5.0.14

Exploring signaling events surrounding extracellular amastigote invasion processes of *Trypanosoma cruzi*

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Trypanosoma cruzi is a protozoan pathogen that infects humans and other mammals, producing a pathology called Chagas disease. The disease is endemic in most of central and South America affecting ~18 million people, with an increasing number of cases in North America. The parasite's life cycle alternates between vertebrates and insects and comprises distinct developmental stages. Amastigotes which are generated by the extracellular differentiation of trypomastigotes are referred to as Extracellular Amastigotes (EA) and are able to invade cultured cells. EA of the G strain promptly aggregate actin filaments by attaching to dorsal microvilli of HeLa cells and, as a result, cup-like structures are formed underneath the parasite. EA is therefore dependent on host actin filaments polymerization to invade cells. EA invasion can be easily detected by several techniques, such as freeze-fracture replicas of recently infected HeLa cells. However, signaling events surrounding these processes are still obscure. In the present study, we aim to examine these events and EA invasion features by focusing on the following molecules: cortactin, ezrin, Protein Kinase D (PKD) and a set of kinase inhibitors. Cortactin has emerged as a key signaling protein in cellular processes such as endocytosis and tumor invasion. The ability of cortactin to interact with and alter the cortical actin network is central to its role in regulating these processes. Ezrin is characterized by an N-terminal FERM domain and a C-terminal actin-binding domain. Once activated, ezrin dissociates and acts as a plasma membrane-cytoskeletal linker and thereby affects a variety of cellular activities, such as actin cytoskeleton regulation, control of cell shape, cellular adherence and migration and the modulation of intracellular signaling pathways. It has been recently demonstrated that cortactin is a substrate of PKD phosphorylation *in vivo* and it also colocalizes with ezrin. Hela, Vero and CHO cells were transfected with the following markers — cortactin, ezrin, RhoA and PKD GFP-vectors — infected with EA and examined for the acquisition of these markers. Cells were previously treated with kinase (PKC, MAP kinase, Src, PI3K and Rho) inhibitors and control cells were left untreated. Several initial assays have yielded encouraging results. For example, PKD is recruited to sites of actin remodeling at the leading edge of EA invasion, which also recruits cortactin. Colocalization of PKD and cortactin may be an indication that PKD plays a role in cytoskeletal reorganization. PKC—PKD signaling cascade is crucial to PKD function in cells. PI3K seems to interfere with neither ezrin nor cortactin recruitment. Here, we propose a signaling pathway model of EA entry. This pathway includes the above-mentioned kinases as upstream molecules.

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5.0.15

Investigation of differences in intestinal microbial composition between breast-fed and infant prebiotic formula-fed infants

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Breast-feeding is the best way of feeding a newborn baby. The kind of delivery and feeding have important influence on the composition of the intestinal flora of newborns.

The aim of this study was investigation of possible differences between the composition of gut microbiota of breast-fed and formula (supplemented with prebiotic)-fed newborns (younger than six months).

Healthy, term born infants enrolled in a four-week study and were divided in two groups — prebiotic formula-fed group and breast-fed group.

Fecal samples were obtained before formula administration (0 day) and during formula administration (14 and 28 days). At study days 0, 14 and 28 total aerobic and anaerobic bacteria, fungi, *Lactobacillus* and *Bifidobacterium* counts were performed on the fecal samples of both groups.

Before (0 day) and after 14th day of feeding the median number of bifidobacteria did not differ among the group. At the end of the 28th day feeding period, the population of bifidobacteria was significantly higher in formula-fed infants versus breast-fed infants. There (during four-week feeding period) was no statistically significant difference in the number of lactobacillus between the babies and no statistically significant difference in the total number of aerobic and anaerobic bacteria and fungi between formula-fed and breast-fed babies.

Infant milk formula containing prebiotic is able to induce intestinal flora of newborns that closely resembles the microbiota of the breast-fed infants. According to obtained results we concluded that addition of prebiotic to infant formula have beneficial effect for the baby and thus minimizes differences in the composition of the intestinal flora between the breast-fed and formula-fed infants.

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5.0.16

Similar bifidogenic effects of the infant formulae with added inuline and breastfeeding on gut microflora

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At birth and in the next few days begins the microbial colonization of human intestine. The composition of the intestinal flora newborns is influenced by the kind of delivery and feeding. *Bifi-*