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Porcine T lymphocytes and NK cells – An update

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ABSTRACT

Natural killer (NK) cells represent an important cell population of the innate immune system with the ability to attack spontaneously pathogen-infected and malignant body cells as well as to produce immune-regulatory cytokines. T lymphocytes belong to the adaptive immune system and perform a wide array of functions in immune regulation, inflammation and protective immune responses. In this review we summarize the current knowledge about the phenotype and functional characteristics of these two cell populations in swine.

Porcine NK cells can be distinguished from T cells by the complex phenotype perforin$^+$ CD3$^-$CD4$^-$CD5$^-$CD6$^-$CD8α^+CD8β^-CD11b$^+$CD16$^+$. Investigations so far show that these cells have the capacity to lyse virus-infected target cells and respond to various regulatory cytokines. Such cytokines can induce interferon-γ (IFN-γ) production in porcine NK cells, as well as the up-regulation of effector/activation molecules like perforin and CD25. Porcine T cells can be divided into a number of subpopulations, including a prominent fraction of T cells expressing T-cell receptors (TCR) with $\gamma\delta$-chains. Like TCR-$\alpha\beta$ T cells, these TCR-$\gamma\delta$ T cells can express CD8α and MHC class II, two molecules which in swine seem to be correlated with an activation status of T cells. Functional properties of these cells seem to include cytolytic activity as well as antigen presentation; however, both aspects require further investigation. Like in other species, TCR-$\alpha\beta$ T cells in swine comprise MHC class-I restricted cytolytic T cells, T-helper cells and recently identified regulatory T cells. We summarize data on the phenotype and function of these cells including memory cell formation. Current knowledge suggests that MHC class-I restricted cytolytic T cells can be identified by the expression of CD8$\alpha\beta$ heterodimers. T-helper cells express CD4 as well as other activation-related markers, including CD8α, MHC class II and CD45RC. Porcine regulatory T cells have a phenotype similar to that of mouse and humans: CD4$^+$CD25$^+$Foxp3$^+$. First results indicate that these cells can suppress proliferation of other T cells and produce IL-10. Finally, the abundant expression of swine-specific activation markers CD8α and MHC class II on T cells and NK cells is discussed in more detail.

1. Introduction

During the last three decades the importance of swine in agricultural and biomedical research has resulted in a substantial increase in the research efforts on the porcine immune system. In agricultural research the interaction of pathogens with the immune system plays the most important role, and detailed knowledge of the host immune system is required to identify protective immune mechanisms and to define immuno-correlates of protection. Knowledge about protective immune mechanisms gives the chance for a better monitoring of vaccination efforts and for an improvement of vaccination strategies. Studies on the interaction of the host immune response with particular pathogens and the identification of pathogen-specific epitopes are often aimed to design new or more efficient vaccines.

The adaptive immune system based on the function of T and B lymphocytes is therefore one of the major targets of research. Stimulated by an interaction of cells belonging to the innate immune system, like dendritic cells and monocytes, B and T lymphocytes generate pathogen-specific reactions and, furthermore, develop long-lasting immunological memory.

The activity of B lymphocytes can be easily detected by their soluble products, specific antibodies, whereas monitoring of antigen-specific T-cell responses needs a detailed knowledge about the phenotypes of responding T lymphocytes and the respective T-cell subpopulations.

Monoclonal antibodies (mAbs) directed against porcine leukocyte differentiation antigens have been developed (summarized by [1,2]), and especially mAbs directed against antigens selectively expressed on T lymphocytes represent now appropriate tools for a

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Table 1
Overview of porcine lymphocyte subsets.

Functional lymphocyte subset	Phenotype	Frequency within PBL	References
NK cells	Perforin$^+$CD2$^+$CD3$^-$CD4$^-$CD5$^-$CD6$^-$CD8α$^+$ CD8β$^-$CD11β$^+$CD16$^+$	2–10%, age-dependent decrease	[8,9] [a]
NKT cells	Perforin$^+$CD3$^+$CD6$^-$CD11β$^+$CD16$^+$	0.5–3%	[8]
TCR-γδ T cells	CD3$^+$TCR-γδ$^+$	5–50%, age-dependent decrease	[30,46]
CTLs	CD3$^+$CD8α$^+$CD8β$^+$	8–21%, up to 40%	[91] [b]
T-helper cells	CD3$^+$CD4$^+$	19–60%, variable portions of CD4$^+$CD8α$^-$ and CD4$^+$CD8α$^+$ subpopulations	[30,31,92]
T_H1	CD3$^+$CD4$^+$, poorly investigated so far	Not yet investigated	
T_H2	CD3$^+$CD4$^+$, poorly investigated so far	Not yet investigated	
T_H17	Not yet identified	Not yet investigated	
Treg	CD4$^+$CD25^{high}Foxp3$^+$	1–3%	[81,82]

[a] Gerner, unpublished results.
[b] Saalmüller, unpublished results.

detailed identification of T cells and T-cell subpopulations. Combined with functional analyses, functionally distinct T-cell subsets which play a role in various immune reactions were defined.

In this article we aim to summarize the current knowledge about porcine T lymphocytes and porcine T-lymphocyte subpopulations (Table 1). In addition, we will discuss some peculiarities of porcine T lymphocytes with regard to unusual and unexpected T-cell phenotypes.

Additional chapters of the article deal with the current knowledge about natural killer (NK) cells in swine. This cell population distributed to the innate immune system gained in importance during recent years because of its role as a central player of the immune system, emerging as a leukocyte population with key functions in the interaction between cells of the innate and adaptive immune system.

2. Porcine NK cells

2.1. Phenotype of porcine NK cells

Porcine NK cells were firstly described as CD2$^+$CD4$^-$CD5$^-$CD8α$^+$ cells [3,4]. Other studies identified a NK-inhibitory antigen, PNK-1, which showed effective blocking of NK-cell function [5]. This antigen was later defined as CD18, a differentiation antigen involved in NK-cell function but without any selective expression on NK cells. Another more selective marker for NK cells seemed to be CD16 [6,7], but CD16 is also expressed on cells of the myeloid lineage, e.g. on monocytes. So far selective markers for porcine NK cells are missing and NK cells can be defined only by distinct marker combinations, e.g. CD8α vs. CD16, CD8α vs. CD3 (Gerner, unpublished results), or as presented in Fig. 1D as CD3$^-$CD4$^-$CD8α$^+$ subset of peripheral blood lymphocytes (PBLs). A recent study from Denyer et al. [8] defined NK cells of swine as cells with the phenotype perforin$^+$CD2$^+$CD3$^-$CD4$^-$CD5$^-$CD6$^-$CD8α$^+$CD8β$^-$CD11b$^+$CD16$^+$.

In our analyses we could confirm these data [9] and further show that with regard to MHC-class II DR expression (Fig. 2C) this lymphocyte subset represents a heterogeneous fraction. Porcine NK cells can be MHC-class II$^-$ as depicted in Fig. 2C, but we also have analyzed NK cells from swine showing a weak to distinct MHC-class II DR expression, both *ex vivo* and after *in vitro* stimulation ([9] and data not shown). We have to date no convincing explanation for this phenomenon, but one might speculate that this MHC-class II DR expression defines an activation status of the respective NK-cell fractions, since a correlation of MHC-class II DR expression with activation could be shown for porcine T cells in previous publications [10,11].

The homogeneous high expression of CD45RC as presented in Fig. 2D also seems to be unexplained. In contrast to the expression pattern on T lymphocytes, the expression of CD45RC on NK cells seems to be constitutive and was not down regulated upon stimulation with cytokines [9]. Therefore, CD45RC expression on porcine NK cells appears to be independent of a prior activation step.

2.2. Functional characterization of porcine NK cells

Experiments characterizing the spontaneous cytolytic activity of porcine NK cells against various cell lines have been reported by different groups [3,4,12,13]. Furthermore, cytolytic activity against target cells infected with pseu-dorabies virus (PRV) and transmissible gasteroenteritis virus was described [14,15]. Increased *ex vivo* NK-cell activity in swine with chronical infectious diseases was also observed [16] and lethal infection with classical swine fever virus (CSFV) led to a tremendous decrease of NK-cell activity during the course of infection [17].

Some *in vitro* studies showed that NK-cell activity could be enhanced by interferon-γ (IFN-γ) [18] and interleukin-2 (IL-2) [19]. Administration of IL-12 resulted in an increased cytolytic activity and secretion of tumor necrosis factor-α (TNF-α) [20], whereas addition of IL-4 diminished the NK-cell activity of IL-2-stimulated NK cells [21]. As demonstrated by Domeika et al. [22], a combination of IL-12 and IL-18 was able to stimulate NK cells for an enhanced IFN-γ production. Recently, our laboratory showed a synergistic effect of IL-2, IL-12 and IL-18 on the cytolytic activity, perforin expression and IFN-γ production of NK cells, and, furthermore, the ability of cytokine-stimulated NK cells for CD25 and additional MHC-class II DR surface expression [9].

3. Porcine T cells

3.1. T-cell development in thymus

As in other higher vertebrates, the thymus of swine is a highly specialized lymphatic organ for T-cell maturation. In the early prenatal period this organ is colonized by T-cell precursors, originating from the bone marrow. During early ontogeny Sinkora et al. [23] postulated a sequential colonization of porcine thymus by CD3ε-bearing thymocytes. The early thymocytes appeared on day 40 of gestation and showed high CD3ε expression. They belonged to the TCR-γδ lineage. In a second wave – 15 days later – mature TCR-αβ thymocytes with high CD3ε expression could be observed, but their appearance was preceded by TCR-αβ thymocytes with low CD3ε antigen density. In the prenatal period only a small percentage of thymocytes was positive for CD25 and belonged mainly to the TCR-αβ thymocytes, whereas in the postnatal period the percentage of CD25$^+$ thymocytes continuously increased [23]. Analyses of the TCR-γδ thymocyte subset