

## Shifts in porcine PBMC populations from adolescence to adulthood

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### ABSTRACT

In mammals, the immune system is undergoing significant changes during development, which has many impacts on the individual's capacity to cope with infectious diseases or other pathologic conditions, where the immune system is involved. Especially in livestock, it is important to know in detail about these changes, including shifts in the composition of systemic leukocyte populations, as this knowledge may help to focus on relevant cell populations when developing novel vaccines for use in juvenile versus adult animals. In this mini-review, a synoptic comparison of published PBMC populations, which were analysed in healthy weaned piglets as well as multiparous non-gestating sows, shows remarkable shifts within leukocyte populations.  $\gamma\delta$  T cells increase by factor 1.5, plasmacytoid dendritic cells and T helper cells more than double, and cytotoxic T cells as well as regulatory T cells increase more than four fold, whereas NK cells as well as B cells in adult sows comprise only 40% and monocytes 70% of the relative population sizes in weaned piglets. In summary, these insights into age-dependent shifts of porcine leukocyte populations indicate a principal increase of acquired immunity-associated leukocyte populations, whereas primarily innate immunity-associated cell types (NK cells, monocytes) are diminished.

### 1. Introduction

Reliable physiologic, including hematologic and immunological, data of the species of interest are the basis for clinical as well as translational veterinary research. Meanwhile, the pig's importance not only as a classical veterinary species but also for biomedical research has been fully accepted. Remarkably, only one recent scientific work has been published dealing with profound systemic cellular immunological reference values for adult pigs (Sipos et al., 2011a). The reason for this is that most research in pigs in the field of applied immunology, i.e. vaccinology and translational research, is done using juvenile pigs. Even in reports dealing with the ontogeny of the porcine immune system, analyses end when animals reach an age of one year at maximum (Denyer et al., 2006; Talker et al., 2013). In fact, pigs – as most other mammals – have to be regarded as entering adulthood when maturation of their skeletal system is finished, which happens at an age of approximately 2.5 years in the porcine species. This age also coincides with full reproductive performance in sows (Peinhart et al., 2011; Sipos et al., 2011b). Therefore it is more accurate to include data of that age when making statements on the development of the pig's immune system including adulthood. From a practical point of view, this is also of importance as developing novel vaccines is perhaps the most efficient preventive measure to cope with infectious diseases, especially in times of increased awareness of the necessity to use antibiotics responsibly.

### 2. New insights into the ontogeny of the porcine immune system

As outlined in the introduction section, much of literature describing ontogeny of the porcine immune system comes from studies during the prenatal and the suckling and weaning periods (Sinkora et al., 2005; Talker et al., 2013). Here, the main focus is on the further development of the systemic cellular immune system by connecting data of two representative studies giving the respective data of 3 and 6 weeks old piglets (Bauer et al., 2018; Worliczek et al., 2010) with the ones of adult non-gestating sows at an age of approximately 3 years (Sipos et al., 2011a), thus adding the analysis of pigs as adults. These three studies are also compatible for such a comparison because all flow cytometric analyses were performed in one laboratory at the University of Veterinary Medicine Vienna, thus minimizing inter-laboratory biases. However, it has to be mentioned that the selection of surface markers for the respective immunocyte populations differed between the studies as staining protocols changed over time, but this should not have a major impact on the overall observations.

The following peripheral blood mononuclear cell (PBMC) populations are included: monocytes, plasmacytoid dendritic cells (pDCs), CD21<sup>+</sup> B cells, T helper cells (naïve and activated/memory Th cells), cytotoxic T cells (CTLs), regulatory T cells (Tregs),  $\gamma\delta$  T cells (CD4<sup>-</sup> subset), and natural killer cells (NK cells). All data discussions are based on relative changes of the respective immunocyte population with

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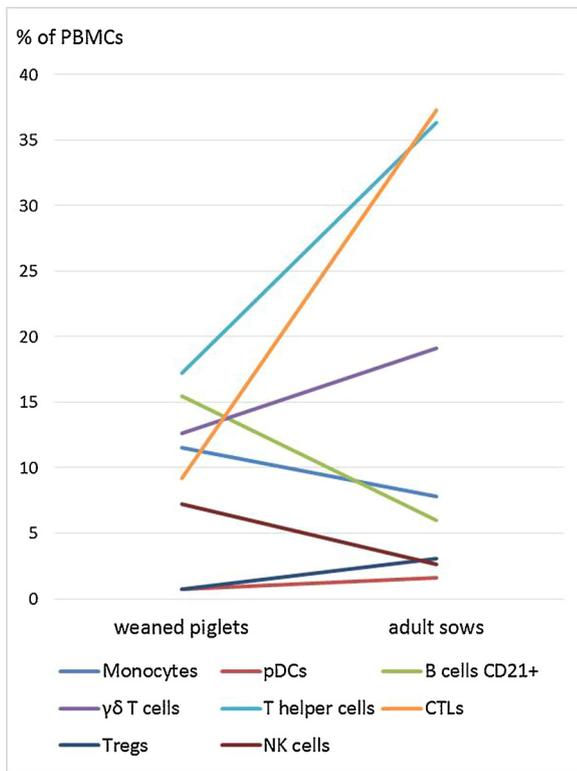


Fig. 1. Age-dependent shifts in PBMC populations in weaned (6-weeks old) versus adult (3-years old) pigs.

regard to total PBMCs. During the first weeks of life (week 3–6), there seems to be a nearly threefold increase in B cells and a doubling of CTLs and  $\gamma\delta$  T cells, whereas proportions of Th cells, Tregs, and NK cells are approximately the same at both time points. These observations largely correspond with the findings described by Talker et al. (2013) with the exception that they found remarkable elevations for all lymphocyte populations during that period of life. However, it has to be mentioned that the piglets in the above mentioned study were vaccinated against *M. hyopneumoniae* and PCV2 on day 14, whereas the animals used as reference for suckling piglets herein (Worliczek et al., 2010) were vaccinated against *M. hyopneumoniae* already at day 2, which may explain the observed differences. However, the principal observation is the same in all studies, namely, that lymphocytes and NK cells rise during the neonatal period and childhood in pigs with a huge increase during the first weeks of life.

The main period of interest reviewed here is the transition from adolescence to adulthood. Fig. 1 gives an overview of the changes in PBMC populations during that period of life. Plasmacytoid DCs and Th cells more than double, CTLs increase by factor 4.1, and regulatory T cells by factor 4.4. Of special notion,  $\gamma\delta$  T cells increase by factor 1.5. This observation is in contrast to Gerner et al. (2009), who state in their review article that  $\gamma\delta$  T cells decline with age. The discrepancy is likely the age difference in the cited studies, which included animals aged 16 months at maximum (Yang and Parkhouse, 1996), thus not reaching adulthood by the above definition. Further, the sample size of only four animals in that publication is very low and does not allow general conclusions. Gerner et al. (2009) also referred to unpublished data of an unknown number of sows aged 2 years, but details concerning the animals examined, such as reproductive and health/vaccination states, were lacking.

Taking into account the increase of  $\gamma\delta$  T cells as described here based on the analysis of 32 healthy sows, the data show no decline. So these cells seem to be of major importance also in the immune defense strategies of adult pigs. This makes sense, as  $\gamma\delta$  T cells are known to also

recognize non-peptide antigens and thus have functions unique from  $\alpha\beta$  T cells (Hayday, 2000). Especially in pigs, subsets of  $\gamma\delta$  T cells seem to also have antigen-presenting and cytolytic functions (Olin et al., 2005; Takamatsu et al., 2002). Future studies, for example dealing with the development of novel vaccines for the use in sows, should clearly include these cells in the analysis of duration of immunity due to their ability to form memory subsets, as has been reported by Lee et al. (2004).

Porcine pDCs are, as in other species, the main producers of IFN- $\alpha$ . Additionally they produce significant amounts of TNF- $\alpha$  and IL-12p40, which is mainly induced by CpG. These cells also express TLR7 and 9, but, interestingly, also TLR3, which in mice and humans is restricted to a conventional DC subset. A remarkable peculiarity is the high expression of complement-related genes (C2, C3, C5, and CD93) (Auray et al., 2016). As one of the most important immunocytes in the defense against viral infections, the increase of pDCs with age is logical.

B cells in adult pigs drop by 60% and monocytes by 30%. The decrease of B cells might be explained by a presumably higher number of plasma cells in the bone marrow of adult sows, already providing a sufficient number of antibody clones for common pathogens (Sipos et al., 2011a). Also, NK cell number is decreased in adult pigs and constitutes only 40% of the relative number in weaners. Interestingly, in humans NK cell number is quite constant from childhood over adolescence to adulthood, but then there is a rise in elderly subjects older than 70 years (Valiathan et al., 2016). However, NK cell function declines simultaneously, which has implications not only on the restricted elimination of transformed cells but is also reflected in part by the slower resolution of inflammatory responses and an increased incidence of bacterial and fungal infections in the elderly (Hazeldine and Lord, 2013).

Most immunocytes investigated so far change their phenotype as well as functional capacity (and sometimes also orientation) during ontogeny. This may be exemplified by a study of Reutner et al. (2013), showing that all naive  $CD8\alpha^-$  Th cells in pigs express CD27, but divide  $CD8\alpha^+$  Th cells into a  $CD27^+$  and a  $CD27^-$  subset thus resembling terminally differentiated effector memory cells and central memory cells, respectively, as described in humans. In pigs,  $CD4^+$  T cells have been traditionally investigated very intensely for a long time, also because of the interesting early observation that part of them form the well known  $CD4^+CD8^+$  phenotype corresponding to memory function as described above with an increase from less than 2% of PBMCs in neonatal piglets to 30–55% in pigs aged 3 years (Zuckermann and Husmann, 1996). Since then, much detailed knowledge has been gathered concerning different phenotypes and functional capacities of porcine  $CD4$  T cells (Gerner et al., 2009). It should be mentioned, that also mucosal immunocyte subpopulations change their phenotypical and functional characteristics. As an example, mucosal  $\gamma\delta$  T cells in pigs are restricted with age and are highly oligoclonal in adult 2 to 5.5 years old pigs. These cells exhibit a striking compartmentalization, meaning, that different  $\gamma\delta$  T cell repertoires are present at different mucosal sites, such as lungs and different sites within the intestinal tract (Holtmeier et al., 2002). This most probably is a consequence of the fact that different anatomical sites are sensitive to different pathogens. Mucosal immunisation, when applicable, is therefore a valuable tool in pig herd management. Therefore, also other mucosal immunocyte populations, such as intraepithelial and stromal T cells, should be analysed concerning developmental changes in phenotype and/or functionality in future studies.

In summary, these insights into age-dependent shifts of porcine PBMC populations indicate a principal increase of acquired immunity-associated leukocyte populations with the exception of peripheral B cells, whereas primarily innate immunity-associated cell types (NK cells and monocytes) decrease. This makes sense, as the cumulating contact with pathogens continuously pushes acquired immunity and thus the majority of encountered pathogens can be defended by more efficient mechanisms. Conversely, such changes may contribute to pathologies,

such as malignancies, in aged pigs, as was observed in minipigs that can reach ages a commercial pig seldom does (Sipos et al., 2007). For future studies, efforts should be laid on the detailed functional characterization of the respective cell types also in sows, as these animals have to mount the reproductive and thus very energy-consuming part of pork industry, which makes them susceptible to a wide range of infectious diseases and thus disease-related losses, mainly by reproductive disorders. These conditions require more efficient vaccines, which can only be developed when the underlying immune mechanisms are fully understood. A first step towards that aim may be the intensified inclusion of  $\gamma\delta$  T cells in scientific work as well as the analysis of select indicator cytokines of immunocytes, especially Th cell and dendritic cell (sub)populations, also in sows, as the cytokine expression profiles can indicate the involvement of diverse “candidates” in the induction of a desired immune response, thus pointing towards preferential targets for novel vaccines.

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