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Expanding the tools for identifying mononuclear phagocyte subsets in swine: Reagents to porcine CD11c and XCR1

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ABSTRACT

Pig is a domestic species of major importance in the agro-economy and in biomedical research. Mononuclear phagocytes (MNP) are organized in subsets with specialized roles in the orchestration of the immune response and new tools are awaited to improve MNP subset identification in the pig. We cloned pig CD11c cDNA and generated a monoclonal antibody to pig CD11c which showed a pattern of expression by blood and skin MNP subsets similar to humans. We also developed a porcine XCL1-mCherry dimer which specifically reacted with the XCR1-expressing dendritic cell subset of the type 1 lineage in blood and skin. These original reagents will allow the efficient identification of pig MNP subsets to study their role in physiological and pathological processes and also to target these cells in novel intervention and vaccine strategies for veterinary applications and preclinical evaluations.

1. Introduction

Pig is an animal model system used to study a variety of immunological and pathological processes where mononuclear phagocytes are implicated. The mononuclear phagocyte family encompasses tissue macrophages (MP), monocytes and dendritic cells (DC) which include plasmacytoid DC (pDC), conventional DC (cDC) 1 and 2 lineages (Guilliams et al., 2014). These cells all have a protective role in immunity against pathogens but they differ in their ontogeny and their functional specificities. Grossly, MP are central in tissue remodeling and repair, monocytes potently stimulate inflammation upon encountering pathogens, pDC excel in type 1 interferon (IFN) synthesis upon viral stimulation, cDC2 primarily induce Th2 and Th17 responses whereas cDC1 appear as the most potent antigen presenting cells to initiate Th1 and $CD8^+$ T cell responses (Guilliams et al., 2014; Mildner et al., 2013; Schlitzer and Ginhoux, 2014; Vu Manh et al., 2015a). Whereas these cell types have been mostly studied in the mouse and human species, recent reports reveal that homologous mononuclear phagocyte subsets exist in pig blood (Vu Manh et al., 2015b), skin (Marquet et al., 2014) and lung (Maisonnasse et al., 2016). However, the limited reagents available against surface markers in swine allow only for enrichment of certain MNP subsets. Complementary markers are therefore needed to improve subset characterization and isolation and to target these cells in prophylactic and therapeutic strategies (Vu Manh et al., 2015b).

Notably, two markers of major importance emerged from studies on mouse and human mononuclear phagocytes, namely CD11c and the XC-chemokine receptor 1 (XCR1), for which no swine-specific tools are yet available. CD11c, also known as complement receptor 4, makes a heterodimer with CD18. CD11c is

Abbreviations: MNP, Mononuclear phagocytes; MP, Macrophages; DC, Dendritic cells; pDC, plasmacytoid DC; cDC, Conventional DC; mAb, Monoclonal antibody; pCD11c, Pig CD11c; pXCL1, Pig XCL1; CHO-huCD18, CHO expressing human CD18.

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involved in phagocytosis and it has a diverse array of ligands that include cell adhesion molecules, LPS, fibrinogen, and collagen. CD11c is used as a prime marker for DC identification as it is expressed very early in DC development in the bone marrow (Schlitzer et al., 2015) as well as in blood and after seeding in different tissues (Bachem et al., 2010; Haniffa et al., 2013; Neyt and Lambrecht, 2013; Yu et al., 2013). MP and other hematopoietic cells also express CD11c, therefore other markers such as Flt3 (CD135) and/or MHC class II should be combined with CD11c to identify DC (Helft et al., 2015). This molecule was efficiently used as a molecular target on DC for vaccination purposes in mouse models and for improving antigen presentation to human T cells *in vitro* (Castro et al., 2008; Cohn et al., 2013; Wang et al., 2000; Wei et al., 2009).

On the other hand XCR1 appears to be specifically expressed by the cDC1 subset and not by any other known population even outside the hematopoietic system (Crozat et al., 2010; Gurka et al., 2014), as found at the protein level in the mouse (Crozat et al., 2011; Dorner et al., 2009), human (Crozat et al., 2010) and monkey (Dutertre et al., 2014a) and at the RNA level in sheep (Crozat et al., 2010) and pig (Vu Manh et al., 2015b). XCR1 binds to XCL1, a chemokine secreted by activated NK, Th1-polarised $CD4^+$ T and $CD8^+$ T cells. The XCR1/XCL1 axis controls the $CD8^+$ T cell differentiation into cytotoxic T cells (Dorner et al., 2009). Until now, pig cDC1 have been distinguished from cDC2 and monocytes/MP by their lower expression of CD172a and their higher expression of CADM1. However, CADM1 expression is promiscuous across cell types (Crozat et al., 2010) and is particularly high on epithelial cells as well as on intra-epithelial DC such as skin Langerhans cells (Marquet et al., 2011) and lung cDC2 (Maisonnasse et al., 2016). Finally, targeting of XCR1 in the mouse has recently been shown to be very efficient at inducing antigen-specific $CD4^+$ and $CD8^+$ T cell response both by protein vaccines (Hartung et al., 2014; Terhorst et al., 2015) and by DNA vaccine (Fossum et al., 2015).

In this work, we developed a monoclonal antibody (mAb) directed to porcine CD11c (pCD11c) and a vaccibody structure made of porcine XCL1 fused to mCherry (pXCL1-mCherry). CD11c and XCR1 expression patterns were analyzed on pig blood cells and on migrated myeloid cells from skin explants and revealed to be very similar to the expression of the orthologous molecules on human cells.

2. Material and methods

2.1. Porcine CD11c (pCD11c) cDNA sequence

The cDNA of the porcine *CD11c* gene, also called Sus scrofa integrin alpha-X (*ITGAX*), was obtained from pig alveolar macrophage total RNA purified according to the Arcturus PicoPure RNA Isolation Kit (Arcturus Life Technologies). For elucidating the 5′ and 3′ end cDNA sequences, the RNA was reversed-transcribed using the SMARTer RACE cDNA amplification kit (Clontech) in order to perform rapid amplification of cDNA end (Race) reactions. For elucidating the inner cDNA sequences, the RNA (5 μg) was reversed-transcribed using the SuperScript II Reverse Transcriptase (InVitrogen) and oligo-dT primers. The PCR strategy to obtain the full length pCD11c coding sequence and part of the UTR sequences is shown Fig. 1. PCR fragments were obtained with the PCR Advantage 2 proof reading polymerase (Clontech) and were sequenced by the Sanger's method. The Af-Fr primers were designed based on the partial LOC100623303 Sus scrofa *ITGAX*-like sequence that has been derived by automated computational analysis using gene prediction method (GenBank XM_013991722.1). Alignment of LOC100623303 on sheep, human and horse ITGAX cDNA sequences revealed that large portions of the coding sequences were missing, i.e. 1–166, 1120–1729, 1817–2093, 2520-end (1 is the nucleotide start of the final pig ITGAX coding sequence). The Af-Er and Br-Fr PCR fragments provided identical sequences corresponding to the 1120–2039 portions. The Df-Fr PCR confirmed the corresponding LOC100623303 sequence. The 5′ end RACE was done with the Cr primer derived from the LOC100623303 sequence and the universal primer mix (UPM). The 3′ end RACE was done with the UPM and the Hf primer which was deduced from a sequence located 69 nt upstream of the STOP codon, conserved between sheep, human and horse ITGAX cDNA. Most of the 2520-STOP region was sequenced from the Ef-Ir PCR fragment, with Ir being deduced from a sequence encompassing the STOP codon and conserved between sheep, human and horse ITGAX cDNA. From these sequences, Gf and Jr primers were deduced and used to generate a PCR fragment which completed and confirmed the sequencing of the STOP region. Our PCR strategy allowed to re-sequence the overlapping regions between our pCD11c and the LOC100623303 and revealed over 99% nucleotide identity. The final pCD11c cDNA sequence has been submitted to GenBank (http://www.ncbi.nlm.nih.gov/) under the accession number KT897895.

2.2. Phylograms of the CD11c and XCL1 protein sequences from different mammalian species

CD11c (isoform 1) from different species were aligned to the pCD11c ORF using CLC sequence Viewer version 7. The selected CD11c sequences are from *Homo sapiens* (NP_001273304.1), *Macaca nemestrina* (XM_011743485.1), *Mus musculus* (NP_067309.1), *Rattus norvegicus* (XP_574569.3), *Ovis aries* (NP_001292819.1), *Equus caballus* CBH29316.3). XCL1 amino-acid sequences from the same species were similarly aligned. The selected XCL1 sequences are from *Sus scrofa* (EU743945.1), *Homo sapiens* (NM_002995.2), *Macaca mulatta* (NM_001032947.1), *Mus musculus* (NM_008510.1), *Rattus norvegicus* (NM_134361.1), *Ovis aries* (NM_001009427.1), *Equus caballus* (XM_001490960.2). Phylograms were generated

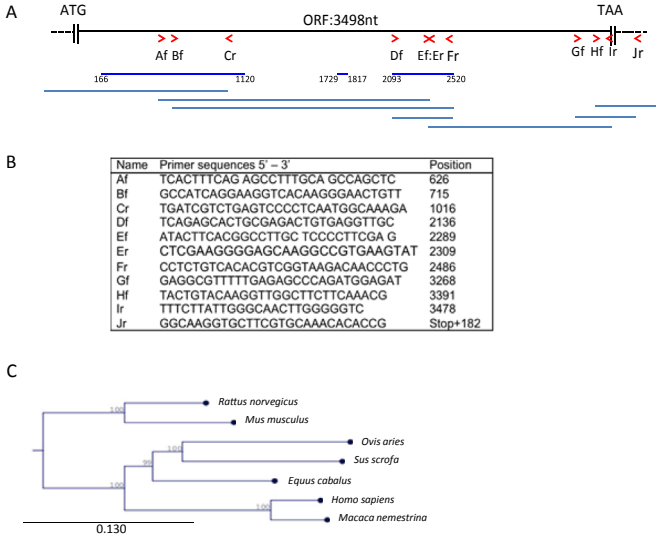

Fig. 1. Sequencing strategy of pCD11c cDNA and phylogenetic analysis of the protein sequence. (A) Location of the primers (Af to Jr, red arrows) used for generating the PCR fragments (pale blue) for sequencing pCD11c cDNA. The partial LOC100623303 Sus scrofa *ITGAX*-like (alternative name of the CD11c gene) sequence is shown is deep blue and aligned on the re-constructed porcine ITGAX coding sequence with the numbering beginning at the START codon. (B) List of the primers used in PCR. (C) Phylogram representation of selected mammalian CD11c protein sequences. Bootstrap values are indicated. Branch length scale is shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)