Abstract

Allergic diseases belong to one of the most common diseases and their incidence is increasing worldwide, especially among children and adolescents. The development of an allergic disorder occurs already in the early postnatal period and several factors have been described that can predispose the newborn to an allergic disease. However, the mechanism of allergy development has not yet been fully elucidated. The important immune cells involved in this mechanism are regulatory T cells (Treg). Their function is the formation of central tolerance to autoantigens and peripheral tolerance towards harmless antigens present in the external environment, including allergens.

The aim of this work is to compare the proportion and functional properties of Treg in children of healthy mothers (children with low risk of allergic disease development) and children of allergic mothers (children at high risk of allergic disease development). Both the total Treg population and individual subpopulations (natural (n) Treg and induced (i)Treg) in the whole umbilical cord blood of both groups of children were monitored. To assess the functional properties, Treg were isolated from cord blood by magnetic separation and subsequently co-cultured with CD4+CD25-T cells or cord blood mononuclear cells (CBMC) stained with carboxyfluorescein succinimidyl ester (CFSE), polyclonally stimulated with phytohemagglutinin and cultured for 3 days. Cell culture supernatants from coculture of Treg with CD4+CD25- T lymphocytes or CBMC were harvested to determine production of regulatory cytokines interleukin (IL) 10 and transforming growth factor beta (TGF-β). From the isolated Treg RNA was extracted for subsequent detection of the gene expression of the regulatory cytokines IL-10, TGF-β and IL-35.

There was no significant difference in the frequency of Treg defined based on expression of cell surface markers CD4, CD25 and intracellular expression of FoxP3 between children of healthy and allergic mothers. No significant difference was observed either in the representation of nTreg and iTreg determined based on the intracellular expression of FoxP3 and Helios in the cord blood of children of healthy and allergic mothers. An immunosuppressive assay revealed a higher proliferation capacity of both CBMC and CD4+ T cells of children of allergic mothers but suppressive properties od Treg of cord blood children of healthy and allergic mothers were not observed. IL-10 production in supernatant of co-culture of Treg with effector cells was lower in children

of allergic mothers compared to children of healthy mothers. No significant difference in the gene expression of regulatory cytokines in Treg of cord blood between children of healthy and allergic mothers was observed.

Higher effector cell proliferation capacity and lower IL-10 production in the Treg cell culture supernatant demonstrate decreased Treg immunoregulatory properties in the cord blood of children of allergic mothers that may contribute to the easier development of undesirable immune responses to relatively harmless environmental antigens (allergens) resulting in easier allergy development in these children. The suppressive properties of Treg in the cord blood of healthy and allergic mothers have not been proven. This finding could indicate a general immaturity of the newborn's immune system compared to the immune system of adults.

Key words: regulatory T cells, Treg, allergic diseases, cord blood, FoxP3, Helios, IL-10, TGF-β, immunosuppressive essay