Table 1. Composition of the experimental diets (g/kg)

Component	Arg suppl. group	Gly suppl. group
Arginine	24	
Glycine		40
Soybean oil	50	50
Casein	200	200
Corn starch	470	470
Salt mixture[a]	35	35
Vitamin mixture[b]	10	10
Methyl cellulose	30	30
Choline chloride	1	1
DL-methionine	3	3
Sucrose	200	200

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

[b]The vitamin mixture contains the following (mg/g): thiamin hydrochloride 0.6 mg, riboflavin 0.6 mg, pyridoxine hydrochloride 0.7 mg, nicotinic acid 3 mg, calcium pantothenate 1.6 mg, D-biotin 0.02 mg, cyanocobalamin 0.001 mg, retinyl palmitate 1.6mg, DL-α-tocopherol ace-tate 20 mg, cholecalciferol 0.25 mg, menaquinone 0.005 mg.

After 8 weeks, a modification of a burned rat procedure was used.[22] Rats were anesthetized with sodium pentobarbitol (0.71 μg/g body weight), and all dorsal hair was clipped prior to burning. Rats were placed in a heat-insulated mold that left approximately 30% of their body surface area exposed. These exposed surfaces were immersed in boiling water for 10 s. After burning, the animals were immediately resuscitated with an intraperitoneal injection of sterile 0.9% saline (10 mL/100 g body weight).[23] The burned rats were anesthetized and were exsanguinated by drawing arterial blood from the aorta 24 h after the burn. Blood samples were collected into heparinized tubes. Fresh blood was used to determine the proportion of T cell subpopulations, and plasma samples were stored at –70 °C until measurements for NO and antibody.

Experiment 2

Thirty rats were divided into 2 experimental groups, with each group containing 15 rats. Rats in the experimental groups were fed Gly or Arg diets for 8 weeks and vaccinated twice with purified recombinant PEIF protein on days 1 and 28 as described in experiment 1. After 8 weeks, a burn injury was induced, and *P. aeruginosa* strain PAO1 (ATCC 15692; 5×10^6 CFU in 2 mL PBS at $2 \times LD_{50}$) was immediately subcutaneously injected into the burned area. Survival of the burned rats was noted every 6 h in the first 3 days, and then every 12 h until 2 weeks.

Measurements of NO Concentrations

Plasma NO is highly unstable in solution and cannot be readily assayed. However, NO is converted to stable nitrite and nitrate ions in aqueous solution. After conversion of nitrate to nitrite using nitrate reductase, nitrite concentrations were measured using the Griess reagent. Plasma NO_2^-/NO_3^- concentrations were determined with a commercial kit (R&D Systems, Minneapolis, MN, USA). Procedures are described in the manufacturer's instructions.

Analysis of Specific Antibody Production Against PEIF

The specific antibody production of vaccinated rats was measured using ELISA as described previously.[24] Purified recombinant PEIF protein was coated on polyvinylchloride, flat-bottom, 96-well Falcon microtiter plates overnight at 4 °C with a protein concentration of 3 μg/mL in the coating buffer (pH 9.6 carbonate buffer). The coated plates were then blocked with 0.5% BSA-PBS. Rat 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 1 h at 37 °C. Bound antibodies were detected using peroxidase-conjugated goat anti-rat secondary antibody (Sigma, St. Louis, MO, USA). After washing 3 times, 100 μL of the 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 at 405 nm. Normal rat serum was taken as the negative control (background). Background signals were subtracted from serum signals.

Analysis of T Lymphocyte Subpopulations

Flow cytometry was used to determine the propor-