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Short Communication

CD4$^+$ and γδTCR$^+$ T lymphocytes are sources of interleukin-17 in swine

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ABSTRACT

In the veterinary field, only limited information is available about interleukin-17A (IL-17), despite the fact that this cytokine plays an important role during pro-inflammatory immune responses and induces the production of chemotactic factors for neutrophils. The aim of this study was to characterize porcine IL-17-producing cells. We tested the cross-reactivity of five anti-human IL-17 monoclonal antibodies because such antibodies against porcine IL-17 are currently unavailable. Whole blood cells (WBCs) were stimulated with phorbol-myristate-acetate (PMA) and ionomycin and subsequently analyzed by flow cytometry. The antibody clone SCPL1362 was found to cross-react with porcine IL-17, whereas the other four antibodies tested did not recognize this cytokine. Using this antibody, we characterized porcine WBC-secreting IL-17 after PMA and ionomycin stimulation. All IL-17-producing WBCs were positive for the T lymphocyte marker CD3. Myeloid cells (CD172α$^+$) and B lymphocytes (CD79α$^+$) were IL-17 negative. The major subset of IL-17 positive T lymphocytes was the CD4$^+$ lymphocytes (about 60% of all IL-17 positive WBCs). The remaining IL-17 positive WBCs were γδTCR$^+$ lymphocytes. CD8 positive and CD8 negative cells were found within both CD4$^+$ and γδTCR$^+$ cells producing the cytokine. Moreover, IL-17 positive cells were mostly CD45RA negative, therefore activated cells or memory cells. Flow cytometry data were confirmed using sorted cells. Both sorted CD4$^+$ and γδTCR$^+$ cells produced IL-17 at mRNA level after PMA and ionomycin stimulation while double negative CD4$^-$γδTCR$^-$ cells were negative for IL-17. We can conclude that only two subpopulations of porcine WBCs are sources of IL-17 after non-specific stimulation: CD3$^+$CD4$^+$ and CD3$^+$γδTCR$^+$.

1. Introduction

Interleukin-17A (IL-17) is pro-inflammatory cytokine that has been described as being involved in immunity against extracellular bacteria, viruses and intracellular bacteria [1]. The main effect of this cytokine is an induction of tissue inflammation. Interleukin-17 is mainly produced by polarized Th17 lymphocytes. The discovery and characterization of this subset changed the Th1/Th2 paradigm [2,3]. The Th17 lymphocytes are involved in the adaptive part of immunity; however, IL-17 is produced early after infection by pathogens, which indicates the presence of innate pathways of IL-17 secretion. For example, the expression of IL-17 was found to occur 5 h post-inoculation of rhesus macaque ileal loops and 48 h post-oral infection of mice with *Salmonella* [4,5]. In these cases, γδ T lymphocytes were found to be the main source of IL-17 [4,6]. So far, NKT cells [7] and so-called lymphoid tissue-like cells have been described as the other innate sources of IL-17 [8].

The biological function of IL-17 and its role in immunity against several pathogens has been described in mice and humans. However, there is limited information about IL-17 in other species, including swine [9]. Recombinant porcine IL-17 showed similar *in vitro* effects as human and murine IL-17 [10]. IL-17 mRNA expression was detected in the mesenteric lymph nodes of ultra-early weaned piglets, which could be related to the alterations of the intestinal mucosa [11]. The presence of IL-17 secreting cells was only very recently found in lungs and peripheral blood of pigs using Western blot and anti-chicken IL-17 polyclonal antibody [12]. Nevertheless, phenotype of IL-17-producing cells has not yet been described. The proportional representation of porcine lymphocyte subsets is distinct from humans and mice [13,14]. For example, γδ T lymphocytes can represent up to 30% of all lymphocytes in the blood [15]. Therefore, the aim of the study was to characterize lymphocyte subpopulations that produce IL-17. On account of the unavailability of commercial antibodies against porcine IL-17 for flow cytometry, we tested the cross-reactivity of anti-human IL-17 antibodies with porcine IL-17.

2. Materials and methods

2.1. Sample collection and stimulation

Conventional, clinically healthy Large White pigs of both sexes, at the age of about 6 months, kept at the Veterinary Research

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Institute, were used in the study. Peripheral blood taken from the *vena jugularis* was immediately heparinized (25 IU/ml). Human blood taken from one donor was used as positive control. Whole blood (0.5 ml) was diluted in the proportion of 1:1 with RPMI-1640 medium (PAA, Pasching, Austria) supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin and 4 μg/ml gentamicin. Samples were stimulated by 15 nM phorbol-myristate-acetate (PMA; Sigma–Aldrich, St. Louis, MO, USA) and 1 μg/ml ionomycin (Sigma–Aldrich) and cultivated in the presence of a protein transport inhibitor, brefeldin A (10 μg/ml, Sigma–Aldrich), for 5 h at 37 °C in 5% CO_2. Unstimulated control samples were cultivated with brefeldin A only. Following this, the samples were harvested and the erythrocytes were lysed with hemolytic solution (8.26 g NH_4Cl, 1 g $KHCO_3$ and 0.037 g Na_4EDTA per liter of distilled water), followed by two subsequent washing steps with cell-wash solution (PBS containing 1.84 g/l EDTA, 1 g/l sodium azide and 4 ml/l gelatine). The whole blood cells (WBCs) obtained were resuspended in cell-wash solution and stained for flow cytometry analyses.

2.2. Cross-reactivity of anti-human IL-17 antibodies with porcine IL-17

In order to test the cross-reactivity of the antibodies, intracellular staining of IL-17 inside stimulated WBCs was used as described previously [16]. A commercially available kit for fixation and per-

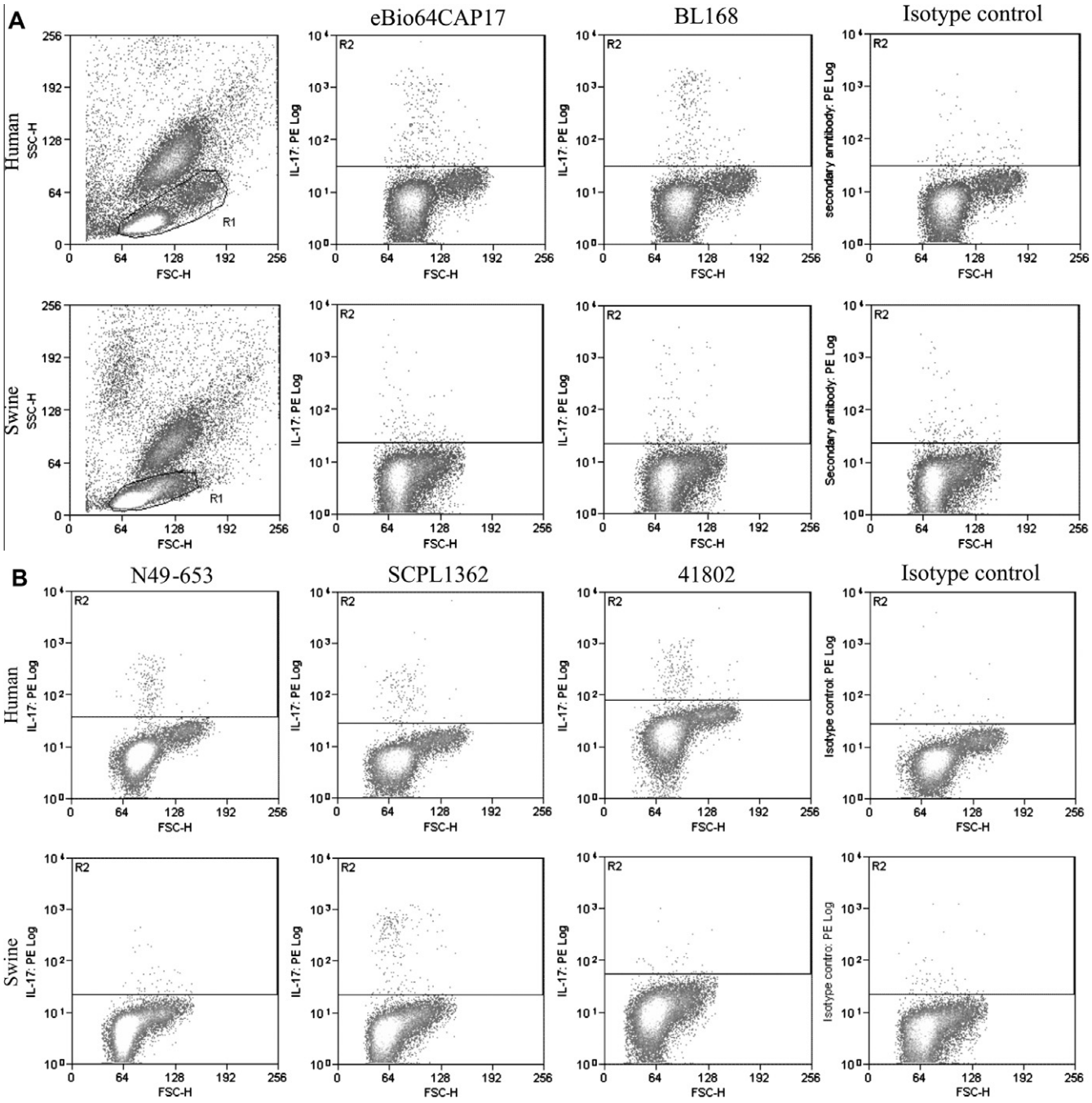

Fig. 1. Cross-reactivity of anti-human IL-17 antibodies with porcine IL-17. (A) Unlabeled anti-human IL-17 antibodies. (B) PE-conjugated anti-human IL-17 antibodies. (Samples of human and swine blood stimulated with PMA and ionomycin were stained with different clones of anti-human IL-17 antibodies. Only cells in region R1 were included into the analyses.)