Supplementary Material S1. Immune characteristics

Lymphoblastogenesis

Isolated live PBMCs were diluted in 1×10^6/mL and seeded onto a 96-well plate. Specific mitogens, all purchased from Sigma, USA, including 25 μg/mL concanavalin A, 20 μg/mL lipopolysaccharide, or 50 ng/mL phorbol 12-myristate 13-acetate plus 250 ng/mL ionomycin, were added to stimulate specific lymphocyte proliferation. Alamar Blue (Serotec Co., Oxford, UK) was added in the last 24 h of an entire 72 h culture at 37°C in a 5% CO_2 humidified incubator. The changes of specific fluorescence were measured by a microplate reader (FLX800, Bio-Tek Instruments, Inc., Winooski, VT, USA).

Cytokine production

Heparinized whole blood was diluted to 1:50 for the detection of interferon-γ (IFN-γ) by the culture medium (Roswell Park Memorial Institute-1640; RPMI-1640) containing 50 μM 2-mercaptoethanol, 10 Mm 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 10 U/mL penicillin and 100 μg/mL streptomycin. The diluted whole blood samples were seeded into 24-well plates and cultured in a humid incubator. The incubator was maintained at 37°C and 5% CO_2 gas. The culture supernatant was collected after 72 h for determination of IFN-γ. The cytokine levels were determined by commercial ELISA reagents (R&D Systems, Minneapolis, MN, USA and IFN-γ; PharMingen, San Diego, CA, USA) according to the manufacturer's procedures. Color changes were detected at OD = 450 nm and 550 nm with a microplate reader (Multiskan Ex Microplate Reader, Thermo, Waltham, MA, USA).

Phagocytosis of granulocyte

DioC18 (0.25 mg/mL; Invitrogen, Waltham, MA, USA) labeled *Escherichia coli* (ATCC 25922) was suspended in 0.5 mL of Hank's balanced salt solution (HBSS) and used for the analysis of phagocytosis. Granulocytes of 1×10^6 each were preseeded in a 96-well plate and then cocultured with fluorescently labeled bacteria at 1×10^7 DioC18-labeled *E. coli* in a PBS solution at 37°C for 90 min. By the end of the coincubation, 100 μL of trypan blue (1.25 mg/mL) were added to quench the residual DioC18-labeled *E. coli*. Phagocytosis of the granulocytes was determined by flow cytometry (Becton Dickinson FACSCalibur™, Franklin Lakes, NJ, USA).

Oxidative burst measurement

At a similar setting as described in the phagocytosis, the granulocytes were co-incubated with unlabeled *E. coli* in a 37°C incubator for 90 min, and the intracellular reactive oxygen species (ROS) was determined by adding 2′,7′-dichlorofluorescin-diacetate (DCF-DA). The generated DCF was directly proportional to ROS as the process of the oxidative burst of the granulocytes was measured by flow cytometer.

Blood immunoglobulin level

The jugular blood was collected in an EDTA vacutainer tube and then centrifuged at 10,000 × g for 2 min after clotting and stored individually at −80°C. The serum antibodies containing immunoglobulin M (IgM), immunoglobulin G (IgG), and immunoglobulin A (IgA) were measured by a sandwich enzyme-linked immunosorbent assay (ELISA). Briefly, microtiter plate wells (Maxisorp; Nunc, Roskilde, Denmark) were coated overnight at 4°C with goat anti-porcine IgM, IgG, or IgA (Bethyl Laboratories, Montgomery, TX, USA). The coated wells were washed with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBST) and blocked with 1% bovine serum albumin (BSA) in PBS for 30 min.

After washing with PBST, the properly diluted samples were added and incubated for 2 h at room temperature (RT). Subsequently, the wells were treated with horseradish peroxidase-conjugated goat anti-porcine IgM, IgG, or IgA (Bethyl Laboratories, USA) for 1 h at RT. The wells were washed, and a 3,3′,5,5′-tetramethylbenzidine (TMB) solution (KPL, Gaithersburg, MD, USA) was added to each well as a substrate. After 30 min of incubation at RT, the reaction was stopped by adding 1 M dihydrogen phosphate (H_2PO_3). The absorbance was measured at 450 nm using a microplate reader (Original multi scan, Thermo, Waltham, MA, USA).

Lymphocyte subpopulation analysis

Fluorescence-labeled primary antibodies were used for the swine lymphocyte subpopulation, including the total T cells, T helper cells, and cytotoxic T cells, and the CD4CD8 double positive population by a flow cytometry. All the fluorescence-labeled monoclonal antibodies were purchased from Serotec Company (Oxford, UK). Briefly, each of 1×10^6 PBMCs samples was incubated with 10 μL of specific fluorescence-conjugated monoclonal antibodies in refrigeration avoiding light for 90 min, and procedures were followed as recommended by the manufacturer. The lymphocyte subpopulations were then determined by flow cytometry and analyzed by CellQuest software (Becton Dickinson FACS Calibur^{TM}, CA, USA).