Effects of Arginine Supplementation on the Mucosal Immunity in Rats with Gut-derived Sepsis

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Running title: effect of arginine on mucosal immunity in sepsis

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ABSTRACT

Background and specific aim：Total parenteral nutrition （TPN）is usually a major nutrient administration method for those critically ill patients. However, previous researches have shown that TPN caused small intestinal gut-associated lymphoid tissue（GALT）atropy, lowered small intestinal immunoglobulin A levels. Since the GALT not only provides indispensable immunologic protection against resident microbial flora and infectious pathogens, but also provides significant immunologic protection for distant mucosal sites, the specific aims of these experiments was to examine the effect of an isonitrogenous 2% Arginine-supplemented diets before or/and Arginine-enriched TPN solution after sepsis on the intestinal immunity in rats with gut-derived sepsis.

Experimental designs： Male Wistar rats were assigned to four groups. Group 1 and 2 fed with semipurified diet, group 3 and 4 replaced part of casein with 2% of total calorie as Arg. After feeding the experimental diets for 10 days, sepsis was induced by cecal ligation and puncture (CLP), at the same time internal jugular vein was cannulated. All rats were maintained with total parenteral nutrition (TPN) for 3 days. Group 1 and 3 were infused conventional TPN, while group 2 and 4 supplemented with Arg, replacing 10% of total amino acid in TPN solution. All rats were sacrificed 3 days after CLP. The lymphocyte subpopulations of Peyer's patches were analyzed by flow cytometry. Luminal small intestinal IgA levels and cytokines secreted from lymphocytes of Peyer's patches were analyzed by means of sandwich enzyme-linked immunosorbent assay.

Results： Total lymphocyte yield in the Peyer's patches, small intestinal IgA levels,

increased in the enteral diets supplemented with Arg groups before sepsis in comparison to the other two groups, but the ratios of lymphocyte subpopulation did not change between the four groups.

Conclusions：Isonitrogenous diet supplementation with 2% Arginine improves IgA-mediated protection in the intestinal immunity.

Keywords: sepsis, arginine, Peyer's patches, immunoglobulin A, mucosal immunity

建議要把營養的部分加在此篇，否則份量似顯不足！但是如何把營養和 mucosal immunity 連在一起，也很困難！

INTRODUCTION

The gut-associated lymphoid tissue（GALT）comprises Peyer's patches, the appendix, and lamina propria lymphocytes. Peyer's patches have attracted special interest, because in this intestinal lymphoid organ large population of antibody-producing plasma cells present, whose number far exceeds that of plasma cells in the spleen, lymph nodes combined. In addition, Peyer's patches are primed against intestinal antigens and subsequently seeded as memory or effector cells to distant mucosal sites, such as the nasopharynx, breast, salivary glands, and lungs（Salmi M, 1991）. Previous researches have shown that total parenteral nutrition（TPN）caused small intestinal gut-associated lymphoid tissue atropy, lowered small intestinal immunoglobulin A levels（Jian Li, 1995）, increased intestinal bacterial overgrowth, and bacterial translocation to mesenteric lymph nodes（Alverdy JC, 1988；Mainous M, 1991）. However, TPN is usually a major nutrient administration method for those critically ill patients. The specific aims of these experiments were to examine whether an isonitrogenous 2% Arg-enriched TPN solution may improve these limits on rats with gut-derived sepsis.

1. Salmi M, Jalkanen S. Regulation of lymphocyte traffic to mucosa-associated lymphatic tissues. Gastroenterol Clin North Am 1991; 20: 495-510.
2. Jian Li, Kenneth A. Kudsk, Barbara Gocinski, Daniel Dent, Jeffrey Glezer, Bobbi Langkamp-Henken, Effects of Parenteral and Enteral Nutrition on Gut-Associated Lymphoid Tissue. J. Trauma 1995；39（1）：44-52.
3. Alverdy JC, Aoys E, Moss GS. Total parenteral nutrition promotes bacterial translocation from the gut. Surgery 1988; 104:185-90.
4. Mainous M, Xu D, Lu Q, et al: Oral-TPN-induced bacterial translocation and impaired immune defenses are reversed with refeeding. Surgery 1991；110:277,
5.

MATERIALS AND METHODS

***Animals and Diets*：**

Male Wistar rats were assigned to four groups. Group 1 and 2 fed with semipurified diet, group 3 and 4 replaced part of casein with 2% of total calorie as Arg. After feeding the experimental diets for 10 days, sepsis was induced by cecal ligation and puncture (CLP), at the same time internal jugular vein was cannulated. All rats were maintained with total parenteral nutrition (TPN) for 3 days. Group 1 and 3 were infused conventional TPN, while group 2 and 4 supplemented with Arg, replacing 10% of total amino acid in TPN solution. The 4 groups were: group 1: without Arg supplementation before and after CLP, group 2: Arg supplemented after CLP, group 3: Arg supplemented before CLP, group 4: Arg supplemented before and after CLP. The experimental diets and TPN solutions were isonitogenous among the

groups.

Surgical Procedures and Sepsis:

Removing of Peyer's patches and isolation of cells：

On day 3 after the CLP operation, surviving rats were weighed and anesthetized. A middle abdominal incision was made, and the intestine from each rat was carefully removed from the stomach-duodenum junction to the ileum ascending-colon junction. Total number of Peyer's patches （PP） was counted from each removed small intestine, and lymphocytes isolation from PP were performed as described by（Janu P, et al 1997 in論文）. Briefly, the PPs were excised from the serosal side of the intestine and teased apart with 18-gauge needles, and then treated with 50 units/ml of type 1 collagenase（Gibco Life Technologies, USA）in RPMI-1640 supplemented with 100μg/mL of penicillin and streptomycin for 60 minutes at 37℃ with constant shaking. After collagenase digestion, the cell suspensions were passed through nylon filters, washed three times with HBSS and adjusted the cell numbers to 2×10^6 cells/ml with complete RPMI-1640 supplemented with 10% fetal calf serum, 2mM glutamine and antibiotics.

Lymphocytes subpopulation analysis by flow cytometry：

To determine the phenotypes of lymphocytes isolated from the Peyer's patches, 10^5 cells were suspended in 100μl HBSS containing fluorescein-conjugated mouse anti-rat CD3（Serotec Co.,UK）and phycoerythrin-conjugated mouse anti-rat CD45Ra（Serotec Co.,UK） to distinguish T cells and B cells, respectively, or in fluorescein-conjugated mouse anti-rat CD8 and phycoerythrin-conjugated mouse anti-rat CD4（Serotec Co.,UK）to identify T helper cells and CTL cells, respectively. After staining for 15 minutes, 1ml red blood cell lysing buffer was added to lyse the

red blood cells and fixed the stained lymphocytes. Flow cytometry analysis was performed on a Profile I（Coulter Co.）.

***Mitogen stimulation assay for T- and B-Lymphocytes* activity：**

The responses of PP cells to mitogenic stimulation were assessed by cytokines secretion as previously described（）. Quadruplicate wells of 96-well flat-bottom microtiter plates（Corning Glass Works, Corning, NY）were seeded with 100μL of PP lymphocytes （2×10^6 cells/ml）and 100μL of mitogen（200ng/ml of PHA or 1μg of LPS）. Control wells contained cells plus 100μL of medium. PP lymphocytes were cultured for 24 h at 37℃ in an atmosphere of 95% air and 5% CO_2. Supernatants were clarified by centrifugation（600×g for 20 minutes）and stored at -80℃ until assayed for cytokines.

***Cytokine Assay*：**

The concentrations of TNF-α, IFN-γ, IL-2, IL4 and IL-10 in the supernatant of mitogens stimulated PP cells were determined by a sandwich enzyme-linked immunosorbent assay（ELISA）. Procedures were described in the manufacturer's instruction（Amersham Pharmacia Biotech, U.K.）

Cytokine immunoassays:

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cytokines. The concentrations of TNF-α, IFN-γ, IL-2, IL4 and IL-10 in the supernatant were determined by a sandwich enzyme-linked immunosorbent assay (ELISA) (Amersham Pharmacia Biotech Inc., U.K.)

Detection of the intestinal sIgA：

The collection of intestinal fluid was modified from the procedure described by（Perdigon G.et al., 1990）. After excised the Peyer's patches, 12cm small intestine fragment from the cecum was removed, the intestinal contents were washed out using 5ml PBS with 1% protease inhibitor（Sigma, USA）. Debris was removed by centrifugation for 10 min at 3000 rpm to harvest the supernatant for analysis of intestinal IgA after appropriate dilution. The total intestinal IgA level was determined by sandwich-type ELISA, in which anti-rat IgA capture antibody（clone A93-3；PharMingen, San Diego, CA） was coated on an ELISA plate and detected with peroxidase-labeled anti-human IgA detecting antibody（clone A93-2；PharMingen, San Diego, CA）. The intestinal washing IgA was normalized for the length of the respective intestinal segment（IgA/cm intestine）.

Statistical Analysis：

References:

1. Böhmer RH, Trinkle LS, Staneck JL. Dose effects of LPS on neutrophils in a whole blood flow cytometric assay of phagocytosis and oxidative burst. Cytometry 1992; 13: 525-31.
2. Schiffrin, E.J., Rochat, F., Aeschlimann, J.M. and A Donnet-Hughes. 1995. Immunomodulation of human blood cells following ingestion of lactic acid bacteria. J. Diary Sci（USA）78: 491-497.
3. Leh Chang, Gary A, Gusewitch, Douglas B.W. Chritton, Judith C. Folz, Lauralynn K. Lebeck and Sandra L.Nehlsen-Cannarella. Rapid flow cytometric assay for the assessment of natural killer cell activity. J. Immu.Methods 1993；

166: 45-54.

4. Perdigon G., Alvarez S., Nader de Macias ME., Maria ER., Pesce de Ruiz Holgado AA. The oral administration of lactic acid bacteria increase the mucosal intestinal immunity in response to enteropathogens. J. Food Protection 1990；53:404-410.

RESULTS

Total lymphocytes yields：

Arg significantly increased the total numbers of Peyer's patches and the total lymphocytes numbers（Fig.11 and 12）

Lymphocyte subpopulations：（Fig.15）

IgA levels: Small intestinal IgA and serum IgA concentration among the groups are summarized in Fig.19. Animals fed with TPN solution supplemented with 2% Arg had a significant increase in intestinal IgA recovery compared with-----. There was no significant difference between the groups in serum IgA.

Production of Inflammatory Cytokines by Splenic Lymphocytes：（Fig.26, 27 and 28）

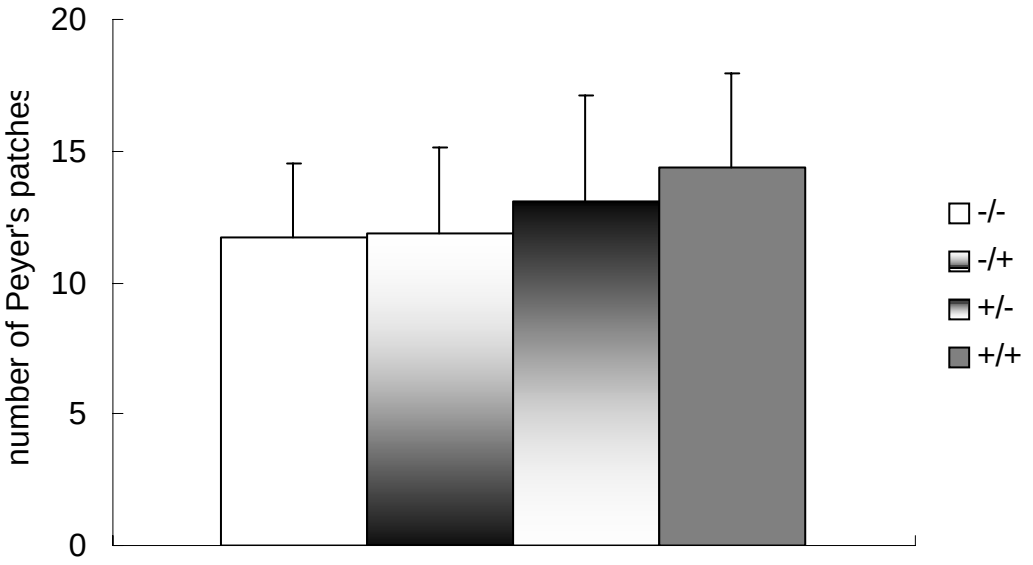


Figure 11. The number of Peyer's patches on the whole small intestine in the four groups 3 days after CLP

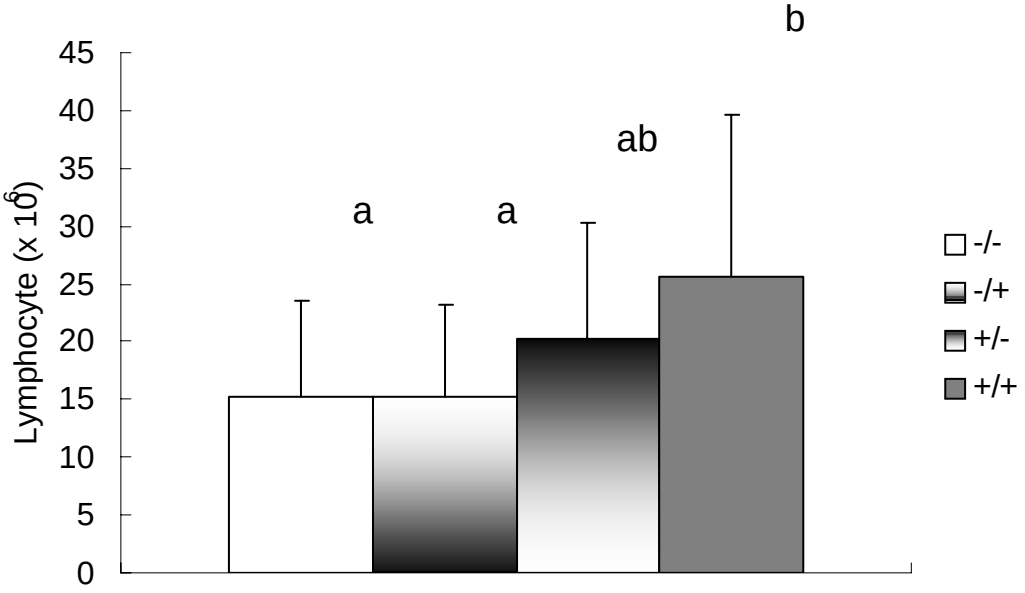


Figure 12. The number of lymphocyte in the Peyer's patches in the four groups 3 days after CLP

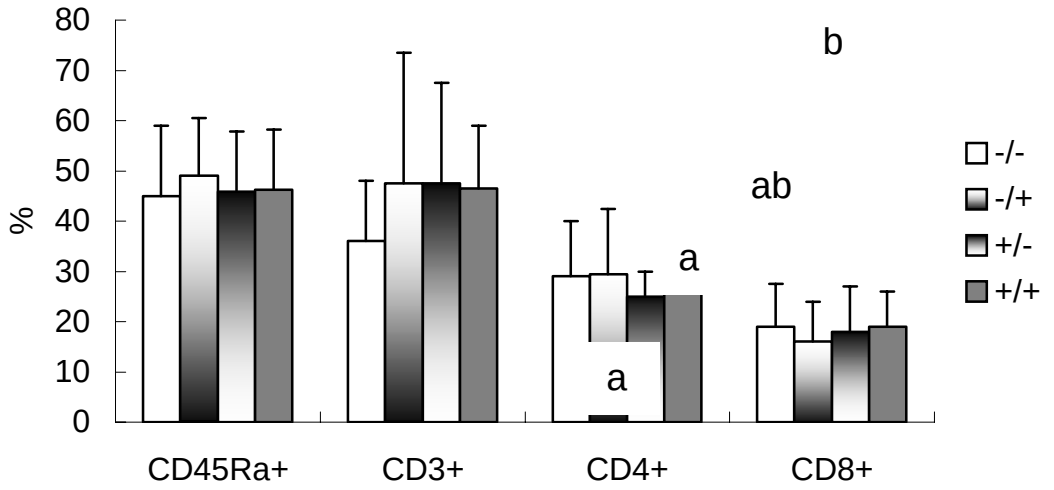
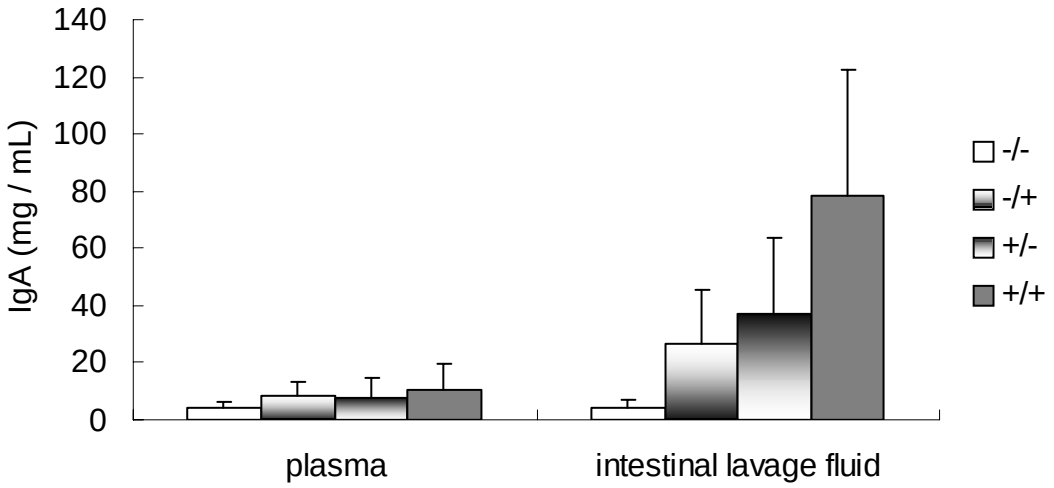


Figure 15. The distribution of $CD45Ra^+$, $CD3^+$, $CD4^+$ and $CD8^+$ lymphocytes in the Peyer's patches in the four groups 3 days after CLP



Total immunoglobulin A (IgA) concentrations in the plasma and intestinal lavage fluid in the four groups 3 days after CLP

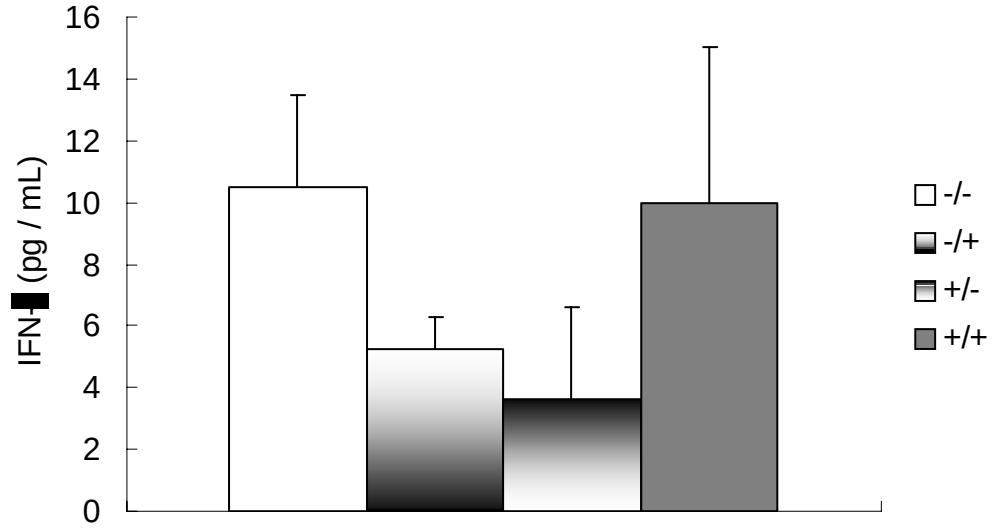


Figure 25. The concentrations of IFN-γ released by PHA-stimulated splenocyte for 24 hours

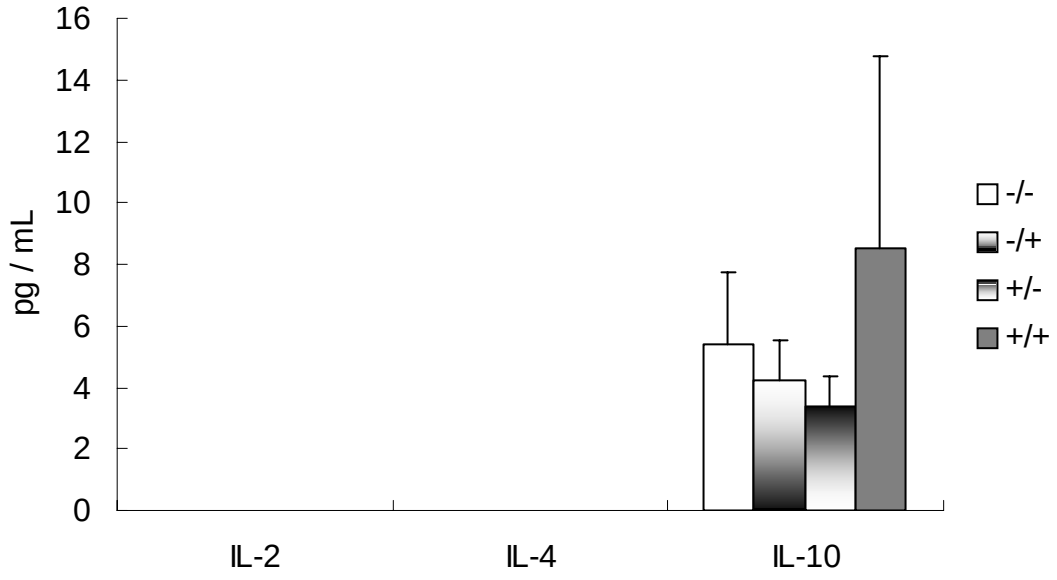


Figure 26. The concentrations of cytokines released Peyer's patches lymphocytes after PHA stimulation for 24 hours

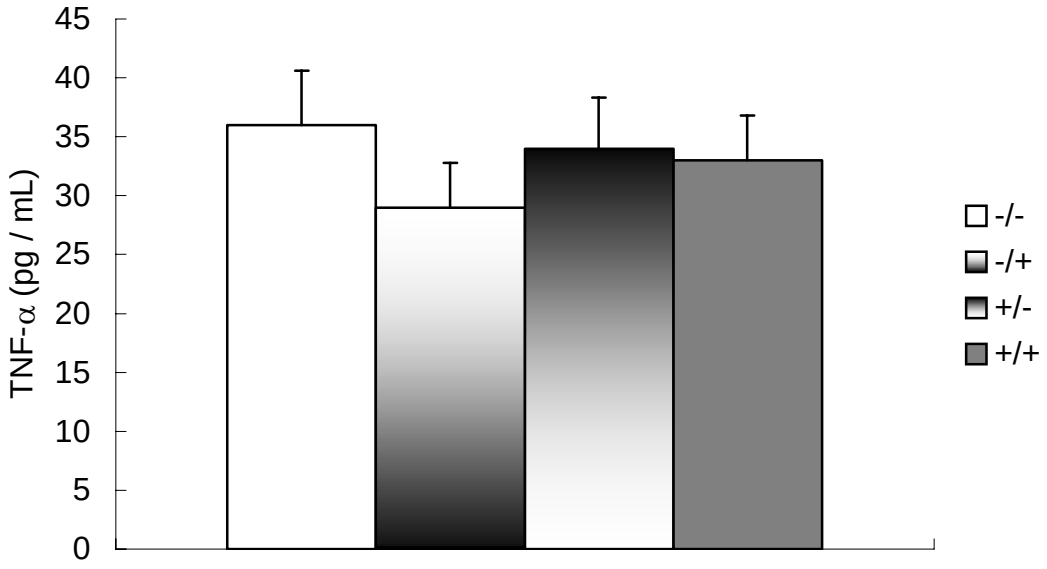


Figure 27. The concentrations of TNF-α released Peyer's patches lymphocytes after LPS stimulation for 24 hours

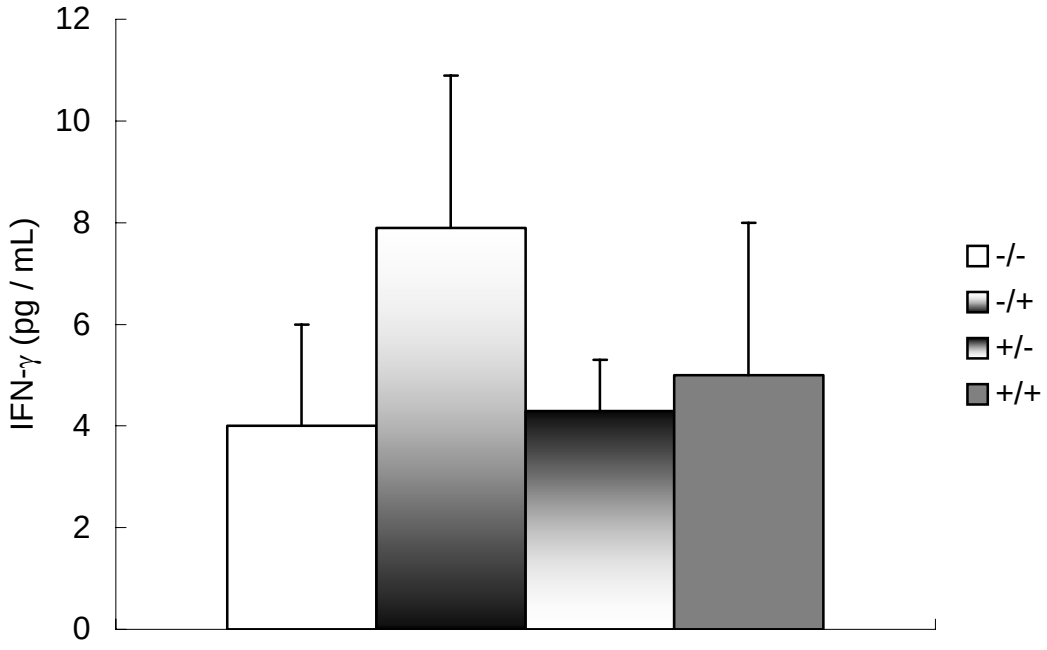


Figure 28. The concentrations of IFN-γ released Peyer's patches lymphocytes after LPS stimulation for 24 hours