critically ill patients.[1] Arginine (Arg) is of particular interest in this regard as accumulating experimental and clinical evidence suggests that Arg not only improves protein metabolism but also enhances immune function in severely injured animal models and patients.[2-6] In addition, Arg is the sole precursor of nitric oxide (NO) which may be involved in various bioactivities.[7]

Burn injury is a post-traumatic inflammatory disease with increased oxidative stress; the pathophysiologic alterations include extensive nitrogen loss, an increased metabolic rate, changes in hormones secretion, and immunologic deficiency.[8] Studies have revealed that Arg improves wound healing and reduces hospital stay when added to the diet of burn patients.[9-11] A study by Cui et al.[12] showed that dietary Arg supplementation improves protein anabolism and attenuates muscle protein catabolism after thermal injury. Previous work in our laboratory demonstrated that dietary Arg supplementation attenuates the oxidative stress induced by burn injury, and a better in vitro macrophage response was observed when Arg was administered.[13] Studies have also shown that after severe burn injury, plasma Arg declines by 30%-40%, and dietary Arg supplementation can replenish the Arg level in plasma.[13] Arg is considered a conditionally essential amino acid in burn patients.[14,15]

Pseudomonas aeruginosa (*P. aeruginosa*) is an opportunistic pathogen that often infects burne patients.[16,17] Therapy for *P. aeruginosa* infection is hindered by its well-known antibiotic resistance.[18] Production of specific antibodies is important for resolving bacterial infections, because antibodies may neutralize bacterial toxins and attract phagocytic cells to ingest and kill the bacteria.[19] Saito et al.[20] demonstrated that Arg supplementation improved survival rates in non-infected animals. To our knowledge, there is no study, so far, investigating the effect of Arg supplementation on the production of specific antibodies and on the survival rate in burned rats complicated with infection. We have designed a novel vaccine called PEIF against *P. aeruginosa*, which can effectively block *P. aeruginosa* challenge in burned mice.[21] The chimeric protein is composed of the receptor binding and membrane translocation domains of *Pseudomonas* exotoxin A (PE) linked with the outer membrane proteins I and F, which together is designated PEIF.[21] In this study, we immunized rats with this novel vaccine against *P. aeruginosa* before burn injury to investigate whether dietary Arg supplementation enhances specific antibody production against PEIF or alters T lymphocyte populations. In addition, the survival rate in burned rats complicated with a lethal dose of *P. aeruginosa* was also evaluated.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 80-100 g (4 weeks of age) were used in this study. All rats were housed in temperature- and humidity-controlled rooms, and were allowed free access to standard chow for 1 week prior to the experiment. The animals included in this study were kept under standard experimental animal care protocols.

Study Protocol

Experiment 1

Twenty rats were randomly assigned to 2 groups, with 10 rats in each group. One group was fed with 2.3% of total calories as Arg supplementation in addition to casein (Arg group). The other group was fed glycine instead of Arg (Gly group). The 2 diets were isonitrogenous (Table 1). Blood was obtained by tail venipuncture before immunization with the novel PEIF vaccine against *P. aeruginosa*. The production and purification of the recombinant PEIF protein followed a procedure described previously.[21] The purified recombinant PEIF protein was emulsified with an equal volume of complete Freund's adjuvant, and then each rat was vaccinated subcutaneously at a dose of 30 μg/0.5 mL on day 1. A booster injection at 50 μg/0.5 mL of PEIF emulsified with an equal volume of incomplete Freund's adjuvant was given on day 28. The immunized rats were bled by tail venipuncture on days 21, 28, 35, 42, 49, and 56 after the first injection and before the burn experiment. Respective sera were isolated and stored at -70 °C until assay.