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Effects of Protein Restriction on Number of Mast Cells in the Intestine of Mature and Immature Rats

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Abstract: This study was designed to evaluate changes in the number of mast cells in rats under different levels of protein deficiency. Twenty-four 20-day-old (immature group) and twenty-four 65-day-old (mature group) male Wistar Albino rats were used. They were divided into six experimental groups, each containing 8 animals. The small intestine tissue samples were obtained under deep anaesthesia. Intestine tissues were fixed in Mota's fixative (Basic Lead Acetate - BLA) for 24 h and embedded in paraffin. Sections of 6 µm thickness were cut and stained with 0.5% toluidine blue in 0.5 N hydrochloric acid at pH 1.0 for 30 min. The numbers of mast cells in small intestine tissues were lower in immature rats fed 3% crude protein diet compared with, except duodenum, rats fed 10% crude protein and control group ($p > 0.005$). In mature rats, the numbers of mast cells were similar among fed 3% crude protein diet and control ($p > 0.05$), but fewer than in the 10% crude protein ($p > 0.05$).

Key words: Protein restriction, mast cell, small intestine, rat

Introduction

The gastrointestinal tract contains many different types of endocrine cells producing different regulatory peptides and amines. These cell types are not randomly distributed along the gastrointestinal tract but occur in different patterns and constellations in different specialized regions. The gut, with its enormous mucosal surface, plays an important role in preventing the circulatory system from taking up intestinal microorganisms and endotoxins (Øster *et al.*, 1998). Mast cells have long been recognized as major effector cells of the allergic or type I hypersensitivity reaction by virtue of their high-affinity surface receptors for IgE. Mast cell mediated events have been postulated to play a role in host defence against parasitic diseases of the intestine, in wound healing and scar formation in the skin and in promoting tumour angiogenesis (Irani and Schwartz, 1989). Most experimental studies analyzing the function of intestinal mast cells have been performed on rodents and virtually all studies of isolated dispersed intestinal mast cells have been performed on the rat or mouse small intestine (Enerback, 1981; Lake *et al.*, 1984; Befus *et al.*, 1982). Histochemical studies suggest that there are two types of rodent mast cells, an 'atypical' mucosal mast cell and a 'typical' connective tissue mast cell found in the peritoneum and in other locations (Enerback, 1966a). Mucosal mast cells differ from those in connective tissue in terms of their morphology under light and electron microscope (Enerback, 1966a), their histochemical characteristics (Enerback, 1966b.) and their content of IgE (Mayrhofer *et al.*, 1976).

Protein malnutrition (PM) is an indirect cause of immunodeficiency for a significant proportion of humans worldwide and leads to increased susceptibility to infection (Petro and Bhattacharjee,

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1981; Schoper and Douglas, 1976). PM depresses both cell-mediated immunity and humoral immunity, resulting in thymus, spleen and lymph node atrophy (Chandra, 1980; Bell *et al.*, 1976). However, the immunological effects of PM may vary depending on the severity of protein deprivation (Chandra and Chandra, 1986). Epidemiological studies have shown that protein-energy malnutrition is associated with a higher rate of infection, which may increase morbidity and mortality through impairment of host defence mechanisms, reduced phagocyte function (McCarter *et al.*, 1998; Dai and McMurray, 1998) and altered cytokine production (Dai and McMurray, 1998; Lyouni *et al.*, 1998).

The objective of the present study was to determine the effects of different levels of protein deficiency on number of mast cells in rat small intestine.

Materials and Methods

Animals

Twenty-four 20-day-old (immature group) and twenty-four 60-day-old (adult group) male Wistar Albino rats were selected as the experimental subjects. They were acclimatized for 7 d with laboratory conditions at 22-25°C with a 12 h light/dark cycle. Each age group of rats was randomly divided into three groups of eight animals each (immature = Control I, Group I, Group II; mature = Control II, Group III and Group IV). Eight rats within each group and each treatment were also assigned to two cages of four rats. Animals were fed a diet containing 10% protein (moderate restriction, Group I and Group III), 3% protein (severe restriction, Group II and Group IV) or 20% protein (normal level, Control I and Control II) (Table 1). All rats were fed ad libitum and had free access to clean tapwater throughout the experiment. Rats were killed by decapitation under ether anaesthesia two months after initiation of the experiment. The rats received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Sciences and published by the National Institute of Health.

The small intestines were removed under pentobarbital anaesthesia (50 mg kg⁻¹ b.w., i.p.). When used for histological techniques, tissues fragments were fixed in Mota's Basic Lead Acetate (BLA) (24 h) and ultimately embedded in paraffin. Six-micrometre thick sections were stained with toluidine blue (Merck, CI No. 52040) in 0.5% aqueous solution at pH 1.0 for 5 min for counting MCs.

Microscopic Examination

Microscopic examination was carried out under a magnification of 400 and the counts of mast cells were determined per square millimeter using a standardized ocular grid. Intact or partially degranulated MCs were counted. Tissue sections were examined under light microscopy (x400) and the number of mast cells counted in random high-power fields using a Nikon Optiphot 2 light microscope incorporating a square graticule in the eyepiece (eyepiece x10, objective x40, total side length of 0.225 mm). MC density was assessed by counting the number of cells in 200 high power

Table 1: Composition of experimental diets

Ingredients	% of total diet		
	Control	Group I and III	Group II and IV
Casein	25.0	12.5	3.0
Corn oil	7.5	7.5	7.5
Vitamin-mineral mix	7.0	7.0	7.0
Starch	57.5	70.0	79.5
Peanut hull	3.0	3.0	3.0

fields in intestine preparations of each group. The MC density was calculated and recorded as MC numbers mm². All the results are expressed as mean±SD.

Statistical Evaluation

Statistical analysis was performed using ANOVA analysis and t-test. p<0.05 were considered statistically significant (SPSS, 1999).

Results and Discussion

Mast cells in cross sections of 6 µm thickness taken from tissue samples belonging to various regions were distinguished easily by the metachromatic staining method. They were located around crypts and nerve fibres in lamina propria and submucosa. They were also observed around blood vessels between muscle groups in tunica muscularis. The number of mast cells showed an increase in both Group I and Group III (moderate protein restriction) compared with Control I and Control II (p<0.05). In the small intestine of both the immature and mature age groups, MC density was higher in the lamina propria than in the tunica muscularis and tunica serosa (Table 2 and 3).

The present study may indicate the protein restriction caused activation of mast cells in the intestine of immature and mature rats. On the other hand, the effect on number of mast cells was not clear in immature and mature rats because the number of mast cells increased in the Group I and III (diet with 10% protein), while it decreased in small intestine tissues of Group II and IV (diet with 3% protein). This is surprising because the number of mast cells has previously been found to decrease on a protein-deficient diet (Rickard and Lagunoff, 1989).

Many pathological characteristics of intestinal disease, such as parasitic infection, can be attributed to the actions of mast cell mediators. The number of mucosal mast cells in the small intestine has been reported to be depressed in protein deficiency and the hyperplasia induced by infection with *Nippostrongylus brasiliensis* is poorly sustained (Cummins *et al.*, 1987). In the present study, the number of mast cells showed a decrease in the duodenum and jejunum of adult rats on the severe protein restriction diet (Group IV) compared with the control group. In the ileum, MC numbers increased in all groups. This result is in accordance with the findings of Cummins *et al.* (1987).

Few data are available on the influence of different malnutrition situations on total number of intestinal mast cells. Zinc deficiency has been shown not to affect the mast cell population in the skin,

Table 2: Mast cells (numbers mm) in immature rats maintained on the different diets (Control I, Group I and Group II received 20, 10 and 3% protein, respectively)

Small intestine	Control I (n = 8)	Group I (n = 8)	Group II (n = 8)
Duodenum	28.5±12.1*	95.8±20.2	23.1±9.7
Jejunum	59.5±9.4	84.0±14.5	35.4±11.5
Ileum	53.8±15.8	84.8±23.4	38.6±8.9

* All samples stained with toluidine blue, pH 1.0; Values are mean±Standard deviation (SD)
Mean values in same rows with no common superscripts differ significantly (p<0.05)

Table 3: Mast cells (numbers mm) in mature rats maintained on different diets (Control II, Group III and Group IV received 20, 10 and 3% protein, respectively)

Small intestine	Control II (n = 8)	Group III (n = 8)	Group IV (n = 8)
Duodenum	79.4±14.8*	129.4±32.4	75.5±21.4
Jejunum	81.9±24.9	148.5±27.7	59.9±17.5
Ileum	75.6±19.7	144.0±26.9	64.8±16.7

* All samples stained with toluidine blue, pH 1.0; Values are mean±Standard Deviation (SD)
Mean values in same rows with no common superscripts differ significantly (p<0.05)

thyroid and metaphysis of the tibia (Belanger, 1978). However, it has been shown that zinc deficiency influences the gastric glandular mucosal wall mast cell population under both normal and stress conditions (Cho *et al.*, 1987; Swenerton and Hurley, 1968). Deficiency of this metal in the body could labilize the cells and reduce the number of mast cells in the stomach (Cho *et al.*, 1987; Mann *et al.*, 1981).

Moderate PM (10%) significantly increased the number of MC in both immature and mature rats in the present study. However, previous studies have shown that PM decreases intestinal MC numbers in the peritoneum and small intestines (Cummins *et al.*, 1987; Rickard and Lagunoff, 1989). It could be concluded that exposure to severe protein deficiency decreases the number of MCs in small intestine tissues of rats. The present study indicates that properties of mast cell should be studied further.

References

- Befus, A.D., F.L. Pearce, J. Gauldie, P. Horsewood and J. Bienenstock, 1982. Mucosal mast cells. I. Isolation and functional characteristics of rat intestinal mast cells. *J. Immunol.*, 128: 2475-2480.
- Belanger, L.F., 1978. The influence of zinc-deprivation on the mast cell population of the bone marrow and other tissues. *J. Nutr.*, 108: 1315-1321.
- Bell, R.G., L.A. Hazeli and P. Price, 1976. Influence of dietary protein restriction on immune competence. II. Effect on lymphoid tissue. *Clin. Exp. Immunol.*, 26: 314-326.
- Chandra, R.K., 1980. Cell-mediated immunity in nutritional imbalance. *Fed. Proc.*, 39: 3038-3092.
- Chandra, S. and R.K. Chandra, 1986. Nutrition, immune response and outcome. *Prog. Food Nutr. Sci.*, 10: 1-65.
- Cho, C.H., L.Y.Y. Fong, P.C.C. Ma and C.W. Ogle, 1987. Zinc deficiency: Its role in gastric secretion and stress-induced gastric ulceration in rats. *Pharmacol. Biochem. Be.*, 26, 293-297.
- Cummins, A.G., A.L. Kenny, V.M. Duncombe, D. Bolin and A.E. Davis, 1987. The effect of protein deficiency on systemic release of rat mucosal mast cell protease II during *Nippostrongylus brasiliensis* infection and following systemic anaphylaxis. *Immunol. Cell Biol.*, 65: 357-363.
- Dai, G.X. and D.N. McMurray, 1998. Altered cytokine production and impaired antimycobacterial immunity in protein-malnourished guinea pigs. *Infect. Immunol.*, 66: 3562-3568.
- Enerback, L., 1981. The gut mucosal mast cell monogr. *Allergy*, 17: 22-26.
- Enerback, L., 1966a. Mast cells in rat gastrointestinal mucosa. I. Effects of fixation. *Acta Pathol. Microbiol. Scand.*, 66: 289-302.
- Enerback, L., 1966b. Mast cells in rat gastrointestinal mucosa. 2. dye-binding and metachromatic properties. *Acta. Pathol. Microbiol. Scand.*, 66: 303-12.
- Irani, A.M. and L.B. Schwartz, 1989. Mast cell heterogeneity. *Clin. Exp. Allergy*, 19: 143-155.
- Lake, A.M, A. Kagey-Sobotka, T. Jakubowicz and L.M. Lichtenstein, 1984. Histamine release in acute anaphylactic enteropathy of the rat. *J. Immunol.*, 133: 1529-1534.
- Lyoumi, S., F. Tamion, J. Petit, P. Dechelotte, C. Dauguet, M. Scotte, M. Hiron, A. Leplingard, J.P. Salier, M. Daveau and J.P. Lebreton, 1998. Induction and modulation of acute-phase response by protein malnutrition in rats: Comparative effect of systemic and localized inflammation on interleukin-6 and acute-phase protein synthesis. *J. Nutr.*, 128: 166-174.
- Mann, N.S., J.M. Khan, A.B. Reddy and P. Lathon, 1981. Effect of oral zinc sulphate on experimental gastric ulcer. *Gastroenterology*, 80: 1223-1229.

- Mayrhofer, G., H. Bazin and J.L. Gowans, 1976. Nature of cells binding anti-IgE in rats immunized with *Nippostrongylus brasiliensis*: IgE synthesis in regional nodes and concentration in mucosal mast cells. *Eur. J. Immunol.*, 6: 537-45.
- McCarter, M.D., H.A. Naama, J. Shou, L.X. Kwi, D.A. Evoy, S.E. Calvano and J.M. Daly, 1998. Altered macrophage intracellular signaling induced by protein-calorie malnutrition. *Cell Immunol.*, 183: 131-136.
- Øster, A., J. Jensen, H. Edlund and L.I. Larsson, 1998. Homeobox gene product Nkx 6.1 immunoreactivity in nuclei of endocrine cells of rat and mouse stomach. *J. Histochem. Cytochem.*, 46: 717-721.
- Petro, T.M. and J.K. Bhattacharjee, 1981. Effect of dietary essential amino acid limitations upon the susceptibility of mice to infection with *Salmonella typhimurium*. *Infect. Immun.*, 32: 251-259.
- Rickard, A.L. and D. Lagunoff, 1989. Protein malnutrition: Effect on rat peritoneal mast cell number, histamine content and IgE receptors. *Am. J. Clin. Nutr.*, 49: 641-645.
- Schoper, K. and S.D. Douglas, 1976. *In vitro* studies of lymphocytes from children with Kwashiorkor. *Clin. Immunol. Immunopathol.*, 51: 21-30.
- SPSS, 1999. SPSS 10.0 for Windows. SPSS Inc., Chicago, IL.
- Swenerton, H. and L.S. Hurley, 1968. Severe zinc deficiency in male and female rats. *J. Nutr.*, 95: 8-14.