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Porcine myelomonocytic markers and cell populations

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ABSTRACT

This review focuses in what is currently known about swine myeloid markers, the expression and function of these receptors in the biology of porcine myelomonocytic cells, the regulation of their expression along the different developmental stages of these cells and their utility to investigate the heterogeneity of monocyte and macrophage populations. Although the number of monoclonal antibodies recognizing surface antigens expressed on either swine granulocytes or monocytes is low compared with those available for human or mouse, they have contributed significantly to study the members of myeloid lineages in this species, allowing to discriminate different maturation stages of these cells in bone marrow and to reveal the heterogeneity of blood monocytes and tissue macrophages. Porcine myeloid cells share many similarities with humans, highlighting the relevance of the pig as a biomedical model.

1. Introduction

Myeloid cells, granulocytes and monocyte/macrophages, play important roles in inflammation and host innate and adaptive immune responses. These cells, although belonging to separate lineages, arise from a common myeloid progenitor and share many surface membrane antigens.

Granulocytes, so-called because they have dense staining granules in their cytoplasm, act as the first line of defense against invading pathogens. After maturing in the bone marrow, they are released into the blood as end-stage, short-lived (2–3 days) effector cells, from where they are rapidly recruited into inflamed tissues or sites of acute infection. According to the staining characteristics of their predominant granules, granulocytes are classified into neutrophils, basophils or eosinophils. Neutrophils, the most numerous of granulocytes, are usually the first immune cells which arrive at sites of infection. Activated neutrophils limit infection via the phagocytosis of pathogens and by generating reactive oxygen intermediates and releasing antimicrobial peptides. Through the production of chemokines, pro-inflammatory cytokines and cell–cell contacts, they recruit and activate monocytes, DCs and lymphocytes, which contribute in the clearance of the microbes and infected cells and lead ultimately to the initiation of an adaptive immune response [1,2].

Eosinophils are thought to be important in host defense against parasites. However, they have also a protective role in other infections, especially against RNA viruses, and act as modulators of innate and adaptive immunity through their capacity to release a range of cytokines and lipid mediators. Moreover, they have been involved in a variety of physiological and developmental processes such as estrus cycling or mammary gland development [3].

Basophils not only are important effector cells in IgE-associated type I hypersensitivity reactions, but they may also mediate immunoregulatory functions through their ability to produce certain cytokines (e.g. IL-4, IL-13) and other mediators that orchestrate local inflammatory responses [4,5].

Macrophage precursors differentiate in the bone marrow into monocytes which enter the blood stream. Then, they migrate to different tissues where they further differentiate into specific macrophages [6]. Macrophages and their precursors constitute the so-called mononuclear phagocyte system (MPS). Besides playing critical roles in host innate immune responses, macrophages have important regulatory and effector functions in the specific immune response and in the maintenance of tissue homeostasis. These cells have essential functions in wound healing and resolution of inflammation, being involved in cell migration, matrix remodeling and angiogenesis. To accomplish this wide variety of tasks, macrophages exhibit an enormous heterogeneity and plasticity in their phenotype and functional capabilities. Under specific

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Abbreviations: APC, antigen presenting cell; ASF, Afican swine fever; CSF, classical swine fever; DC, dendritic cell; GPI, glycophosphatidyl inositol; Flt3L, Flt3 ligand; FSC, forward scatter; HSC, hematopoietic stem cell; ITIM, immunoreceptor tyrosine-based inhibitory motif; LBP, LPS-binding protein; LPS, lipopolysaccharide; MPS, mononuclear phagocyte system; PALS, periarteriolar lymphoid sheaths; PCV2, porcine circovirus-2; PRRS, porcine reproductive and respiratory syndrome; PWMS, post-weaning multisystemic wasting syndrome; SCF, stem cell factor; SIRP, signal-regulatory protein; Sn, sialoadhesin; SRCR, scavenger receptor cysteine rich; SSC, side scatter; TLR, toll-like receptors.

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conditions monocytes can also differentiate into dendritic cells (DC) [7]. These cells will not be considered in detail in this review as they are the subject of other article of this issue.

2. Swine myelomonocytic differentiation antigens

The differentiation of granulocytes and monocytes/macrophages is accompanied by the acquisition of new cell surface antigens, which correlates with the functional competence of these cells. Surface protein markers defined by monoclonal antibodies have provided a significant impulse to the characterization of different subsets of myelomonocytic cells. They have allowed definition of the different cell types, analysis of their heterogeneity, and the study of cell lineage differentiation. The number of well-characterized markers for the study of myeloid lineage in swine is far behind that available for the human or murine species. However, it is already enough to unveil many aspects of the biology of these cells.

In this section, we intend to review the markers of utility for the study of myelomonocytic lineage cells in the porcine species, as a reference for the following sections that will review our current knowledge of myelopoiesis, heterogeneity of myeloid cells and some aspects of their interaction with pathogens. In this review, we have consciously left apart a very important family of molecules, the Toll-like receptor family (TLR), as far as they have deserved a whole section in this issue.

2.1. Markers for studying the myeloid lineage

2.1.1. SWC3/CD172a

SWC3 was the first porcine myelomonocytic marker established [8]. Since its official definition in the First International Swine Cluster of Differentiation Workshop, this marker has been extensively used as the main marker of myeloid cells in swine, to the point that "SWC3$^+$ cells" has been generally accepted as a synonym of myelomonocytic cells. Expression of SWC3 occurs in the earliest precursors of the myelomonocytic lineage, and this expression is maintained all along the differentiation of these cells [9]. It is also expressed at low levels in plasmacytoid DC [10].

SWC3 has been identified as the porcine homolog of CD172a or SIRPα [11]. Cloning and expression of porcine SIRPα cDNA allowed confirmation of this identity, in that every anti-SWC3 antibody tested reacted with porcine SIRPα expressed in CHO transfected cells [12]. Moreover, cloning and expression of the cDNA coding for a splicing variant of porcine SIRPα, showed that this variant was also recognized by anti-SWC3 antibodies [12].

Signal-regulatory proteins (SIRPs) are a family of transmembrane glycoproteins involved in signal transduction, which are expressed at high levels in neurons and myeloid cells, including macrophages, monocytes, granulocytes and DC [13,14]. Members of this family have been characterized in mouse, rat, cattle and human [14].

Structurally, porcine SIRPα is a 507 amino acids glycoprotein, that has an extracellular region consisting of three immunoglobulin (Ig)-like domains – an N-terminal V-type domain, followed by two C-type domains – and a cytoplasmic domain containing immunoreceptor tyrosine-based inhibitory motifs (ITIMs), very similar to its homologs in other species [12]. The porcine SIRPα splicing variant lacks the two C-type Ig-like domains, having therefore only 291 amino acids, being otherwise identical to the larger form. Other members of the SIRP family have similar extracellular structures, but show large differences in their carboxy-terminal segments, with different types of transmembrane and cytoplasmic regions, or even lacking these [14]. Based on these differences, SIRP proteins have been classified in four groups named with Greek letters [15]. The different types of SIRP proteins are products of different genes, but still share closely related extracellular regions.

Nothing is known about ligands of porcine CD172a, but in mouse [16], rat and human SIRPα has been shown to interact with CD47 through its amino-terminal Ig-like domain [17,18]. The fact that this N-terminal domain is conserved in the two variants suggests that both can be functionally relevant in porcine cells. Signaling through SIRPα leads to tyrosine phosphorylation of ITIM motifs present in the cytoplasmic region of SIRPa and the subsequent recruitment of src homology 2 (SH2) domain-containing tyrosine phosphatases SHP-2 and SHP-1 that negatively regulate signal transduction cascades. Considering the early expression of CD172a in myeloid ontogeny – already detectable on immature myelomonocytic precursors, and increasing upon further differentiation of the cells into the most mature stages of tissue macrophages and blood granulocytes [9,19] – we favor the hypothesis that this molecule might be involved in functions such as the control of proliferation, differentiation and activation of these cells.

2.1.2. β2 integrins

Integrins are heterodimeric glycoproteins composed of α and β subunits, which are expressed at the plasma membrane of different cell types [20]. Each β chain can associate with several α chains, giving name to the different integrin families.

The β2 subfamily is composed in most studied species by four members characterized by their α chains named CD11a, b, c and d, associated with the common β2 chain or CD18. All these molecules are exclusively expressed by leukocytes, but while CD11a is expressed on all leukocytes, the other three have a more restricted pattern of expression, being expressed mainly by myelomonocytic cells [21,22]. In the pig, four CD11 have also been described. CD11a has a pattern of expression similar to other species, and falls out of the scope of this chapter. The other CD11 have been characterized by the use of both swine-specific and crossreactive antibodies [23,24]. The data, compiled and reviewed in the Third International Workshop on Swine Leukocyte Differentiation Antigens, prompted the adoption of a nomenclature different from that previously used for other species, resulting in adopting the terms wCD11R1, wCD11R2 and wCD11R3 for the antigens associated with the three α chains of the porcine β2 integrin family [25]. At the time of writing, only the gene coding for porcine CD11a has been cloned.

2.1.2.1. wCD11R1. This antigen is identified by anti-human CD11b, as well as porcine-specific antibodies. However, unlike human CD11b, it is not expressed by most blood monocytes nor by broncho-alveolar lavage macrophages, being present on 50% of granulocytes, NK cells and some DC [25–28]. The molecule immunoprecipitated by these antibodies has an apparent molecular weight of 165,000 [25].

2.1.2.2. wCD11R2. Anti-human CD11c antibody S-Hcl3 also binds to porcine leukocytes. While human CD11c is expressed by all myeloid cells, the swine molecule is not present on granulocytes, having a moderate expression on monocytes and pulmonary alveolar macrophages [24,25]. It is also expressed on blood DC and plasmacytoid DC [10]. Additionally, similar to humans, a subset of T cells (CD3$^+$) shows variable expression of this antigen [24]. The molecular weight of this molecule was estimated at 160,000 [25].

2.1.2.3. wCD11R3. A series of swine-specific antibodies identify wCD11R3. The distribution of this molecule corresponds almost exactly with the distribution of CD11b in man; the functional aspects studied also resemble human CD11b. In fact, two of the antibodies were originally described as recognizing a putative