Abstract

Cytokines as primary modulators of immune system cells play a key role in their development, maintenance and activity of each cell population. Cytokine profile of peripheral blood mononuclear cells thus reflects the immunopathological events involved in pathogenesis of the disease. **Focus of my thesis was cytokine dysbalance in several immunopathological disorders (type 1 diabetes mellitus, autoimmune thyroiditis, allergic colitis) with the aim to find distinctive cytokine profile of each disorder and to point out common features and differences in these disorders.**

Protein array and enzyme-linked immunosorbent assay (ELISA) method were used for analysis of cytokines in breast milk and cytokines produced *in vitro* by peripheral and cord blood mononuclear cells spontaneously and after stimulation by autoantigens.

First immunological disorder to study was type 1 diabetes mellitus (T1D), where patients with T1D were studied (n = 10) as well as their first degree relatives (n = 9), with particular group of neonates whose one parent suffered from T1D (n = 52). In patients with T1D prediabetic phase was dominated by spontaneous and postimulatory production of Th1-associated cytokines (IFN-γ, TNF-β) which dropped at the time of T1D manifestation and remained suppressed for at least two following years. Immunological profile of newly diagnosed patients with T1D was distinguished by dominant production of Th3/Tr1-associated cytokines (TGF-β and IL-10) and simultaneously a marked production of proinflammatory cytokines (TNF-α and IL-6) and chemokines (MCP-1 and MIG). Exclusively high risk relatives in prediabetic phase were able to induce Th2 type immunological response by increased production of IL-5 after nonspecific stimulation by mitogen (phytohemagglutinin). Immunological response of cord blood mononuclear cells is influenced by hyperglycaemia of the mother with T1D along with her autoimmune environment and this might lead to lower occurrence of T1D in the child. Th1-like immunological response was seen in children of diabetic father and in children of diabetic mother with perfect compensation of their diabetes.

Infectious pathogens might play role in ethiopathogenesis of autoimmune disorders. Mechanisms of immune regulation should avert a chronic infection but at the same time prevent from excessive aggressivity which might lead to pathological autoreactivity. However, antigens of infectious pathogens might induce autoreactivity as was demonstrated on production of chemokines and inflammatory cytokines (IL-6) in patients with autoimmune thyroiditis (AT) after stimulation with specific antigens of bacterium *Helicobacter pylori* (47 patients with AT vs. 17 controls).

Cytokines in breast milk are important biologically active factors which often compensate for missing immune mechanisms of the neonate and influence the developing immune system of the breastfed infant. Concentrations of cytokines in breast milk show great interindividual variability and cytokine dysbalance might contribute to the development of immunopathological disorders in infant. Significantly higher concentration of Th1 cytokine IFN-γ together with lower concentration of regulatory cytokine TGF-β was found in breast milk of mothers whose infants developed allergic colitis (n = 20) compared to healthy infants (n = 20). Adiponectin, leptin and AFABP, otherwise known as regulatory hormones of food intake and glucose and lipid metabolism, are candidate cytokines investigated as possible

factors involved in nutritional programming of infants. These cytokines were detectable in breast milk until 12 months after delivery. Borderline positive correlation of adiponectin in breast milk at 6 months after delivery and weight gain of infant during the first year was found in our study. Adipophilin which is the key factor in production and excretion of lipid droplets into breast milk showed no correlation with nutritional factors of mothers nor infants. In summary, it is concluded that there was a persistent proinflammatory tendency of immunological reactions common for all studied autoimmune disorders. In case of breast milk cytokine analysis it was confirmed that there is apparent association between cytokine spectrum of breast milk and the presence of immunopathological disorder (allergic colitis) in infant.

1. Úvod

1.1. Cytokiny

Cytokiny jsou proteiny podobné hormonům, které hrají integrální roli v regulaci imunitní odpovědi – její iniciaci, udržování a následné downregulaci. Mohou působit autokrinně na buňky, které je produkují, parakrinně na přilehlé buňky, a některé i endokrinně ovlivňují chování vzdálených buněk po vstupu do krevního oběhu. Cytokiny jsou převážně solubilní molekuly, zároveň však existují i membránové formy. Typickým rysem cytokinů je pleiotropní účinek (působení na několik různých druhů buněk), působení v kaskádě (jeden cytokin indukuje tvorbu druhého) a redundance (jednotlivé cytokiny mohou být nahrazeny jinými). Koordinovaným působením různých cytokinů se synergickou nebo antagonickou aktivitou vzniká tzv. cytokinová síť. Cytokiny mohou mít velice široké spektrum účinku včetně účinku na buněčný růst, buněčnou diferenciaci, cytolytickou aktivitu efektorových buněk, apoptózu a chemotaxi. Mezi cytokiny všeobecně řadíme interleukiny, chemokiny, interferony, transformující růstové faktory, faktory stimulující kolonie, faktory nekrotizující nádory a jiné růstové faktory.

1.2. Cytokinová polarizace a současný pohled na dělení Th lymfocytů

Cytokiny hrají zásadní roli v diferenciaci pomocných T buněk, při které se z naivních CD4+ T buněk (Th0) stávají efektorové T buňky. Po aktivaci zprostředkované přes receptor TCR se mohou naivní (prekurzorové) Th0 buňky diferencovat do několika hlavních efektorových linií (Th1, Th2, Th17, Th9, Th22, Tfh a iTreg) podílejících se na různých typech imunitní odpovědi. Každá linie efektorových Th buněk exprimuje specifické transkripční faktory a produkuje specifický profil cytokinů, jež určují jejich efektorové funkce. Důležitým pravidlem diferenciace Th buněk je, že jeden z charakteristických cytokinů produkovaný každou diferencovanou efektorovou buňkou je současně hlavním induktorem své buněčné linie, čímž vytváří pozitivní zpětnou vazbu. Mezi tyto cytokiny s efektem pozitivní zpětné vazby patří IFN-γ pro Th1, IL-4 pro Th2, IL-21 pro Th17 a TGF-β pro iTregs. V podmínkách *in vivo* však rozhoduje o diferenciaci Th buněk složitá cytokinová síť.