# In Vitro Immunomodulatory and Cellular Activities of the Plastein Reaction Products of Bovine Colostrum Hydrolysates


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**ABSTRACT**

In this research the skimmed milk isolated from colostrums collected on the 2nd day postpartum were hydrolyzed by Flavourzyme. The skimmed milk hydrolysates were subjected into a plastein reaction with Flavourzyme under pH 5 and 9 for 4, 8 and 12 hours, respectively. This research investigates the effects of different plastin products on human mononuclear cell (MNC) growth, on the secretion of cytokines (IL-1β, IFN-γ and TNF-α) as well as nitric oxide, and on the growth inhibition of human leukemic U937 cells. The results showed that the products of plastein reaction with Flavourzyme under pH 9 for 8 hours (Pp-9-8) (at 800 μg/mL) had significant effects on the inhibition of U937 cell growth and cytokine secretion. Additionally, Tp-9-8, Lp-9-8 and Gp-9-8 from the plastein reactions with three amino acids (Tyrosine, Leucine and Glycine) under pH 9 for 8 hours were compared with Pp-9-8. It was found that both Pp-9-8 and Lp-9-8 had significant effects on the inhibition of U937 cell growth, and could considerably stimulate the secretion of cytokines. This research also investigated the simulated digestion in the gastrointestinal tract for Pp-9-8 and Lp-9-8 and the effects of the enzymes in the gastrointestinal tracts on the growth inhibition and immunoregulation of U937 cells. Results showed that the plastein products after the simulated digestion in the gastrointestinal tracts had a tendency to decrease both the inhibition of U937 cell growth and the amount of cytokine secreted.



Keywords : Bovine colostrums、Plasteins、Leukemic U937 cells、Cytokines、Flavourzyme


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## REFERENCES

參考文獻 1.宮華婷。2006。初乳之免疫調節作用及對人類白血病細胞(U937)之抑制效果。碩士論文，國立台灣大學食品科技研究所。 2.陳志瑋。2006。微膠囊化牛初乳蛋白質水解物之抗氧化性。碩士論文，大葉大學生物產業科技研究所。 3.江淑華。2005。牛初乳及其酵素水解物之抗氧化性與其蛋白質組成之相關性研究。博士論文，大葉大學生物產業科技系。 4.宋晏仁，周純芬，謝文欽。2006。醫用免疫學。合記圖書出版社，台北。 5.戴達夫。2001。酵素生化科技。華榮圖書有限公司，台北。 6.藍以政。1998。血液疾病簡介。內科新知。 7.Albina, J. E., Cui, S., Mateo R. B., and Reichner, J. S. 1993. Nitric oxide-mediated apoptosis in murine peritoneal macrophages. Journal of Immunology, 150, 5080-5085. 8.Alem?鴨, A., P?臆ez-Sant?瀋, E., Bordenave-Juchereau, S., Arnaudin, I., G?曠ez-Guill?聲, M. C., and Montero, P. 2011. Squid gelatin hydrolysates with antihypertensive, anticancer and antioxidant activity. Journal of Food Research International, 44, 1044 – 1051. 9.Alluwaimi. A. M. 2004. The cytokines of bovine mammary gland:prospects for diagnosis and therapy. Research in Veterinary Science. 77:211-222. 10.Amarowicz, R. and Shahidi, F. 1997. Antioxidant activity of peptide fractions of capelin protein hydrolysates. Food Chemistry, 58, 355 – 359. 11.Anderson, J. W., Johnstone, B. M., and Cook-Newell, M. E. 1995. Meta-analysis of the effects of soy protein intake on serum lipids. New England, Journal of Medicine, 333, 276 – 282. 12.AOAC. 1995. Official method of analysis 14th ed. Association of Official Analytical Chemists, Washington, D.C., U.S.A. 13.Aoyama, T., Fukui, K., Takamatsu, K., Hashimoto, Y., and Yamamoto, T. 2000. Soy protein isolate and its hydrolysate reduce body fat of dietary obese rats and genetically obese mice (yellow KK). Nutrition, 16, 349 – 354. 14.Armstrong, W. B., Kennedy, A. R., Wan, X. S., Atiba, J., Mclaren, C. E., and Meyskens, F. L. 2000. Single-dose administration of Bowman-Birk inhibitor concentrate in patients with oral leukoplakia. Cancer Epidemiology, Biomarkers and Prevention,9, 43 – 47. 15.Aso, K., Yamashita, M., Arai, S., and Fujimak, M. 1974. Hydrophobic Force as a Main Factor Contributing to Plastein Chain Assembly. Journal of Biochemistry., 76, 341-347. 16.Barbara, J. A., Ostade, X., and Lopez, A. 1996. Tumour necrosis factor-alpha (TNF-alpha): the good, the bad and potentially very effective. Immunology Cell Biology, 74, 434 – 43. 17.Belitz, H. D., Gorsch, W., and Schieberle, P. 2009. Food chemistry, 4th edn, 83-86. Heidelberg, Germany: Springer-Verlag Berlin Heidelberg. 18.Beutler, B., and Cerami, A. 1989. The biology of cachectin/TNF-a primary mediator of the host response. Annual Review Immunology, 7, 625 – 55. 19.Blum, J. W., and Hammon, H. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. Livestock Production Science, 66, 151 – 159. 20.Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N., and Williamson, B. 1975. An endotoxin-induced serum factor that causes necrosis of tumors, Proc. Proceedings of the National Academy of Science of USA, 72, 3666 – 3670. 21.Chen, Y. Y., and Chang, H. M. 2004. Antiproliferative and differentiating effects of polysaccharide fraction from fu-ling (Poria cocos) on human leukemic U937 and HL-60 cells. Food and Chemical Toxicology : 759 – 769. 22.Chen, C. W., Chiang, S. H., Wang, S. Y., Lin, Y. T., and Chang, C. Y. 2011. Growth inhibition and differentiating effects of protein hydrolysates frombovine colostrums on human leukemic u937 cells. Journal of Food Biochemistry ISSN 1745-4514. 23.Clemente A. 2000. Enzymatic protein hydrolysates in human nutrition. Trends Food Science and Technology, 11, 254 – 62. 24.Damiens, E., Mazurier, J., Yazidi, I., Masson, M., Duthille, I., Spik, G., and Boilly-Marer, Y. 1998. Effects of human lactoferrin on NK cell cytotoxicity against haematopoietic and epithelial tumour cells. Acta Biochimica and Biophysica, 1402, 277-287. 25.Das, U. N. 2004. Anti-inflammatory nature of exercise. Nutrition, 20 (3), 323-326. 26.Einspanier, R. and Schams, D. 1991. Changes in concentrations of insulin-like growth factors 1 and2 and their binding proteins in human milk: Effect of heat treatment on IGF andIFG binding protein stability. Journal of Dairy Research, 58, 171 – 178. 27.Elfstrand, L., Lindmark-Mansson, L., Paulsson, M., Nyberg, L., and Akesson, B. 2002. Immunoglobulins, growth factors andgrowth hormone in bovine colostrums and the effects of processing. International Dairy Journal, 12, 879 – 887. 28.Estrada, C., Gbmez, C., Martin, C., Moncada S., and Gonzllez, C. 1992. Nitric oxide mediates tumor necrosis factor-alpha cytotoxicity in endothelial cells. Biochemical and Biophysical Research Communications, 186, 475-482. 29.Francis, G. L., Upton, F. M., Ballard, F. J., McNell, K. A., and Wallace, J. C. 1988. Insulin-like growth factors 1 and 2 in bovine colostrum. Sequences andbiological activities comparedwith those of a potent truncatedform. Biochemical Journal, 251, 95 – 103. 30.Fujimaki, M., Arai, S., and Yamashita, M. 1977. Enzymic protein degradation and resynthesis for protein improvement in food proteins. American Chemical Society, 156-187 31.Gamble J. R., Harlan J. M., Klebanoff S. J., and Vadas M. A. 1985. Stimulation of the adherence of

neutrophils to umbilical vein endothelium by human recombinant TNF. Proceedings of the National Academy of Science of USA, USA, 82, 8667 – 71. 32.Gauthier, S. F., Pouliot, Y., and Saint-Sauveur, D. 2006. Immunomodulatory peptides obtained by the enzymatic hydrolysis of whey proteins. International Dairy Journal, 16, 1315 – 1323. 33.Gruen, J. R., and Weissman S. M. 1997. Evolving views of the major histocompatibility complex. Blood, 90, 4252 – 65. 34.Hettiarachchy, N. S., and Kalapathy, U. 1997. Soybean protein products. In K. Liu (Ed.). Soybeans: Chemistry, technology, and utilization, 379 – 411. 35.Hibbs, J. B., Vavrin, Z., and Taintor, R.R. 1987. L-arginine is required for expression of the activated macrophage effector mechanism causing selective metabolic inhibition in target cells. Journal of Immunology, 13X, 550-565. 36.Hsu, K. C., Cheng, M. L., and Hwang, J. S. 2007. Hydrolysates from tuna cooking juice as an anti-hypertensive agent. Journal of Food and Drug Analysis, 15, 169 – 173. 37.Iigo, M., Kuhara T., Ushida, Y., Sekine, K., Moore, M. A., and Tsuda H. 1999. Inhibitory effects of bovine lactoferrin on colon carcinoma 26 lung metastasis in mice. Clinical Experimental Metastasis, 17, 35-40. 38.Jin, Y., Cox, D. A., Knecht, R., Raschdorf, F., and Cerletti, N. 1991. Separation, purification, andsequence identification of TGF-b1 and TGF-b2 from bovine milk. Journal of Protein Chemistry, 10, 3411 – 3416. 39.Jolles P, and Migliore-Samour D. 1986. Preparation of immunological agents by treating lipid-free bovine casein with proteolytic enzyme and fractionating the product. Patent Assignee: Rhone-Poulenc Sante. United States Patent 4, 851, 509, European Patent 170, 550. 40.Jolles P, Migore-Samour D, and Parker F. 1988. Immuno stimulant substances derived from bovine casein and compositions containing the same. Patent Assignee: Rhone-Poulenc Sante. United States Patent 4, 777, 243. 41.Kakuta, I. 1998. Reduction of stress response in carp, Cryprinus carpio L, held under deteriorating environ- mental conditions, by oral administration of bovine lac-toferrin. Journal of Fish Diseases, 21, 161-167. 42.Kapel, N., Benhamou, Y., Buraud, M., Magne, D., Opolon, P., and Gobert, J. G. 1996. Kinetics of mucosal ileal gamma- interferon response during cryptosporidiosis in immuno- competent neonatal mice. Parasitology Reserch, 82, 664 – 667. 43.Kelly, G. S. 2003. Bovine colostrums: a review of clinical uses. Alternative Medicine Review, 8, 378 – 394. 44.Kim, S. E., Kim, H. H., Kim, J. Y., Kang, Y. I., Woo, H. J., and Lee, H. J. 2000. Anticancer activity of hydrophobic peptides from soy proteins. BioFactors, 12, 151 – 155. 45.Kito, M., Moriyama, T., Kimura, Y., and Kambara, H. 1993. Changes in plasma lipid levels in young healthy volunteers by adding an extruder cooked soy protein to conventional meals. Bioscience. Biotechnology and Biochemistry, 57, 354 – 355. 46.Kulkarni, P. R., and Pimpale, N. V. 1989. Colostrum – a review. Indian Journal of Dairy Science, 42, 216. 47.Kuprash, D. V., Tumanov, A. V., Liepinsh, D. J., Koroleva, E. P., Drutskaya, M. S., and Kruglov, A. A. 2005. Novel tumor necrosis factor-knockout mice that lack Peyer ' s patches. European Journal of Immunology, 35, 1592 – 1600. 48.Liao, H. F., Chou, C. J., Wu, S. H., Khoo, K. H., Chen, C. F., and Wang, S. Y. 2001. Isolation and characterization of an active compound from black soybean and its effect on proliferation and differentiation of human leukemic U937 cells. Anticancer Drugs, 841 – 846. 49.Liu, K. 2000. Expanding soybean food utilization. Food Technology, 54, 46 – 58. 50.Lotem, J., and Sachs, L. 1987. Regulation of cell-surface receptors for hematopoietic differentiation-inducing protein MGI-2 on normal and leukemic myeloid cells. International Journal of cancer prevent. 51.Ma, C. Y., Kung, H. T., Chen, J. N., Cheng, C. Y., Chang, H. M,. and Wu, S. B. 2009. Effect of bovine colostrum on the growth inhibition and differentiation of humanleukemic U937 cells. Journal of Food Agricultural, 89, 965-969. 52.Marino, M.W., Dunn, A., Grail, D., Inglese, M., Noguchi, Y., Richards, E., Jungbluth, A., Wada, H., Moore, M., Williamson, B., Basu, S., Old, L. J. 1997. Characterization of TNF-deficient mice. Proceedings of NationalAcademy of Science, 94, 8093 – 8. 53.Manev, V., Maneva, A., and Sirakov, L. 1998. Effect of lactoferrin on the phagocytic activity of polymorphonuclear leucocytes isolated from blood of patients with autoimmune diseases and Staphylococcus aureus allergy. Advances in experimental medicine and biology, 443, 321-330. 54.Makhatadze, N. J. 1998. TNF locus: genetic organisation, and biological implications. Human Immunology, 59, 571 – 9. 55.Marcotty, C., Frankenne, F., van Beeumen, J., Maghuin-Rogister, G., and Hennen, G. 1991. Insulin-like growth factor 1 (IGF-1) from cow colostrum: Purification and characterization. Growth Regulation, 1, 56 – 61. 56.Mead, J. R., and You, X. 1988. Susceptibility differences to Cryptosporidium parvum infection intwostrainsofgamma interferon knockout mice. J. Parasitol. 84, 1045-1048 57.Mendis, E., Rajapakse, N., and Kim, S. K. 2005. Antioxidant properties of a radicalscavenging peptide purified from enzymatically prepared fish skin gelatin hydrolysate. Journal of Agricultural and Food Chemistry, 53(3), 581?587. 58.Mestecky, J., and McGhee, J. R. 1987. Immunoglobulin A molecular, and cellular interactions involved in IgA biosynthesis, and immune response. Advances in Immunology, 40, 135 – 245. 59.Migliore-Samour D, and Jolles P. 1988. Casein, a prohormone with an immunomodulating role for the newborn. Experientia, 44(3), 188-193. 60.Miyauchi, H., Hashimoto, S., Nakajima, M., Shinoda, I., Fukuwatari, Y., and Hayasawa, H. 1998. Bovine lactoferrin stimulates the phagocytic activity of human neutrophils: identification of its active domain, Cellular Immunology, 187, 34-37. 61.Moldoveanu, A. I., Shephard, R. J., and Shek, P. N. 2001. The cytokine response to physical activity and training. Sports Medicine, 31(2), 115-144. 62.Mosmann, T. R., Cherwinski, H., Bond, M.W., Giedlin, M. A., and Coffman, R. L. 1986. Two types of murine helper T cell clone: 1. Definition according to profiles of lymphokine activities and secreted proteins. Journal of Immunology, 136, 2348 – 2357. 63.Mosmann, T. R., and Coffman, R. L., 1989. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology, 7, 145 – 173. 64.Munker, R., and Koeffler, H. P. 1987. Tumor necrosis factor:recent advances. Klin Wochenschr, 65(8), 345-52. 65.Onozaki, K., Urawa, H., Tamatani, T., Iwamura, Y., Hashimoto, T., Baba, T., Suzuki H., Yamada, M., Yamamoto, S. and Oppenheim, J. J. 1988. Synergistic interactions of interleukin 1, inyerferon-bata, and tumor necrosis factor in terminally differentiating a mouse myeloid leukemia cell line(M1). Evidence that interferon-beta is an autocrine differentiating factor. Journal of Immunology. 66.Pakkanen, R., and Aalto, J. 1997. Growth factors andantimic robial factors of bovine colostrum. International Dairy Journal, 7, 285 – 297. 67.Palmar, R. M. J., Bridge, L., Foxwell N. A., and Moncada, S. 1992. The role of nitric oxide in endothelial cell damage and its inhibition by glucocorticoids. British Journal of Pharmacology, 105, 11-12. 68.Pasparakis, M., Alexopoulou, L., Episkopou, V., and Kollias, G. 1996. Immune and inflammatory responses in TNF alpha-deficient mice: a

critical requirement for TNF alpha in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response, Journal of Experimental Medicine, 184, 1397 – 1411. 69.Payne, C. M., Bernstein, C., Bernstein, C., Bernstein, H., Gerner, E. W. and Garewal, H. 1999. Reactive nitrogen species in colon carcinogenesis. Antioxis Redox Signal, 1, 499-67. 70.Petersen, A. M., and Pedersen, B. K. 2005. The anti-inflammatory effect of exercise. Journal of Applied Physiology, 98(4), 1154-1162. 71.Pikarsky, E., Porat, R. M., Stein, I., Abramovitch, R., Amit, S., and Kasem, S. 2004. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature. 431 : 461 – 466. 72.Platanias, L. C., and Vogelzang, N. J. 1990. Interleukin-1:biology, pathophysiology, and clinical prospects. American Journal of Medicine, 89, 621-9. 73.Playford, R. J. 2001. Peptide therapy and the gastroenterologist: colostrums and milk-derived growth factors. Clinical Nutrition, 20, 101 – 106. 74.Puddu, P., Carollo, M.G., Belardelli, F., Valenti, P., and Gessani, S., 2007. Role of endogenous interferon and LPS in the immunomodulatory effects of bovine lactoferrin in murine peritoneal macrophages, Journal of Leukocyte Biology, 82, 347-353. 75.Reiner, S. L., and Seder, R. A., 1995. T helper cell differentiation in immune response. Current Opinion in Immunology, 7, 360 – 366. 76.Rudolf, A., Alkasrawi, M., Zacchi, G., and Lid?聲, G. 2005. A comparison between batch and fed-batch simultaneous saccharification and fermentation of steam pretreated spruce. Enzyme and Microbial Technology, 37(2), 195-204. 77.Rutkowski, P., Kaminska, J., Kowakska, M., and Steffen, J. 2003. Cytokine and cytokine receptor serum levels in adult bone sarcoma patients: correlation with local tumor extent and prognosos. Journal of Surgical Oncology, 84, 151-159. 78.Scammell, A. W. 2001. Production and uses of colostrum. Australian Journal of Dairy Technology, 56, 74 – 82. 79.Sekine, K., Watanabe, E., Nakamura, J., Takasuka, N., Joong, D. K., Asamoto M., Krutovskikh V., Baba-Toriyama H., Ota T., Moore M. A., Masuda M., Sugimoto H., Nishino H., Kakizoe T. and Tsuda H. 1997. Inhibition of azoxymethane-initiated colon tumor by bovine lactoferrin administration in F344 rats. Japan Journal of Cancer Research, 88, 523-526. 80.Shamay. A., Cohen, N., Niwa. M. and Gertler, A. 1988. Effects of insulin-like growth factor I on dcoxyribonuclcic acid synthesis and galactopocsis in bovine undifferentiated and lactating mammary tissue. Endocrinology, 126, 804-809. 81.Shau, H., Kim, A., and Golub, S. H. 1992. Modulation of natural killer and lymphokine- activated killer cell cytotoxicity by lactoferrin, Journal of Leukocyte Biology, 51, 343-349. 82.Siemensma, A. D., Weijer, W. J., and Bak, H. J. 1993. The importance of peptide lengths in hypoallergenic infant formulae. Trends in Food Science and Technology, 4, 16-21. 83.Smith, L. M., Bonafonte, M. T., and Mead, J. R. 2000. Cytokine expression and specific lymphocyte proliferation in two strain of Cryptosporidium parvum-infected gamma-interferon knockout mice. Journal of Parasitology, 86, 300 – 307. 84.Sukan, G., and Andrews, A. T. 1982. Application of the plastein reaction to caseins and to skim milk powder II. Chemical and phy-sical properties of the plasteins. Journal of Dairy Research, 49, 279-293 85.Sun, Y., and Cheng, J. Y. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresource Technology, 83, 1-11. 86.Sutas Y, Soppi E, Korhonen H. 1996. Suppression of lymphocyte proliferation in vitro by bovine caseins hydrolyzed with Lactobacillus casei GG-derived enzymes. Journal of Clinical Immunology, 98(1), 216-224. 87.Takakura, N., Wakabayashi, H., Yamauchi, K., and Takase, M., 2006. Influences of orally administered lactoferrin on IFN-gamma and IL-10 production by intestinal intraepithelial lymphocytes and mesenteric lymph-node cells, Biochemistry Cell Biology, 84, 363-368. 88.Tamatani, T., Kimura, S., Hashimoto, T., and Onozaki. 1989. Purification of guinea pig tumor necrosis factor (TNF) : comparsion of its antiproliferative and differentiative activities for myeloid leukemic cell lines with those of recombinant human TNF. Journal of Biochemistry, 105(1), 55-60. 89.Tokuyama, Y., and Tokuyama, H. 1993. Purification and identification of TGF-b2-relatedgrowth factor from bovine colostrum. Journal of Dairy Research, 60, 99 – 109. 90.Wang, W., and Mejia, E. G. 2005. A new frontier in soy bioactive peptides that may prevent age-related chronic diseases. Comprehensive Reviews in Food Science and Food Safety, 4, 63 – 78. 91.Wang, W. P., Iigo, M., Sato, J., Sekine, K., Adachi, I., and Tsuda, H. 2000. Activation of intestinal mucosal immunity in tumor-bearing mice by lactoferrin. Janpan, Journal of Cancer Reserch, 91, 1022-1027. 92.Wergedahl, H., Liaset, B., Gudbrandsen, O. A., Lied, E., Espe, M., and Muna, Z. 2004. Fish protein hydrolysate reduces plasma total cholesterol, increases the proportion of HDL cholesterol and lowers acyl-CoA: cholesterol acyltransferase activity in liver of Zucker rats. Journal of Nutrition, 134, 1320 – 1327. 93.Wu, H. C., Chen, H. M., and Shiau, C. Y. 2003. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (Scomber austriasicus). Food Research International, 36, 949 – 957. 94.Wyatt, C. R., Brackett, E. J., and Savidge, J. 2001. Evidence for the emergence of a type-1-like immune response in intestinal mucosa of calves recovering from cryptosporidiosis. Journal of Parasitol, 87, 90 – 95. 95.Xu. R. J., Mellor, D. J., Birtles, M. J., Breier, B. H., and Gluckman, P. D. 1994. Effects of oral IGF-I or IGF-II on digestive organ growth in newborn piglets. Biology of the Neonate, 66, 280-287. 96.Yamanaka, H., Hagiwara, k., Kirisawa, R., and Iwai, H. 2003. Proinflammatory cytokines in bovine colostrum potentiate the mitogenic response of peripheral blood mononuclear cells from newborn calves through IL-2 and CD25 expression. Microbiology of Immunology, 47(6), 461 – 468. 97.Zablocka, A., Janusz, M., Macala, J., and Lisowski, J. 2007. A proline-rich polypeptide complex (PRP) isolated from ovine colostrum. Modulation of H2O2 and cytokine induction in human leukocytes. International Immunology, 7,981-988. 98.Zhao, X. H., and Li, Y. Y. 2009. An approach to improve ACE inhibitory activity of casein hydrolysates with plastein reaction catalyzed by Alcalase. European Food Research and Technology, 229, 795 – 805. 99.Zhao, X. H., Wu, D., and Li, T. J. 2010. Preparation and the radical scavenging activity of papain catalyzed casein plasteins. Dairy Science and Technology, 90, 521 – 535. 100.Ziegler, F., Ollivier, J. M., Cynober, L., Masinin, J. P., Coudray-Lucas, C., Levis, E., and Giboudeau, J. 1990. Efficiency of enteral nitrogen support in surgical patients: small peptides v non-degraded proteins. Gut, 31, 1277-1283