third quartile.

FIGURE 2

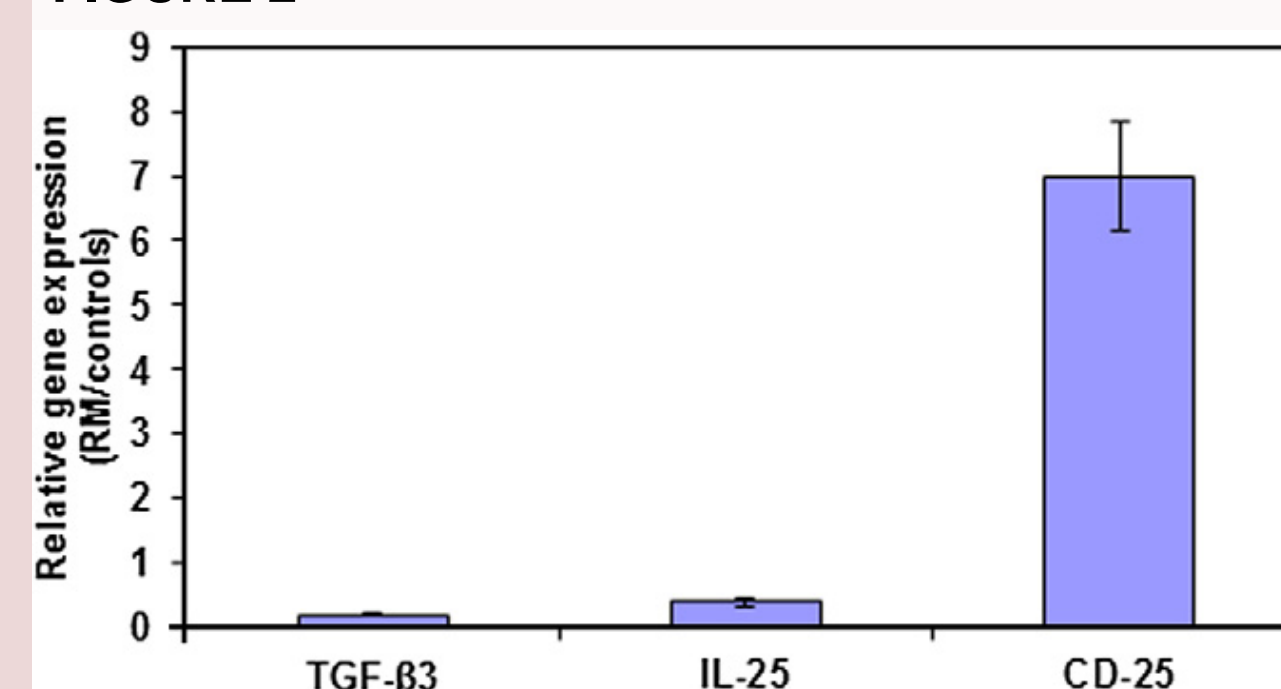
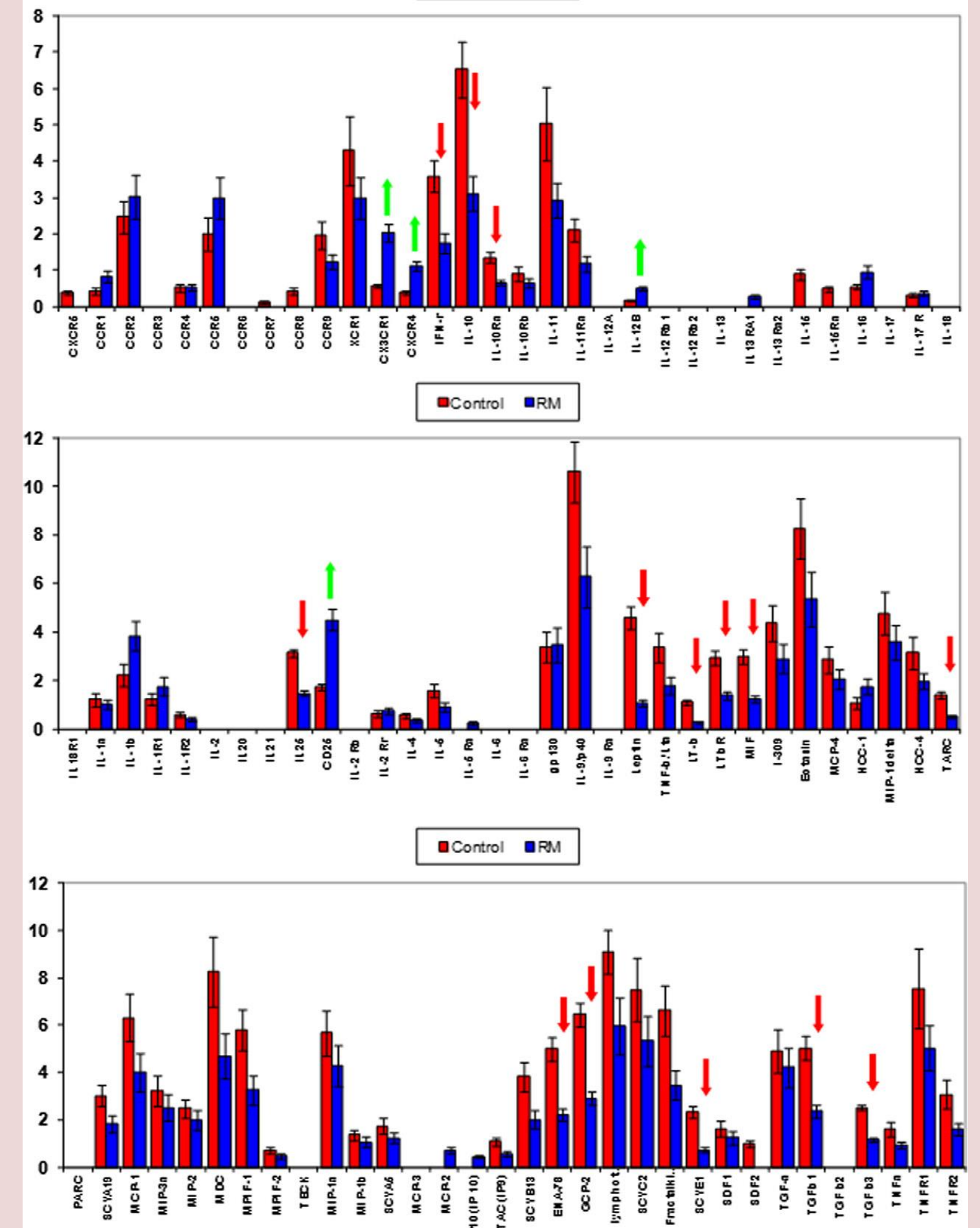
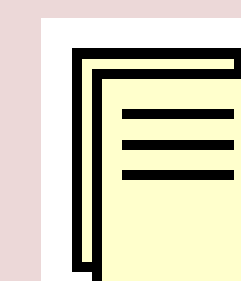


FIGURE 1



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DISCUSSION

Recurrent miscarriage is often an unexplained problem, and many efforts have been made by researchers to provide information that can aid in clinical practice. The immunological aspect has been already investigated, but very often studies analyze the serum levels of single cytokines chosen based on previous studies or a hypothetical pathogenetic mechanism.

Our study evaluated local, rather than systemic, immunological and inflammatory aspects of recurrent miscarriage by analyzing the role of inflammatory cytokines.

Cytokine activation is not widespread, but a cytokine imbalance could be responsible for a fatal alteration of the placental environment.

The results obtained from the analysis of three specific cytokines allowed us to confirm at the maternal–fetal interface Thomas Wegmann's assumptions about the immunotrophic theory; we observed a reduction in the levels of cytokines belonging to the Th2 profile (TGF- β 3 and IL-25) and an increase in the level of CD-25, which belongs to the Th1 profile. In previous studies, our group has evaluated other aspects of the same disease, in particular, the thrombotic–hemostatic and hypoxic aspects, in an attempt to use a more complete approach.

Our intent has been to study the global alterations of the main aspects involved in recurrent miscarriage, which are the inflammatory and immunological patterns, the anticoagulant–procoagulant system and the hypoxic environment.

All of these aspects represent primary pathophysiological mechanisms, likely shared by other obstetric pathologies such as preeclampsia, HELLP syndrome, IUFD and IUGR, all of which are associated with impaired placentation.



*Di chi sarà il mondo di domani?
Di chi oggi canta in coro.*

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IL Di.S.C.O. SI RACCONTA