NUCLEAR FACTOR κB DOWNREGULATION IN HUMAN T-CELLS IS ESSENTIAL FOR THE MAINTENANCE OF THE CYTOKINE PROFILE REQUIRED FOR PREGNANCY SUCCESS

S. A. McCracken, E. D.M. Gallery, J. M. Morris

Perinatal Research, Kolling Institute of Medical Research, St Leonards, NSW, Australia

Human pregnancy is associated with a shift away from Th-1 type and a bias towards Th-2 type immune responses. The molecular mechanisms that regulate this are unknown. We assessed the expression and activity of Nuclear Factor (NF)-κB, a transcription factor that plays a central role in regulating immune responses. Nuclear and cytoplasmic fractions were prepared from isolated T-cells from non-pregnant and pregnant females and subjected to Western blotting to assess NF-κB and its' inhibitors IκBα and β expression. NF-κB activity in nuclear extracts was determined by Electrophoretic Mobility Shift Assays. Isolated T-cells were pre-incubated with/without the NF-κB translocation inhibitor SN50 and subsequently stimulated with PMA/ionomycin in the presence of the protein transport inhibitor Brefeldin A. Cytokine production was determined using flow cytometry.

Our results demonstrated high levels of immunoreactive NF-κB (p65) in all nuclear fractions of T-cells from non-pregnant females. In contrast, low levels of p65 were detected in nuclear fractions of T-cells from pregnant females. Levels of IκBα and β were also higher in cytoplasmic fractions of T-cells from non-pregnant than from pregnant females. The reduction in p65 levels in pregnancy was consistent with reduced levels of active NF-κB in T-cells from pregnant relative to non-pregnant females. Stimulation of T-cells from non-pregnant females with PMA/ionomycin resulted in IκBα degradation, p65 translocation and subsequent production of Th-1 cytokines IFN-γ and IL-2. In contrast, PMA stimulation had no effect on NF-κB activity in T-cells from pregnant females and a reduced effect on IFN-γ and IL-2 production. In the presence of SN50, IFN-γ and IL-2 production by T-cells from non-pregnant females was attenuated demonstrating a specific role for NF-κB in the production of these Th-1 cytokines. We can therefore conclude that, specific down-regulation of NF-κB in T-cells in pregnancy is an essential requirement for maintaining the cytokine profile necessary for pregnancy success.

10.1071/SRB04Ab286