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Huvec: an Experimental Model to Test the Hypothesis that Infection is the Leading Cause of Preeclampsia

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Introduction

During normal pregnancy, endothelial nitric oxide synthase (eNOS) is believed to play an important role in lowering peripheral vascular resistance (Silacci et al. 2000), while in preeclampsia (PE), characterised by pregnancy induced hypertension and proteinuria, there is a lack of vasodilation probably due to a reduced eNOS expression (Seligman et al. 1996). In PE we have found serological evidence of infection (high WBC count, high ferritin and low transferrin) (Todros et al. 2000) and several Authors observed an alteration of other markers of an infectious status (i.e: neopterin and C-reactive protein) (Haeger et al. 1992; Teran et al. 2001). Our objective was to test in vitro the hypothesis that infection negatively affects eNOS expression.

Methods

Human umbilical vein endothelial cells (HUVEC) were obtained from umbilical cords of normal term pregnancies, immediately after delivery. HUVEC were isolated by a collagenase dispersion method within 24 hours after delivery. Confluent HUVEC monolayers were treated for 72 and 144 hours with the bacterial endotoxin lipopolysaccharide (LPS) (1 µg/ml) and the proinflammatory cytokine TNF-α (10 ng/ml). The protein expression of eNOS was evaluated with immunoblot assay after SDS-PAGE electrophoresis and after a Low-Temperature (LT) SDS-PAGE of HUVEC lysates, while eNOS mRNA was examined with semiquantitative RT-PCR.

Results

SDS-PAGE shows a progressive decrease of eNOS protein expression but only the LT SDS-PAGE can discriminate the potentially active dimer. The monomer of eNOS is already decreased after 72h of LPS or TNF-α treatment, while the dimer changes only with both stimuli. After 144h LPS or TNF-α stimulation, eNOS dimer begins to decrease, while monomer is already fading and disappeares with LPS plus TNF-α (Fig.1). The mRNA of eNOS is reduced with LPS+TNF-α after 72h or with the single stimulus after 144h; it disappears after 144h treatment with both stimuli (Fig. 2).

Conclusions

Our data showed that either inflammation and infection decrease mRNA expression and consequently protein expression of eNOS; while dimerization of the protein is not influenced by these stimuli, indeed the dimeric protein seems to diminish only when the monomeric protein amount is not any longer sufficient for the formation of the dimer.
The inflammatory stimuli, such as TNF-α, but overall infective ones, such as LPS plus TNF-α, can lead to a relatively long term decrease of eNOS expression. Our findings suggest that an host inflammatory response to an infection could be the trigger for the appearance of symptoms in preeclamptic patients. If our data will be confirmed by further studies new perspectives in the prevention and therapy of preeclampsia will be opened.

Acknowledgements

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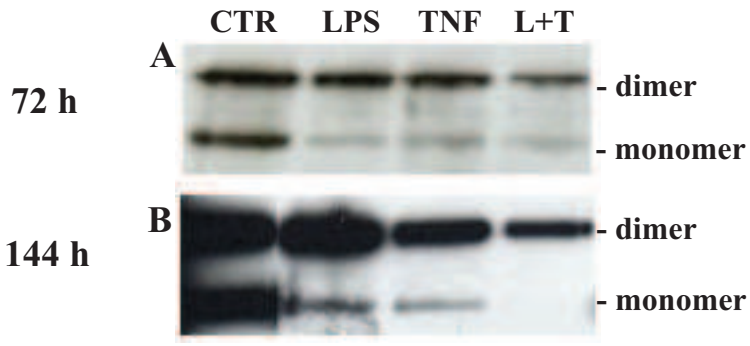

Fig. 1. LT SDS-PAGE analysis of eNOS monomer and dimer expression in of HUVEC not stimulated (CTR) or incubated with LPS or TNF alpha, alone or in combination (L+T), for 72h (panel A) or 144 h (panel B). Results are representative of a set of 5 indipendent experiments.

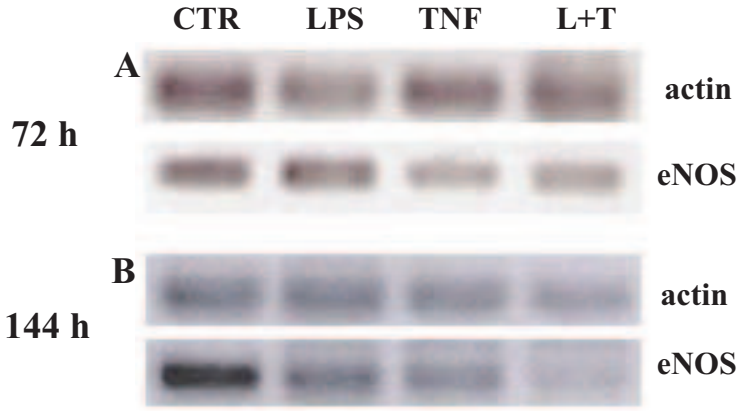

Fig. 2. Expression of eNOS and b-actin mRNA as detected by semiquantitative RT-PCR analysis. HUVEC were incubated as before, and the results are representative of a set of 5 indipendent experiments.