Effects Of Glutamine Supplementation On Antioxidant Enzyme Activity And Immune Responses In Burned Mice

Sung-Ling Yeh, PhD[1], Huey-Fang Shang, PhD[2], Ming-Tsan Lin, MD[3], Chiu-Li Yeh, MS[1], Wei-Jao Chen, MD[3]

[1]Institute of Nutrition and Health Science, [2]Department of Microbiology and Immunology, Taipei Medical University, and [3]Department of Surgery, College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China.

Running title: Effect of glutamine on immune response in burns

Corresponding author: Wei-Jao Chen, MD
Department of Surgery
National Taiwan University Hospital
7 Chung-Shan S. Road
Taipei, Taiwan 100
Republic of China
Tel: 886-2-397-0800, ext. 2122
Fax: 886-2-341-2969
E-mail: sangling@tmu.edu.tw

Abstract

This study investigated the effect of glutamine (Gln) supplementation on specific antibody production and antioxidant enzyme activities in burned mice vaccinated with detoxified *Pseudomonas* exotoxin A linked with the outer membrane proteins I and F (PEIF). Also, the survival rate of non-vaccinated burned mice infected with *Pseudomonas aeruginosa* (*P. aeruginosa)* was evaluated. This study consisted 3 consecutive experiments. Experiment 1: Thirty BALB/c mice were assigned to 2 groups. Control group fed with casein as the protein source; Gln group was supplemented with 4% Gln (w/w) to replace part of casein. The mice were immunized twice with PEIF and the production of specific antibodies against PEIF was measured every week. Eight weeks after immunization, all mice received a 30% body surface area burn injury. Mice were sacrificed 24h after the burn. The antioxidant enzyme activities and lipid peroxides in the tissues as well as specific antibody production were analyzed. Experiment 2: Twelve mice were divided into the control and the Gln groups, and fed with one of the 2 experimental diets for 4 weeks. Then burn injury was induced and mice were sacrificed 24h later. In vitro, splenocyte was cultured and interleukin (IL)-4,IL-10 were measured after mitogen stimulation. Experiment 3: Survival rates of non-vaccinated burned mice complicated with *P. aeruginosa* infection were evaluated. Survival rate was observed for 8 days after the burn. The results demonstrated that antioxidant enzyme activities and lipid peroxides in tissues tended to be lower in the Gln group than in the control group after the burn. Specific antibodies production against *P. aeruginosa* increased significantly in the Gln group at 4 and 7 weeks after immunization, and at 24 h after the burn. IL-4 concentrations in the mitogen stimulated splenocyte was significantly higher in the Gln group than the control group.

Survival rates of non-vaccinated burned mice in the Gln group were significantly higher than the control group after bacterial infection between the 2 groups. These results suggest that vaccinated mice receiving Gln supplemented diet may enhance humoral immunity and attenuate oxidative stress induced by burn injury. Also, Gln supplementation improves survival of burned mice complicated with *P. aeruginosa* infection.

Key words: Burns, Glutamine, Antioxidant enzyme activity, Antibody, vaccination, *P. aeruginosa.*

Introduction

Burn injury is a post-traumatic inflammatory disease. Severe injury, particularly thermal injury leads to a profound depression of both humoral and cell-mediated immunity (1-4). The immunosuppression occurs after burn injury causes increased susceptibility to infection (5). Besides, the response to the initial burn injury and the presence of the burn are often associated with secondary damage to tissues distant from the injured skin (6-9). This response appears to be mediated by both reactive oxygen metabolites and activated neutrophils (10,11). Nishigaki et al. (12) reported that lipid peroxide levels increase in burned rat skin, and lipid peroxide generated in the burn wound may accumulate in the liver, lung, kidney, and gut of injured animals.

Glutamine (Gln) has traditionally been thought as a nonessential amino acid, but laboratory and clinical data suggests that it may be essential during certain inflammatory conditions, such as infection and injury (13-14). A number of studies have

demonstrated beneficial effects of supplying Gln or its precursors (ornithin α-ketoglutarate and α-ketoglutarate) on metabolic stressed conditions by increasing nitrogen retention, preserving integrity of the intestinal mucosa and intestinal permeability, maintaining immunologic function and reducing infections (13-19). Parry-Billings et al. (14) reported that plasma Gln levels decreased and remained depressed for 3 weeks after injury in burn patients. Their experiments showed that the depressed Gln concentrations were associated with reduced proliferation of lymphocytes from healthy volunteers and depressed phagocytosis by peritoneal macrophage from normal mice. Ogle et al. (20) also reported that Gln improved the bactericidal ability of abnormal neutrophils from the pediatric patients after burns. Although Parry-Billings et al. and Ogle et al. suggested the efficacy of Gln supplementation, they did not supply Gln to their patients. Very limited studies of Gln supplementation on immune response have been performed in burn injury. To our knowledge, there is no study investigating the effects of Gln supplementation on humoral immunity and antioxidant status in burn injury.

Pseudomonas bacteremia presents an another medical problem because of its high mortality and its strong antibiotic resistance (21). Patients with extensive thermal injury are at high risk of infection with *Pseudomonas aeruginosa* (*P. aeruginosa)* (22). Thus, these patients are candidates to received *P. aeruginos* vaccine. Chen et al (23) designed a novel vaccine, PEIF, against *P. aeruginosa*, which can enhance specific antibody production and effectively block *P. aeruginosa* challenge in burned mice. In this study, we immunized mice with this novel vaccine against *P. aeruginosa* before burn injury to investigate whether Gln supplementation has beneficial effects on attenuating oxidative stess induced by burn injury and the specific antibody production against PEIF. In addition, in vitro cytokine secretion

and the survival rates in non-vaccinated burned mice complicated with a lethal dose of *P. aeruginosa* were also evaluated.

2. Materials and methods

2.1 Animals

Male BALB/c mice weighing 10-15 g (4 weeks of age) were used in this study. All mice were housed in temperature- and humidity-controlled rooms, and allowed free access to standard chow for 1 week prior to the experiment. Animals included in this study were kept under standard experimental animal care protocols.

2.2 Study protocol

Experiment 1: Thirty mice were randomly assigned to 2 groups, 15 in each group. One group was fed a control diet (control), in which all amino acids were provided by casein. The other group was fed Gln (Gln) supplemented diet, by which 4% Gln (w/w) was used to replace part of casein, corresponding to 40% of the dietary nitrogen source. Two diets were isonitrogenous (Table 1). Mice were anesthesized with ether, and blood was taken from the retrobulbar vessels before immunizing with the novel PEIF vaccine against *P. aeruginosa*. The production and purification of the recombinant PEIF protein followed procedures described previously (23). The emulsified vaccine was prepared by mixing the purified recombinant PEIF protein with an equal volume of complete Freund's adjuvant, and then each mouse was vaccinated subcutaneously at a dose of 2 μg/mouse on day 1. A booster injection was given at a dose of 4 μg/mouse of PEIF emulsified with an equal volume of incomplete Freund's adjuvant on day 28. Before the burn, immunized mice were

bled from retrobulbar vessels on days 21, 28, 35, 42, 49, and 56. The sera were isolated and stored at –70℃ until assay. After 8 weeks, a modification of the burned mouse procedure was used (24,25). Mice were anesthetized with sodium pentobarbitol（0.71 μg/g body wt）and shaved dorsally prior to burning. A Teflon template with a precisely cut window （2.5 x 3-cm^2）was pressed firmly against the shaved back. Ethanol (95% v/v, 0.5 ml) was evenly spread over the area of the back outlined by the window, ignited, and allowed to burn for 15 s (24,25). Animals were immediately resuscitated by an intraperitoneal injection of sterile 0.9% saline (10 ml/100 g body wt.) (15). This procedure produced a full-thickness burn injury on approximately 30% of the total body surface area. All mice were deprived of food for 24 h with only free access to water, in order to induce a hypermetabolic state simulated in burn patients (16,26). Mice were anesthetized and sacrificed by cardiac puncture 24 h after the burn. Blood samples were collected in tubes containing heparin for analysis of T lymphocyte subpopulations. The remaining blood samples were centrifuged to isolate the sera. Amino acid was analyzed by the standard ninhydrin technology (Beckman Instrument, model 6300, Palo Alto, CA), after deproteinization of the plasma with 50% salicylic acid (27). Tissues including liver, and kidney were rapidly excised. All samples were stored at –70 ^{o}C until being assayed for antioxidant enzyme activities and lipid peroxide levels.

Experiment 2: Twelve mice were divided into the control and Gln groups, and fed either with control diet or Gln supplemented diet for 4 weeks. After that, burn injury was induced and mice were sacrificed 24h after the burn. Spleens of the mice were removed aseptically, and were placed in phosphate buffer saline (PBS) for the pretreatment of in vitro splenocyte cultures.

Experiment 3: Thirty mice were allocated to the non-vaccinated group and were

further divided into 2 experimental groups, with each group containing 15 mice. Mice were fed with control or Gln diets for 8 weeks. After that, burn injury was induced and immediately after the burn, 3.2×10^5 CFU (1x LD_{50}) *P. aeruginosa* strain PAO1 (ATCC 15692) was injected to the burned skin of the mice. Survival of the burned mice was recorded every 6 h in the first 3 days, and then every 12 h until the end of 8 days. The mice were also deprived of food except water for 24 h, then the experimental diets were given for the remaining days.

2.3 Measurements of antioxidant enzymes and TBARS

Fifteen percent tissue homogenates were prepared at 4 ^{o}C in a 250-mM sucrose solution, containing 10 mM-Hepes (pH 7.4) using a homogenizer. The homogenates were centrifuged to discard cell debris and mitochondria (28). The supernatant was used for analysis of superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) activities (enzyme kits from Randox). Protein concentrations of supernatants were measured by Lowry's method. The production of thiobarbituric acid-reactive substances (TBARS, assumed to be mainly malondialdehyde and its precursors) in mouse liver, and kidney homogenates was determined by the method of Uchiyama and Mihara (29). The molar extinction coefficient of malondialdehyde was assumed to be 156,000 (30).

2.4 Analysis of specific antibody production against PEIF

The specific antibody production of vaccinated mice was measured by ELISA as described previously (23). Briefly, purified recombinant PEIF protein was coated on polyvinylchloride, flat-bottom, 96-well Falcon microtiter plates overnight at 4℃ with a protein concentration of 3 μg/ml in coating buffer（pH 9.6 carbonate buffer）. The coated plates were then blocked with 0.5% BSA-PBS. Mouse sera from each group were diluted

1000-fold with 0.5% BSA-PBS, and 50 μl of diluted sera was added to the coated well and incubated for 1h at 37 ℃. Bound specific antibodies were detected using peroxidase-conjugated goat anti-mouse secondary antibody（Sigma,）. After 3 washings, 100 μl of substrate solution (0.54 mg/ml 2,2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid, ABTS, and 0.03% H_2O_2 in 0.1 M citric acid) was added to each well, and the absorbance was read after 15 min using a microplate reader at 405nm. Normal mouse serum was used as the negative control.

2.5 Analysis of T lymphocyte subpopulations

Flow cytometry was used to determine the proportions of CD4+, CD8+ and CD3 T lymphocytes in fresh blood. One hundred microliters of blood was incubated for 15 min at 4 ^{o}C with 10 ul of fluorescein-conjugated (FITC) rat monoclonal anti-mouse CD4+, phycoerythrin-conjugated (PE) rat anti-mouse CD8+ and FITC rat anti-mouse CD3 (Serotec, Oxford, UK). After that, red blood cells were lysed with lysing buffer (Serotec). Fluorescence data were collected on 5 x 10^4 viable cells and analyzed by flow cytometry (Coulter, Miami, FL, USA).

2.6. In vitro cultures of splenocytes and cytokine assay

Splenocytes were obtained by mechanical disruption using the homogenizer. Cell suspensions were passed through sterile nylon mesh to remove debris. RBC were lysed by sterile distill water for 15 sec, and immediately neutralized to isotonic cell suspensions. After washing with PBS for 3 times (300 x g for 5 min), splenocytes were resuspended in RPMI-1640 with supplements. The number of isolated splenocytes was determined by hemacytometer count using trypan blue dye exclusion. Phytohemagglutinin (PHA, 10 ng/mL; Sigma Co., USA) were used to stimulate cytokine production by isolated splenocyte in culture. Triplicate wells of 96-well

flat-bottomed microtiter plate (Falcon, Becton Dickinson, Co., Oxford, CA.) were seeded with 100 ul of splenocytes (2.5 x 10^6 cells/ml in RPMI-1640) and 100 ul mitogen. Control wells contained cells plus 100 ul of medium. The final volume in all wells was 200 ul. Splenocytes were incubated in the presence or absence of PHA for 24 hr at 37°C in an atmosphere of 95% air and 5% CO_2. Supernatants were centrifuged and stored at –70°C until for the analysis of cytokine. Interleukin (IL)-4, IL-10 concentrations in splenocyte supernatant were determined with commercially available enzyme-linked immunosorbent assay (ELISA) in microtiter plate, antibodies specific for mice cytokines were coated onto the wells of the microtiter strips provided (Amersham Pharmacia Biotech Inc., UK).

2.6 Statistics

Data are expressed as the means ± SD. Differences between groups were analyzed by student's *t* test. Survival rate was measured by Kaplan-Meier survival analysis. A p value < 0.05 was considered statistically significant.

3. Results

There were no differences in initial body weights and weights after experimental diets between the 2 experimental groups in either experiment 1, 2, or 3 (data not shown). The results in experiment 1 showed Gln-supplemented group had significantly higher plasma Gln levels than did the control group (462.2 ± 82.6 vs. 332.5 ± 37.7 nmol/mL, $p < 0.05$). No significant differences in the percentages of CD4, CD8, CD3 T cells or the CD4/CD8 T cell ratio were observed between the Gln and control groups after the burn. (Table 2). Antibody production increased

logarithmically after the second booster and reached a plateau after 7 weeks. The specific antibody production in the Gln group was significantly higher than in the control group at various time schedule (Fig.1). The SOD activities and lipid peroxide concentrations in kidney, and GSHPx activities in liver were significantly lower in the Gln group than in the control group after the burn of the vaccinated mice. (Table 3).

In experiment 2, stimulation of splenocytes with PHA significantly increased the production of IL-4 in Gln group. However, the production of IL-10 did not differ between the 2 groups when the same concentration of mitogen was treated (Fig. 2A,B).

In experiment 3, the survival rate of non-vaccinated burned mice in the Gln group was significantly higher than that of the control group after bacterial infection ($p < 0.001$) (Fig. 3).

4. Discussion

In this study, we administered Gln before burn injury to investigate the effect of Gln supplementation on burned mice, because rodents are known to be relatively resistant to trauma and recover rapidly after the burn (16). Since very few studies have investigated the effect of Gln on burn injury, the results of this study may provide basic information on the possible roles of Gln in burns. We did not include a non-burn control group in this study, because numerous studies have already shown that burn injury results in oxidative stress and impaired immune function (1-4, 6-9).

Gln is of major importance for muscle metabolism and is a preferred energy

source for cells of the intestinal mucosa and of the immune system, particularly macrophage and lymphocytes (20). An in vitro study by Rohde et al. (31) showed that Gln influences the production of T cell-derived cytokines and is important for optimal T lymphocyte proliferation. Yaqoob and Calder (32) revealed that IL-2 production and IL-2 receptor expression depend on the concentration of Gln present in the culture medium. Gismondo et al. (33) also showed that orally administered Gln to nude mice significantly increased intestinal CD3, CD4, CD8 lymphocytes when compared to the group without Gln supplementation. In this study, blood CD4, CD8 T cells, and the CD4/CD8 ratio did not differ between the 2 groups. Also, there were no differences in CD3 populations between the 2 groups. Our finding was inconsistent with the reports mentioned above. There are 2 possible explanations: 1) the result of in vitro studies may not actually reflect the in vivo situations. 2) sample used for evaluating the effect of Gln on lymphocyte functions were derived from healthy volunteer or normal rodents. It may differ from the stressed metabolic condition observed in this study, and may consequently lead to different immune response.

The finding in this study showed that the production of specific antibodies against *P. aeruginosa* was significantly higher in the Gln group than in the control group after immunization and after the burn. In order to understand the possible role of cytokine secretion on antibody production, IL-4 and IL-10 production in mitogen-stimulated splenocyte cultures were analyzed. IL-4 and IL-10 are produced by Th2 lymphocytes. These 2 cytokines activate B cells and promotes humoral immunity (34). Although IL-10 did not differ between the 2 groups, IL-4 production was significantly higher in the Gln group than the control group when the same concentration of mitogen was treated. This result suggests that orally supplemented

Gln may enhance IL-4 secretion which may consequently promote antibody production in burned mice.

Burn injury is a trauma with high oxidative stress (35). After burn injury, generalized tissue inflammation is present in uninjured organs within hours (36). Organ injury remote from the region of thermal injury has been shown to be due to intravascular action of complements, resulting in stimulation of intravascular neutrophils, leading to the formation of toxic oxygen products (8). Lipid peroxide is thought to be one of the most harmful substances produced after burns (37). SOD and GSHPx are enzymes which protect tissues from the effects of free radicals and lipid peroxides, and the activities of both SOD and GSHPx increase after free-radical-mediated injury and lipid peroxidation (38). Saitoh et al. (37) demonstrated that Mn-SOD activities in lung and kidney were significantly higher than in the control group after a burn. The results of this study reveal that SOD activities in kidney and GSHPx activities in liver were significantly lower in the Gln group when compared with the control group. Also, lipid peroxide concentrations in kidney were lower in the Gln group than in the control group after the burn of the vaccinated mice. This finding may indicate that Gln supplementation tended to attenuate the oxidative stress induced by burn injury. Study by Morlion et al. (39) showed that generation of cysteinyl-leukotrienes was enhanced after Gln supplementation in surgical patients. Hong et al. (40) also revealed that Gln preserves liver glutathione after lethal hepatic injury. Because glutathione is a potent antioxidant that protects tissue from free radical injury, cysteinyl-leukotrienes also contain glutathione, it is possible that Gln supplementation enhances the antioxidant protection after the burn.

Animal study by Gianotti et al. (41) showed that Gln supplementation decreases

bacterial translocation and increases survival rates in septic rats. Wischmeyer et al. (42) demonstrated that the incidence of gram-negative bacteremia was significantly reduced in the intravenous Gln administered burn patients. In addition, a trend toward lower mortality rate in the Gln group was observed. In this study we also observed a higher survival rates in the Gln group than the control group in burned mice complicated with *P. aeruginosa* infection. The survival rates of the Gln and control groups were 23% and 0% at 8 days after the burn. Since the defense mechanism against bacteria is depended on the neutralization and opsonization of antibodies (24), the result in this study may indicate that Gln augment host defense by enhancing antibody production, and consequently improve survival in burned infected mice.

In conclusion, the findings of this study suggest that Gln supplementation did not influence T cell population, however, humoral immunity was enhanced and oxidative stress induced by burn injury was attenuated in vaccinated burned mice. Also, Gln administration before burn injury significantly increased survival rates in burned mice complicated with *P. aeruginosa* infection.

Acknowledgements

This study was supported by research grant NSC 90-2314-B-002-305 from the National Science Council, ROC.

References

1. Farrell MF, Day NK, Tsakraklides V, et al. Study of lymphocyte depletion and serum complement perturbations following acute burn trauma. Surgery 1973;73:697-705.

2. Goldman AS, Rudloff HB, McNamee R, et al. Deficiency of plasma humoral recognition factor activity following burn injury. J Reticuloendothel Soc 1974;15:193-8.

3. Miller CL, Baker CC. Changes in lymphocyte activity after thermal injury: the role of suppressor cells. J Clin Invest 1979;63:202-10.

4. Ninnemann JL. Immunosuppression following thermal injury through B cell activation of suppressor T cells. J Trauma 1980;20:206-13.

5. O'Sullivan ST, O'Connor TPF. Immunosuppression following thermal injury: the pathogenesis of immunodysfunction. Br J Plastic Surg 1997;50:615-23.

6. Till GO, Beauchamp C, Menapace D, et al. Oxygen radical dependent lung damage following thermal injury of rat skin. J Trauma 1983;23:269-77.

7. Till GO, Hatherill JR, Tourtellotte WW, et al. Lipid peroxidation and acute lung injury after thermal trauma to skin. Am J Pathol 1985;119:376-84.

8. Cetinkale O, Konukoglu D, Senel O, et al. Modulating the functions of neutrophils and lipid peroxidation by FK506 in a rat model of thermal injury. Burns 1999;25:105-12.

9. Gurbur V, Corak A, Yegen BC, et al. Oxidative organ damage in a rat model of thermal injury: the effect of cyclosporin A. Burns 1997;23:37-42.

10. Simpson R, Alon R, Kobzik L et al. Neutrophil and neutrophil-mediated injury in

intestinal ischemia-reperfusion. Ann Surg 1993;218:444-54

11. Hernandez LA, Grisham MB, Twohig B et al. Role of neutrophils in ischemia-reperfusion induced microvascular injury. Am J Physiol 1987;253:H699-703

12. Nishigaki I, Hagihara M, Hiramatsu M, et al. Effect of thermal injury on lipid peroxide levels of rat. Biochem Med 1980;24:185-9.

13. Willmore DW. The effects of glutamine supplementation in patients following elective surgery and accidental injury. J Nutr 131:2543S-2549S, 2001

14. Parry-Billings M, Evans J, Calder PC, Newsholme EA. Does glutamine contribute to immunosuppression after major burns? Lancet 336:523-525, 1990

15. Boucher JL, Obled C, Farges MC, et al. Ornithine α-ketoglutarate modulates tissue protein metabolism in burn-injured rats. Am J Physiol 1997;273:E557-63.

16. Vaubourdolle M, Coudray-Lucas C, Jardel A, et al. Action of enterally administered ornithine α-ketoglutarate on protein breakdown in skeletal muscle and liver of the burned rats. J Parenter Enter Nutr 1991;15:517-20.

17. Stehle P, Wurste N, Puchestein C, Zander J, Albers S, Lawin P. Effect of parneteral glutamine peptide supplements on muscle glutamine loss and nitrogen balance after major surgery. Lancet 1:231-233, 1989

18. van der Hulst, RRWJ, van Kreel BK, von Meyenfeldt MF, Brummer RM, Arends J, Deutz NEP, Soeters PB. Glutamine and the preservation of gut integrity. Lancet 341:1363-1365, 1993

19. Calder PC. Glutamine and the immune system. Clin Nutr 13:2-8, 1994

20. Ogle CK, Ogle JD, Mao JX, Simon J, Noel JG, Li BG, Alexander JW. Effect of glutamine on phagocytosis and bacterial killing in normal and pediatric burn patient neutrophils. J Parenter Enter Nutr 18:128-133, 1994

21. Hancock REW. Intrinsic antibiotic resistance of *Pseudomonas aeruginosa*. J Antimicrob Chemother 1986;18:653-6.

22. Liberman MM, Walker HL, Ayala E, Chapa I. Active and passive immunization with *Pseudomonas aeruginosa* ribosomal vaccines and antisera in the burned rat model. J Surg Res 40:138-144, 1986

23. Chen TY, Shang HF, Chen TL, et al. Recombinant protein composed of *Pseudomonas* exotoxin A, outer membrane protein I and F as vaccine against *P. aeruginosa* infection. Appl Microbiol Biotechnol 1999;52:524-33.

24. Holder IA. The pathogenesis of infection owing to *Pseudomonas aeruginosa* using the burned mouse model: experimental studies from the Shriners Burns Institute, Cincinnatti. Can J Microbiol 1985;31:393-402.

25. Holder IA, Jogan M. Enhanced survival in burned mice treated with antiserum prepared against normal and burned skin. J Trauma 1971;11:1041-6.

26. Cynober L. Amino acid metabolism in the thermal burns. J Parenter Enter Nutr 1989;13:196-205.

27. Smith RJ, Panico K: Automated analysis of o-phthalaldehyde derivatives of amino acids in physiological fluids of reverse phase high performance liquid chromatography. J Liq Chromatogr 8:1783-1795, 1985

28. Yeh SL, Chang KY, Huang PC, Chen WJ. Effects of n-3 and n-6 fatty acids on

plasma eicosanoids and liver antioxidant enzymes in rats receiving toal parenteral nutrition. Nutrition 13:32-36, 1997

29. Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Chem 1978;86:271-8.

30. Hu ML, Frankel EN, Leibovitz BE, et al. Effect of dietary lipids and vitamin E on in vitro lipid peroxidation in rat liver and kidney homogenates. J Nutr 1989;119:1574-82.

31. Rohde T, Maclean DA, Klarlund Pedersen B. glutamine, lymphocyte proliferation and cytokine production. Scand J Immunol 44:648-650, 1996

32. Yaqoob P, Calder PC. Glutamine requirement of proliferating T lymphocytes. Nutrition 13:646-651, 1997

33. Gismondo MR, Drago L, Fassina MC, Vaghi I, Abbiati R, Grossi E. immunostimulating effect of oral glutamine. Dig Dis Sci 43:1752-1754, 1998

34. Dipiro JT. Cytokine networks with infection: mycobacterial infections, leishmaniasis, human immunodeficiency virus infection, and sepsis. Pharmacotherapy 1997;17:205-23.

35. Tredget EE, Yu YM. The metabolic effects of thermal injury. World J Surg 1992;16: 68-79.

36. Youn YK, LaLonde C, Demling R. The role of mediators in the response to thermal injury. World J Surg 1992;16:30-6.

37. Saitoh D, Okada Y, Ookawara T, et al. Prevention of ongoing lipid peroxidation by wound excision and superoxide dismutase treatment in the burned rat. Am J

Emerg Med 1994;12:142-6.

38. Gonzales R, Auclair C, Voisin E, et al. Superoxide dismultase, catalase, and glutathione peroxidase in red blood cells from patients with malignant disease. Cancer Res 1984;44:4137-9.

39. Morlion BJ, Stehle P, Wachtler P, Siedhoff HP, Kohler M, Konig W, Furst P, Puchstein C. Total parenteral nutrition with glutamine dipeptide after major abdominal surgery. Ann Surg 227:302-308, 1998

40. Hong RW, Rounds JD, Helton WS, Robinson MK, Wilmore DW. Glutamine preserves liver glutathione after lethal hepatic injury. Ann Surg 215:114-119, 1992

41. Gianotti L, Alexander JW, Gennari R, Pyles T, Babcock GF. Oral glutamine decreases bacterial translocation and improves survival in experimental gut-origin sepsis. J Parenter Enter Nutr 19:69-74, 1995

42. Wischmeyer PE, Lynch J, Liedel J, Wolfson R, Riehm J, Gottieb L, Kahana M. glutamine administration reduces gram-negative bacteria in severely burned patients: a prospective, radomized, double blind trial verus isonitrogenous control. Crit Care Med 29:2075-2080, 2001

FIGURE LEGENDS

Fig. 1. Production of PEIF-specific antibodies in the control and the Gln groups. Mice were immunized twice with recombinant PEIF protein on days 1 and 28, and sera antibody titers were measured by ELISA at weeks 0, 3, 4, 5, 6, 7, and 8. The dilution of mice antiserum was 1:1000. * Significant difference between the 2 groups ($p < 0.05$).

Fig. 2. A) Interleukin (IL)-4 and B) IL-10 concentrations in the PHA-stimulated spenicyte cultures. * $p < 0.05$ by student's t test. There was no difference in IL-10 concentrations between the 2 groups after mitogen stimulation.

Fig.3 Survival curves of non-vaccinated burned mice challenged with $1xLD_{50}$ *P. aeruginos*. The survival rates in the Gln group were significantly higher than the control group ($p < 0.001$).

Table 1

Composition of the experimental diets (g/kg)

Component	Control	Gln
Casein	200	151
Glutamine	--	40
Protein N	31.3	31.3
Soybean oil	50	50
Corn starch	636	645
Salt mixture[1]	40	40
Vitamin mixture[2]	10	10
Methylcellulose	60	60
Choline chloride	1	1
DL-Methionine	3	3

[1]Salt mixture contains the following (mg/g): calcium phosphate diabasic 500mg, sodium chloride 74mg, potassium sulfate 52mg, potassium citrate monohydrate 220mg, magnesium oxide 24mg, manganese carbonate 3.5mg, ferric citrate 6mg, zinc carbonate 1.6mg, curpric carbonate 0.3mg, potassium iodate 0.01mg, sodium selenite 0.01mg, and chromium potassium sulfate 0.55mg.

[2]Vitamin mixture contains the following (mg/g): thiamin hydrochloride 0.6mg, riboflavin 0.6mg, pyridoxine hydrochloride 0.7mg, nicotinic acid 3mg, calcium pantothenate 1.6mg, D-biotin 0.02mg, cyanocobalamin0.001mg, retinyl palmitate 1.6mg, DL-α-tocopherol acetate 20mg, cholecalciferol 0.25mg, menaquinone 0.005mg.

Table 2. Superoxide dismutase (SOD), glutathione peroxidase (GSHPx) activities and thiobarbituric acid-reactive substances (TBARS) in liver, and kidney homogenate after the burn

	SOD(U/g tissue)		GSH-Px(U/g tissue)		TBARS(nmol/g tissue)	
	Control	Gln	Control	Gln	Control	Gln
Liver	6.85 ± 1.34	10.38 ± 8.99	1.91 ± 0.32	1.64 ± 0.28*	2.91 ± 0.90	2.55 ± 0.58
Kidney	7.84 ± 1.45	5.32 ± 3.14*	1.11 ± 0.31	1.20 ± 0.3	2.34 ± 0.74	1.85 ± 0.61*

Data are expressed as the Mean ± SD.

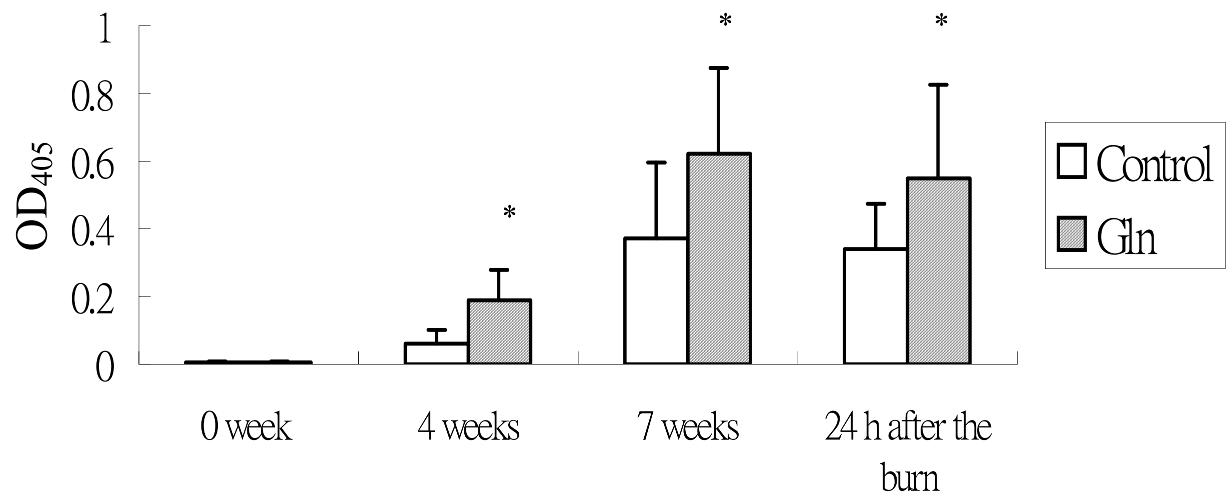
*Significantly different from the Control group at $p < 0.05$

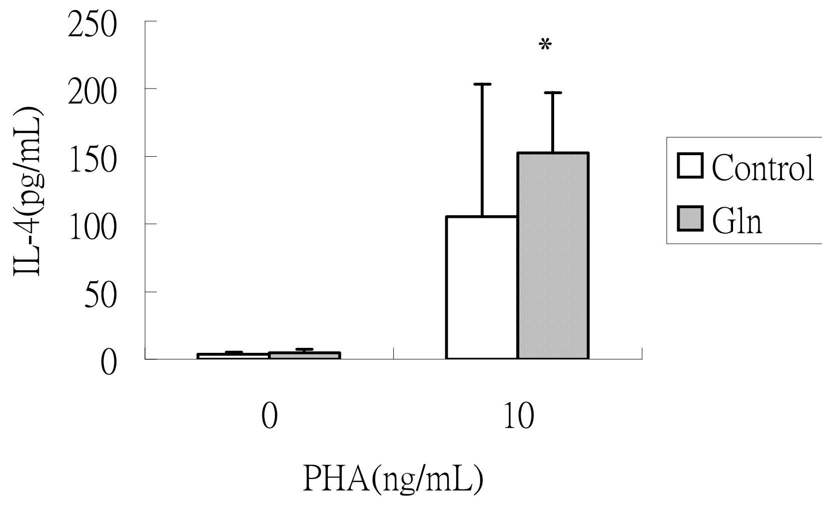
Table 3

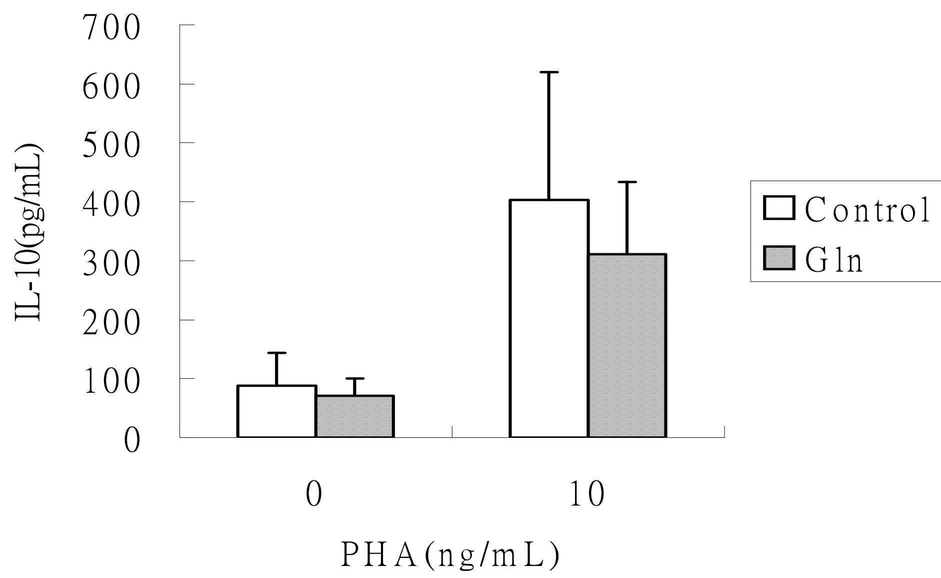
Blood CD4, CD8, CD3 cells and the CD4/CD8 ratio between the 2 groups after the burn

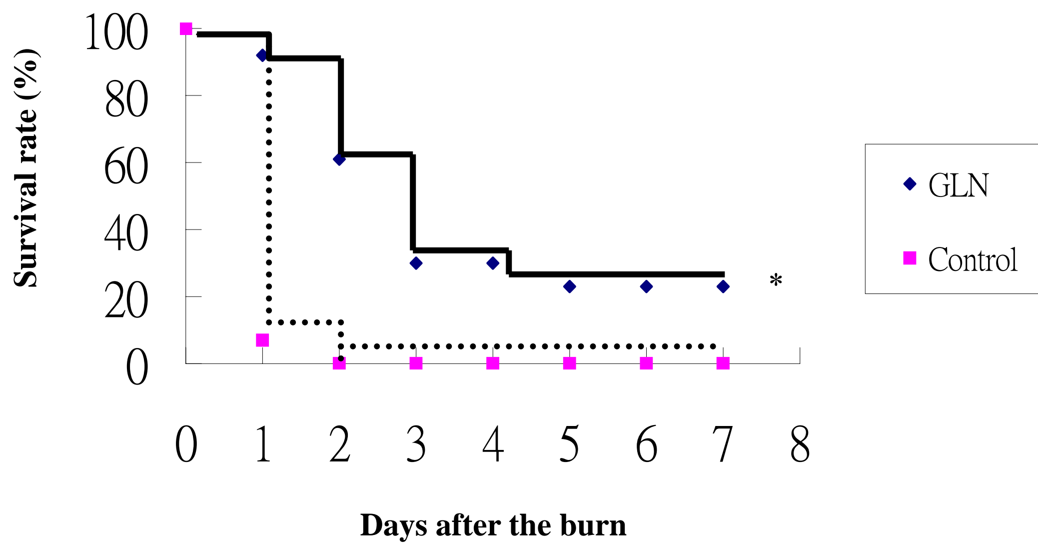
	CD4 (%)	CD8 (%)	CD4/CD8	CD3 (%)
Control	32.4 ± 6.9	14.8 ± 2.4	2.18 ± 0.23	45.3 ± 7.0
Gln	31.3 ± 4.8	12.8 ± 2.2	2.46 ± 0.25	38.5 ± 5.8

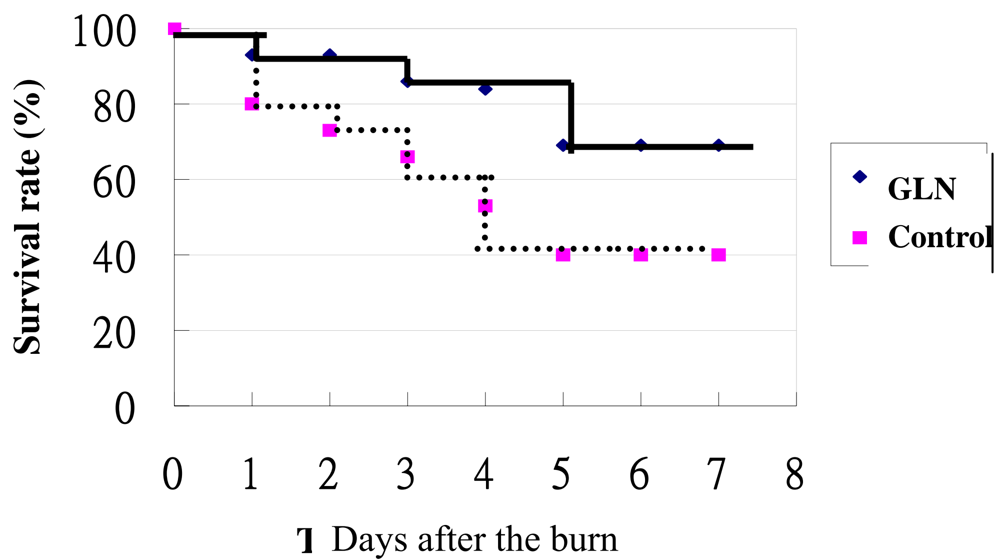
There were no significant differences in the CD4, CD8, or CD3 populations or the CD4/CD8 ratio between the 2 groups.


1
0.8
0.6
0.4
0.2
0
OD405
*
*
*
Control
Gln
0 week
4 weeks
7 weeks
24 h after the burn


250
200
150
100
50
0
IL-4(pg/mL)
*
Control
Gln
0
10
PHA(ng/mL)


700
600
500
400
300
200
100
0
IL-10(pg/mL)
Control
Gln
0
10
PHA(ng/mL)


100
80
60
40
20
0
Survival rate (%)
0
1
2
3
4
5
6
7
8
Days after the burn
*
GLN
Control


100
80
60
40
20
0
Survival rate (%)
0
1
2
3
4
5
6
7
8
Days after the burn
GLN
Control