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Review

Phenotypic and functional differentiation of porcine αβ T cells: Current knowledge and available tools☆

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ABSTRACT

Domestic pigs are considered as a valuable large animal model because of their close relation to humans in regard to anatomy, genetics and physiology. This includes their potential use as organ donors in xenotransplantation but also studies on various zoonotic infections affecting pigs and humans. Such work also requires a thorough understanding of the porcine immune system which was partially hampered in the past by restrictions on available immunological tools compared to rodent models. However, progress has been made during recent years in the study of both, the innate and the adaptive immune system of pigs. In this review we will summarize the current knowledge on porcine αβ T cells, which comprise two major lymphocyte subsets of the adaptive immune system: $CD4^+$ T cells with important immunoregulatory functions and $CD8^+$ T cells, also designated as cytolytic T cells. Aspects on their functional and phenotypic differentiation are presented. In addition, we summarize currently available tools to study these subsets which may support a more widespread use of swine as a large animal model.

1. Introduction

Large animal models offer certain advantages for immunological research in comparison to the frequently used rodent models. For example, cattle and sheep have been used for lymph cannulation studies, enabling unique insights into lymphocyte recirculation throughout the body (Pabst and Reynolds, 1987; Hein and Griebel, 2003; Hope et al., 2006; Vrieling et al., 2012). Pigs are also considered as an attractive model, especially for various zoonotic infections including influenza virus and *Mycobacterium tuberculosis* for the respiratory tract or hepatitis E virus, norovirus and rotavirus in the digestive tract (reviewed in Meurens et al., 2012). Moreover, transgenic pigs are deemed as promising candidates for xenotransplantation (Griesemer et al., 2014). Studies on these topics benefit from a detailed knowledge on basic aspects of the porcine immune system but also require a well-equipped immunological tool box. Compared to the knowledge on the murine immune system, pig-specific data are still lacking behind and also the immunological tool box still has considerable gaps. However, progress has been made during recent years and this review will address these two aspects for porcine αβ T cells.

T cells can be separated into two major subsets by the polypeptides that form their T-cell receptors (TCRs). Accordingly, αβ T cells have TCRs consisting of α and β-chains, while a further subset of T cells with a TCR-γδ exists. Together with B cells, αβ T cells are the major cellular component of the adaptive immune system while γδ T cells appear to perform a plethora of different functions attributable to both the innate and the adaptive immune system (Vantourout and Hayday, 2013). For αβ T cells it has become clear that they play an essential role within protective immune responses and contribute to long-lasting immune memory (Bonilla and Oettgen, 2010). Therefore, their study is a central aspect in analyses on host-pathogen interactions of the infectious diseases mentioned above but is also indispensable in the development of novel vaccines to combat these pathogens.

2. Porcine T cells: major subsets and their phenotypic peculiarities

The development of porcine αβ T cells in the thymus appears to follow the generally accepted model derived from other species, starting from a $CD4^-CD8^-$ stage *via* a $CD4^+CD8^+$ double positive

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stage to mature CD4+ and CD8+ single positive stages (Sinkora et al., 2000; Sinkora and Butler, 2009). However, analyses of porcine T cells in blood and secondary lymphatic organs revealed several peculiarities compared to humans and rodent species. Already in the 1980s, when the first monoclonal antibodies (mAbs) for porcine CD4 and CD8 became available, it was observed that a substantial proportion of blood-derived CD4+ porcine T cells co-expresses CD8 (Pescovitz et al., 1985; Saalmüller et al., 1987). Later on it was discovered that the mAb clones used in these studies bind specifically to the CD8α chain and it was concluded that a subpopulation of porcine CD4+ T cells expresses CD8αα homodimers (Zuckermann et al., 1998). The early studies with these CD8α-specific mAbs also indicated the existence of a considerable CD4−CD8α− population of T cells which were later identified as γδ T cells (Hirt et al., 1990; Yang and Parkhouse, 1996). Indeed, swine belong together with other ungulates and chicken to the so-called "γδ-high" species (Holderness et al., 2013), displaying high frequencies of this T cell subset in blood and spleen. For example, numerous reports describe frequencies of up to 50% of γδ T cells within total T cells in the blood of pigs during an age of 4–12 months, but this high number declines in older animals (Yang and Parkhouse, 1996; Takamatsu et al., 2006; Gerner et al., 2009; Talker et al., 2013; Sedlak et al., 2014). These studies also revealed that a subset of porcine γδ T cells expresses CD8α, highlighting the abundant expression of this molecule on porcine T cells. For a more detailed description of porcine γδ T cells the reader is referred to a number of reviews on this topic: (Takamatsu et al., 2006; Gerner et al., 2009; Mair et al., 2014).

MAbs for a direct detection of porcine αβ T cells are still missing. However, a combination of CD3 and TCR-γδ specific mAbs enables an indirect identification which revealed that the vast majority of these putative αβ T cells in the pig are either CD4+CD8α−, CD4+CD8α+ or CD4−CD8α^{bright} (Gerner et al., 2009) and it is currently assumed that CD3+TCR-γδ−CD4+CD8α$^{-/+}$ cells represent porcine CD4+ T(-helper) cells whereas CD3+TCR-γδ−CD4−CD8α^{bright} cells represent CD8+ (cytolytic) T cells. This notion is supported by various functional studies which are outlined below. Existing minor populations of CD3+TCR-γδ−CD4−CD8α^{dim} and CD3+TCR-γδ−CD4−CD8α− T cells have – to our knowledge – not been further studied so far.

3. Differentiation of T cells in humans and mice

Much of the existing knowledge on memory T cell formation and persistence as well as functional differentiation is derived from studies with human and murine T cells. Therefore, in the following a brief overview on this knowledge is provided and currently available knowledge in the pig will be compared with this in the following sections.

T cells exiting from thymus are considered as mature but naïve in relation to their cognate antigen. Following antigen encounter T cells proliferate, differentiate into effector cells and migrate to peripheral tissues and inflamed sites. After clearance of the pathogen, the vast majority of effector T cells dies, but a small pool of T cells develops into long-lived memory cells (Harty and Badovinac, 2008; McKinstry et al., 2010). Numerous studies in mice and humans revealed that these memory cells survive in different stages of differentiation which can be separated according to surface marker expression and cytokine production profiles. This started with the seminal description of central memory (T_{CM}) and effector memory T cells (T_{EM}) in human PBMC (Sallusto et al., 1999). T_{CM} cells are characterized by a CD45RA−CCR7+CD62L+ phenotype. The expression of CCR7 and CD62L enables extravasation through high endothelial venules and after a secondary antigen contact the cells still have a high capacity for proliferation and production of IL-2. In contrast, CD45RA−CCR7−CD62L− T_{EM} cells have a reduced proliferative capacity and rapidly produce high amounts of effector cytokines such as IFN-γ. This concept of central and effector memory T cells was soon confirmed in studies with murine T cells (Reinhardt et al., 2001; Masopust et al., 2001) and it was also revealed that a CD127^{high}CD62L^{high} and a CD127^{high}CD62L^{low} phenotype corresponds with murine T_{CM} and T_{EM} cells, respectively (Huster et al., 2004). Moreover, the analysis of killer lectin-like receptor G1 (KLRG1) expression serves in the identification of short-lived effector CD8+ T cells in mice which show a CD127^{low}KLRG1^{high} phenotype, whereas T_{CM} and T_{EM} have been described as CD127^{high}KLRG1^{low} (Obar and Lefrancois, 2010). In addition to T_{CM}, T_{EM} and short-lived effector T cells recently the existence of three further differentiation stages of human memory T cells has been postulated (Fig. 1; Mahnke et al., 2013), namely stem cell memory T cells (T_{SCM}), transitional memory T cells (T_{TM}) and terminal effector (T_{TE}) memory T cells. The phenotypic classification of these subsets is based on differential expression of CD45R0, CCR7, CD28 and CD95. For T_{SCM} cells, defined by a CD45R0−CCR7+CD28+CD95+ phenotype, it was shown that they have a high proliferative capacity and can give rise to T_{CM}, T_{EM} and short-lived effector T cells but also have the capacity for self-renewal, a feature typical of stem cells (Gattinoni et al., 2011). T_{TM} cells have been placed between T_{CM} and T_{EM} cells. The lack of CCR7 expression differentiates them from T_{CM} cells and their CD28 expression distinguishes them from T_{EM} cells. T_{TM} cells seem to have a higher responsiveness to IL-15 than T_{EM} cells (Lugli et al., 2010). T_{TE} cells, distinguished by a CD45R0−CCR7−CD28−CD95+ phenotype seem to represent the terminal stage of differentiation and were previously designated as T_{EMRA} cells because of their re-expression of CD45RA (Sallusto et al., 2004). In addition to these five subsets introduced by Mahnke et al. (2013), tissue-resident memory T cells (T_{RM}) are now considered to be a separate subset of memory T cells. T_{RM} cells display substantial phenotypic similarities to T_{EM} cells, as they are CD45RA−CD62L−CCR7−, but express CD69 and partially CD103. They reside permanently in peripheral tissues after an infection is cleared (Gebhardt et al., 2009; Masopust et al., 2010; Mueller et al., 2013).

4. Porcine CD4+ T cells

4.1. Differentiation of porcine CD4+ T cells following antigen contact

As outlined above, a peculiarity of porcine CD4+ T cells is the abundant expression of CD8α. Initial analyses with CD1-specific mAbs revealed that these CD4+CD8α+ T cells were indeed of extrathymic origin (Saalmüller et al., 1989) and ontogenetic studies showed a continuous expansion of this T cell population in blood from birth to adulthood (Zuckermann and Husmann, 1996; Borghetti et al., 2006; Grierson et al., 2007; Talker et al., 2013). These data provided strong hints that CD8α expression on porcine CD4+ T cells is related to antigen contact. This notion was further corroborated by the finding that *in vitro* stimulation of CD4+ T cells *via* allogeneic mixed leukocyte reactions (Saalmüller et al., 1987), Staphylococcus enterotoxin B (Saalmüller et al., 2002) or ConA + IL-2 (Reutner et al., 2013) resulted in an up-regulation of CD8α. In addition, in *in vitro* restimulation experiments with pseudorabies virus (PRV) (Summerfield et al., 1996), PRV-derived peptides (Ober et al., 1998), classical swine fever virus (CSFV) (Saalmüller et al., 2002), lysozyme (Revilla et al., 2005a), foot and mouth disease virus (FMDV)-derived peptides (Blanco et al., 2000; Gerner et al., 2006) and an influenza A(H1N1)pdm/09 vaccine (Lefevre et al., 2012) it could be shown that only CD4+CD8α+ but not CD4+CD8α− T cells respond by proliferation to antigenic restimulation. Similarly, IFN-γ production was only found within CD4+CD8α+ T cells following

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