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Influence of SCF on Enzyme Expression During Small Bowel Murine Development

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

Key words: SCF, enzyme expression, alkaline phosphatase, lactase, DPP IV.

Introduction

Colostrum and milk are essential for the development and growth of mammals. Postnatal development and maturation of their gastrointestinal tract is influenced by supply of maternal milk. Among the substances present in colostrum and milk stem cell factor (SCF) has major role in gut maturation and development. SCF is a cytokine which binds the c-kit. It exists in two forms – cell surface bound and free (soluble) form. Its main role is to maintain the proliferation and differentiation of hematopoietic stem cells and other progenitor cells. Data demonstrates the major role for SCF in the generation of intestinal mastocytosis and the host protective immune response following parasitic infection [2]. The later factor interacting with c-kit is considered to be important for the homeostasis of epithelial barrier function in the intestinal tract [7].

The aim of our study was to determine whether SCF has influence on the expression of the enzymes alkaline phosphatase, lactase and dipeptidil peptidase IV (DPPIV) at the developing small bowel of 5-day-old mice.

Materials and Methods

Organ culture preparation: Organ cultures were prepared from 5-day-old Balb/c mice from both sexes. A short segment of small intestine extending distally from the pylorus

was removed from each mouse. The segment was cut into 3 parts – duodenum, jejunum and ileum. Samples were taken according to intestinal length by P l a y f o r d et al. [6]. The explants were incubated in culture medium RPMI 1640 containing 10 % fetal calf serum with 20 ng/ml rm SCF (Immunotools /Germany) for 24, 48 and 72 hours at 37° C, 5% CO_2 at 100 % humidity. For enzyme check incubated explants were plunged into Tissue –Tek culture medium obtained from Sakura, USA and frozen at –25° C. Frozen tissue explants were cut by 10 μm sections on cryostat and used without fixation to determine the activity of the enzymes.

Enzyme substrates preparation: Alkaline phosphatase substrate was prepared by the method of Burstone. TRIS/HCl with pH 0.9, naphtol-AS-MX-phosphate and fast Blue B were used. Incubation was implemented for 8 min at 37°C, 5% CO_2. Specimens were post fixed in 4 % FA for four hours, washed and covered with glycerin - gelatin. Lactase substrate was prepared from 5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside, Nitrotetrazolium Blue chloride and citric buffer with pH 6. Specimens were incubated for 2 hours at 37°C, 5 % CO_2. Specimens were postfixed in 4 % FA for 15 min, washed and covered with glycerin-gelatin. For dipeptidil peptidase IV (DPPIV) determination we used phosphate buffer with pH 7.73 and 0.3 mM solution of aldehyde piperonal. Reaction was observed on fluorescent microscope after two hours of incubation at the same conditions [4].

Results

Small intestinal epithelium is one of the most actively renewing tissues in the body [5]. Intestinal enzyme activities are known to respond to changes in dietary composition. Studies in rats and humans suggest that adaptive mechanisms differ between species in response to altered intakes of carbohydrate and fat [3]. Colostrum is additionally a rich and concentrated source of various factors that demonstrate biological activity in vitro [1]. The ingestion of colostrum in neonatal mice has great effect on gastrointestinal tract development and influences digestive enzyme activities. Deposition of reaction products was visualized in thin sections of frozen gut tissue. The distribution of alkaline phosphatase with or without presence of SCF in the light microscope is seen in Fig.1 and Fig. 2. Our observations showed more vivid results on day two of explants' treatment. Hence we show only pictures from the second day of incubation.

Presence of SCF didn't show any significant effect on the availability of alkaline phosphatase. Explants treated with SCF and those, incubated without it showed equal distribution of the reaction product. We also compared the three parts of the small bowel

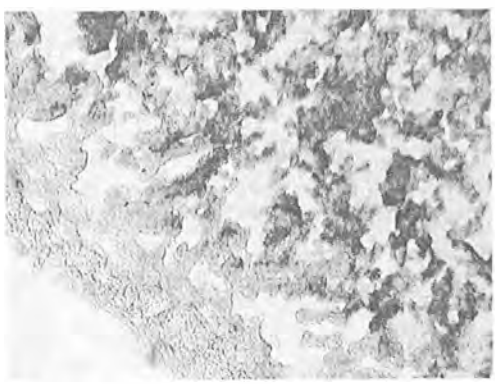

Fig. 1

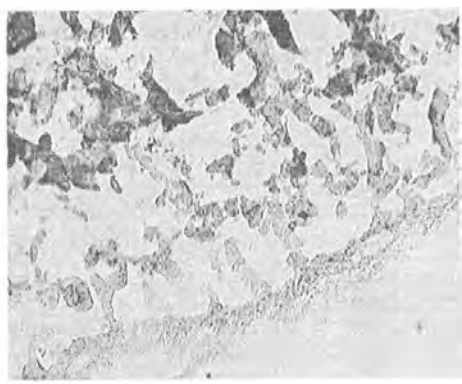

Fig. 2

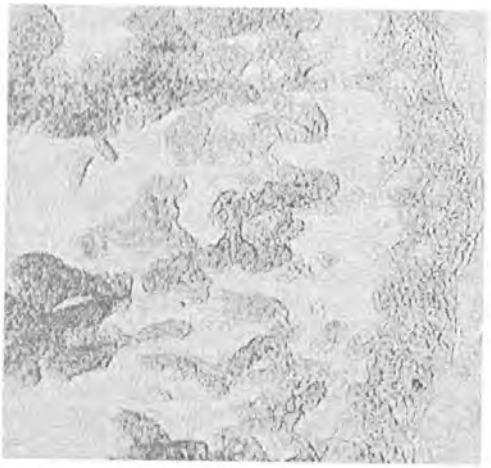

Fig. 3

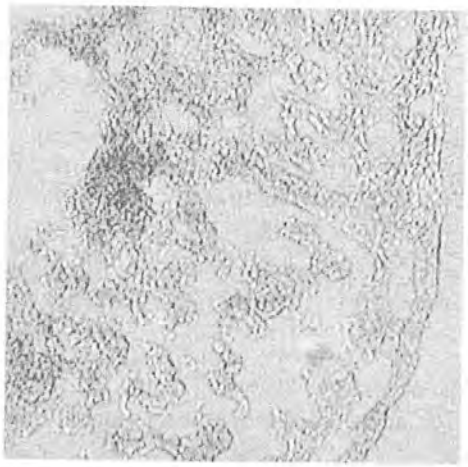

Fig. 4

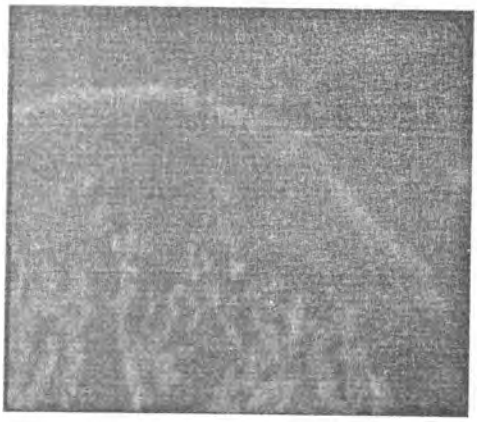

Fig. 5

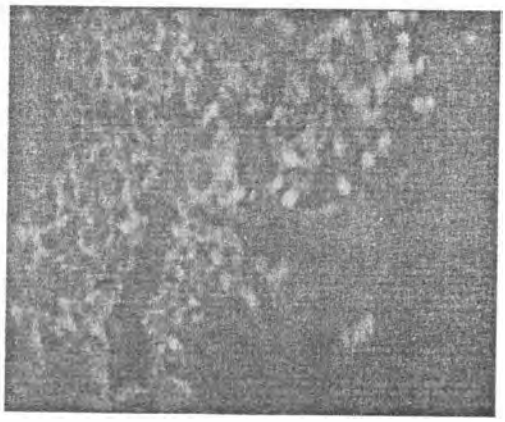

Fig. 6

(namely duodenum, jejunum and ileum) for samples treated with SCF. Staining for alkaline phosphatase was more contrasting for the jejunum and ileum.

Samples stained for lactase in presence of SCF were again more contrasting at day two of incubation. Comparing both stimulated and non stimulated samples we didn't see any significant difference in lactase's distribution (Fig. 3 and Fig. 4.). Lactase availability was lower in jejunum and ileum compared to duodenum. No lactase was found at the gut wall.

Staining for DPP IV in presence of SCF was performed by fluorescent tagging of the enzyme (Fig. 5 and Fig. 6). It showed more contrasting villi at all parts of the small intestine, comparing to non stimulated samples. Our conclusion was that in presence of SCF the expression of DPP IV was increased.

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