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TNF-α Augments Enzyme Expression in the Small Intestine of Developing Mice

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Colostrum and milk are essential for the development and growth of mammals. Among the substances present in colostrum and milk tumor necrosis factor-alpha (TNF-α) has significant role in gut maturation and development. The aim of our study was to determine whether TNF-α has influence on the expression of the enzymes alkaline phosphatase, lactase and dipeptidil peptidase IV (DPP IV). Deposition of reaction products was visualized in thin sections of frozen gut tissue. Our observations showed more vivid results on first day of explants' treatment. Presence of TNF-α didn't show any significant effect on the activity of lactase. However in presence of TNF-α expression of alkaline phosphatase and DPP IV was increased.

Key words: TNF-α, enzyme expression, alkaline phosphatase, lactase, DPP IV.

Introduction

Colostrum and milk are essential for the development and growth of mammals. The composition of human milk provides the infant with all nutritional requirements in its early life [1]. A key cytokine delivered to developing intestine by mammary secretions is tumor necrosis factor alpha (TNF-alpha). Milk TNF-α is secreted by milk macrophages and by mammary epithelium. Levels of the cytokine in colostrum are significantly higher than both transitional and mature milk [8]. Besides participating in humoral and cell immunity, TNF-α also plays an important role in many diseases such as severe hepatitis, septic shock and inflammatory bowel disease [5]. During the first days of lactation TNF-α is physiologically active, enhancing the immune system of the neonate [13]. This includes development of monocytes and IL-1 and IL-10. The effects of milk cytokines and in particular tumor necrosis factor alpha on the maturation and functions of the epithelium, mucosal leukocytes and other specialized cells and structures in the alimentary system is not well documented. For that reason we target our research on TNF- α since it has got protective effect on respiratory system [10], but fewer investigations are held on the alimentary tract. The aim of our study was to trace the enzyme activity of alkaline phosphatase, lactase and DPP IV in the small intestine in organ culture in presence of TNF-α. Quantitative analysis of the number of dividing cells, stimulated or non stimulated with the factor was done.

Material and Methods

Tissue treatment. Balb/c mice 5 days old were used. Segments from duodenum, jejunum and ileum were taken according to the method of Playford et al. [7]. Specimens were incubated in culture medium RPMI 1640 containing 10 % fetal calf serum with 30 pg/ml rm TNF-alpha /Immunotools /Germany/ for 24, 48 and 72 h at 37 °C, 5% CO_2 respectively. All animal procedures were approved by the animal ethics committee at the Institute.

Autoradiography. It was developed by standard procedure and 5 μCi of ^{3}H-Thymidine were added (Amersham, UK) to untreated or TNF-alpha treated cultures 18 h before the end of the culture. Gut explants were put into Tissue Tek embedding medium (Sakura, USA) and frozen at –25 °C. They were cut on cryotom (Reichert Jung, FRG) to 10 μm sections and applied to glass slides. The number of dividing cells was counted using light microscope.

Enzyme localization. For the visualization of alkaline phosphatase activity we followed Burnstone's technique [3]. Sections were incubated in 0.7 mM naphthol-AS-MX-phosphate and 0.8 mg/ml Fast Blue B in 0.1 M TRIS/HCl buffer, pH 9.0 for eight min at 37 °C.

The visualization of lactase activity was performed after Gossrau [4]. We used substrate medium, containing 1 mM 5-Bromo-4-chloro-3-indolyl-beta-D-galactopyranoside and 1.2 mg/ml nitroblue tetrazolium chloride in 0.1 M sodium citrate /citric acid buffer, pH 6.0 for 2 h at 37°C.

For the visualization of DPP IV activity we used a newly developed procedure by Ivanov et al. [6]. The sections were incubated in medium, containing 0.3 mM fluorogenic substrate Gly-Pro-4-hydrazido-N-hexyl-1,8-naphthalimide (Gly-Pro-HHNI) and 0.3 mg/ml piperonal in 0.1 M phosphate buffer, pH 7.7 for two hours at 37°C. Then the sections were post fixed in 4 % neutral formalin for 15 min at room temperature, stained with hematoxylin according to the standard histochemical procedure and embedded in glycerol jelly.

Results and Discussion

The small intestinal mucosa changes rapidly in the first few postnatal weeks. The role of immune system in colostrums and milk is significant in protecting not only the mature, healthy newborn but also premature infant who is more prone to infections and damage caused by inflammatory processes. The human milk-fed preterm infant may experience improved health, such as, lower rate of infection, necrotizing enterocolitis, better gastrointestinal function, and neurodevelopment [14, 9].

TNF-α is synthesized as a 26-kDa transmembrane pro-hormone, which undergoes proteolytic cleavage to yield a 17-kDa soluble TNF-α molecule. Despite the differences in the location, both forms of TNF-α are capable of mediating biological responses [2,18] and together may be responsible for both local and systemic actions of this cytokine [16]. Tumor necrosis factor-alpha that is present in colostrums is a multifunctional cytokine which exerts a myriad of biological actions in different tissues. It has been demonstrated to regulate or interfere with adipocyte metabolism like transcriptional regulation, glucose and fatty acid metabolism and hormone receptor signaling [11]. Other *in vivo* studies demonstrate that elevated levels of serum TNF-α could be an important mediator of bacterial invasion of the intestinal mucosa during acute liver failure [12]. To date most of the cellular actions of TNF-α have been attributed to the activities of two distinct receptors TNFR1 and TNFR2 which are playing primarily a modulatory

role in ligand passing [17]. Both TNF receptors can be released from the cell surface and can exist in soluble form. Elevated levels of soluble TNF receptors are presented in many pathological states like cancers, sepsis, and fever [15].

Murine intestinal mucosa undergoes significant morphological changes like disappearance of large supranuclear vacuoles and decrease in the intestinal permeability towards proteins (gut closure). It has also been observed that activities of some mucosal enzymes are modified during that early postnatal period. For instance lactase which is essential for lactose hydrolysis during the first two postnatal weeks loses part of its activity and concentration [11].

In our previous work we have found positive morphological effect of TNF-α on murine gut development. To complete our study we traced the effect of TNF-α on some small bowel enzymes as described above.

Using autoradiography we counted the number of dividing nuclei. For that purpose we used StatMost for Windows. One-Way ANOVA results were used to prepare graphs. For first day of incubation the number of dividing nuclei was increased for the samples treated with TNF-alpha (Fig.1). On second and third day of incubation we did not see significantly different results for the two groups.

Intestinal alkaline phosphatase (AP) is a brush border protein that hydrolyzes monophosphate esters. It is expressed exclusively in villus enterocytes and is considered as a marker for crypt-villus differentiation. Addition of TNF-alpha to culture medium augmented AP's activity in all parts of the small bowel (Fig. 2a) compared to untreated specimens (Fig. 2b).

Dipeptidyl peptidase (DPP IV) is highly expressed in differentiated enterocytes. It is implicated in the degradation of various peptides and hormones including glucagons, neuropeptides and chemokines. Stimulation of the explants with TNF-alpha resulted in increased activity in all three parts of the small bowel (Fig. 3 a, b – control)

Lactase is essential for lactose hydrolysis during the first postnatal weeks. It also is considered marker of terminal differentiation in enterocytes. In our study addition of TNF-alpha did not affect enzyme's activity (Fig. 4a, b).

The results of our study do raise the possibility that TNF-α can ameliorate the activity of both alkaline phosphatase and dipeptidyl peptidase and has no significant effect on the activity of lactase.

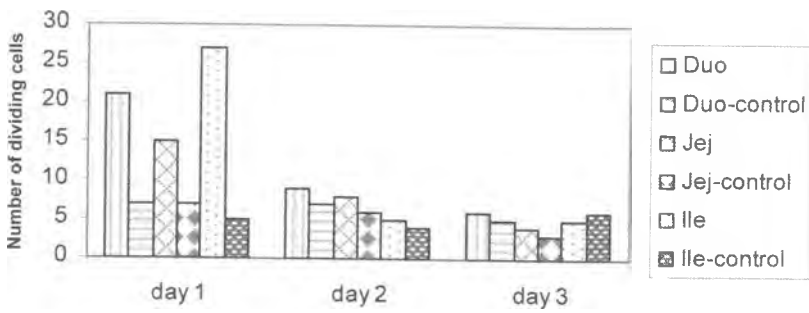
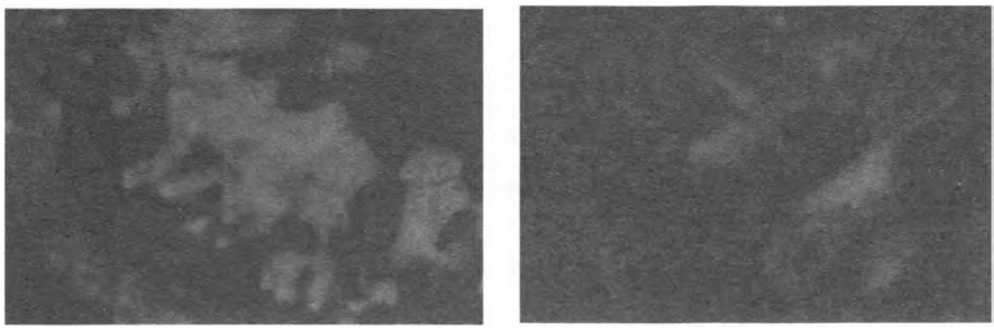


Fig. 1. Incorporation with *^{3}H-Thy* in murine enterocytes, treated with TNF-α and controls. Elevated levels of incorporation for the first 24 h after treatment, especially in jejunums

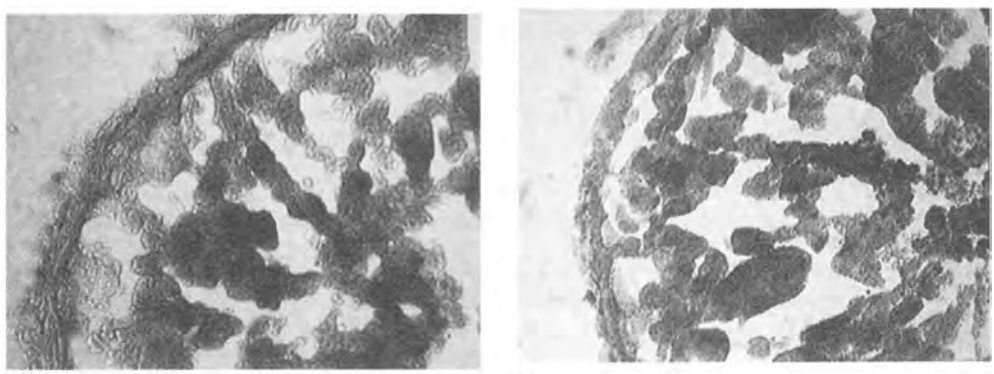
a) b)

Fig.2. Expression of alkaline phosphatase (AP) in cultured intestinal villi of 5 day old mouse, treated with TNF-α (2a). Lower expression of AP in controls in all parts of the small bowel (2b). Original magnification × 40



a) b)

Fig. 3. Expression of dipeptidyl peptidase (DPP IV) in murine intestinal villi. Specimens treated with TNF-α (3a) show more reaction product in comparison to untreated controls (3b).
Originally × 60



a) b)

Fig. 4. Expression of lactase in intestinal microvilli of 5 days old mouse. No significant differences were observed between samples (4a) and controls (4b). Original magnification × 40

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