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

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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 IκBβ 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 IκBβ 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.