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Anatomical particularities of the porcine immune system—A physician's view

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ABSTRACT

In this article the anatomical structure of the porcine immune organs is described. The focus is on their particularities that are related to the use of pigs as an animal model. Key issues of the intrauterine development of the lymphoid organs are presented, such as the specific epithelio-chorial placenta, the appearance of the thymic tissue and the initial development of B cells. The role of the thymus for the development of α/β and γ/δ T cells and the location of tonsillar tissue in the naso-pharynx, in the oral cavity and at the basis of the tongue are described. The porcine spleen is of interest for surgical techniques to treat splenic trauma adequately. The observation of the inverted lymph node structure of pigs is puzzling and it remains unclear why only few species have this distinct morphological organisation. Based on the functional differences in lymphocyte recirculation observed in pigs, specific lymph cannulation experiments are possible in the porcine immune system. The porcine intestinal lymphoid tissue and the lymphocytes in the mucosal epithelium and lamina propria are of interest for studying the gut immune responses. For use as a model the fact that the pig is a monogastric omnivorous animal represents an advantage, although the porcine ileal Peyer's patch has no obvious anatomical equivalent in man. Based on the detailed knowledge of porcine immune morphology the pig is suitable as model animal for immunology—in addition to the various experimental approaches in physiology, pharmacology, surgery, etc. that are applicable to human medicine.

1. Introduction

In recent years experimental interest in the pig's immune system increased remarkably. The pig is important for transplantation research, both for the development of surgical techniques and as xenotransplant donor [1,2]. The pig is an omnivorous monogastric animal, and it is a species well recognised for research in physiology [3,4], e.g. for cardiac function and myocardial ischemia, hemorrhagic shock, hypercholesterolemia, hypertension, vascular pharmacology and blood vessel remodelling [5–8], renal blood flow and function [9,10] and nerve conduction velocity [11]. The pig's skin morphology is comparable to that of humans, therefore the pig is used for studies of skin perfusion and wound healing [12,13], dermal drug absorption [14,15] and treatment of injury such as burns [16]. Further, as the pig is an essential source of human food, research in pig husbandry and nutrition is performed to develop a safe and sustainable meet production from pigs [17]. The respiratory system of pigs has the advantage that diagnostic procedures in the lungs such as the bronchi-alveolar lavage can be performed in the living animal repeatedly [18,19].

For the applied experimental studies in the different areas of physiology or clinical medicine performed in pigs, it is of importance to understand porcine immunology and to obtain insight into the function of the immune system—both systemic and local at the mucosal surfaces [20]. A big advantage of the rodent models in biomedical research is the presence of many different strains of mice and rats, e.g. several mouse strains are preferentially reacting in a more T-helper 1 (Th1) or Th2 immune response pattern. In addition, the genetically modified mouse strains can be used to study, e.g. a specific molecule in the organism using gene knockout approaches. In contrast to rodents where the animals are kept under highly standardised conditions, many pig experiments are carried out in outbred pigs reared in conventional farms. Pig samples taken at the slaughterhouse show a wide variety of immunological results, e.g. the lymphocyte subset distribution described show wide inter-individual differences [21].

So far experimental pigs are often obtained from a local breeder, and the studies are thus dependent on pig strains regionally available. These animals belong mostly to the hybrid pigs, crossbred on the basis of the different land races. However, there have been many efforts to analyse the specific genetic peculiarities of the numerous pig races and breeds (general overview of the different studies: http://www.lib.iastate.edu/services1/ref/agnic/aboutpage.html), and a complete mapping of the pig's genome is

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under work (further information and data: http://www.animalgenome.org/QTLdb/pig.html; http://www.toulouse.inra.fr/lgc/pig/RH/IMpRH.htm, see also [22]).

Several minipig breeds are used for biomedical research, e.g. the Hormel-Hanford minipig [23], the Goettingen minipig (www.minipigs.dk), the Minnesota minipig [24] and the Yucatan minipig [25,26]. The gut development and intestinal immunology has been studied in Goettingen minipigs [27–29]. For use in immunology and transplantation research MHC-homozygous pigs have been bred [30]. Much experience has been acquired in improving the quality of rearing minipigs in terms of the microbiological flora [31,32].

As the pig as experimental animal is large enough to perform even specific surgery on blood and lymph vessels, many data are available about cell migration between lymphoid organs. The immigration and emigration of lymphoid cells have been studied in the thymus as primary lymphoid organ and in all secondary lymphoid organs like the tonsils, spleen, peripheral and mucosal lymph nodes and the gut associated lymphoid tissue (GALT) [33]. The integration of bone marrow and liver in the immune system have been shown in pigs using specific labelling of lymphoid cell populations or in ex vivo studies with in vitro normothermic organ perfusion for several hours [34]. In pigs several lymphocyte quantification studies for all lymphoid organs and for the mucosal immune system have been performed [35,36].

Designing experimental studies in pigs was often affected by the lack of suitable immunological reagents. The growing interest in pig immunology from the field of xenotransplantation, where the pig is the donor of choice [37–39], and from infectiology [40–42] has increased the numbers of immunological markers such as monoclonal antibodies [43]. Analysis of cytokine expression including chemokines by RT-PCR and quantitative PCR are established [44,45] and antibody secretion can be studied using Elisa-spot assays [19].

2. Development of primary and secondary lymphoid organs before birth

The gestation period in pigs lasts 115 days (for a general review about pigs: [46]). As the placenta has six layers (epithelio-chorial placenta), the embryo is separated from the mother's blood supply during the intrauterine period; there is no transfer of maternal cells and immunoglobulins into the embryo/fetus [46,47]. It is not known so far, whether the porcine six-layered placenta is tight for DNA fragments or whether DNA can be transferred to the embryo/fetus as it is the case in mice [48].

In the porcine embryo blood islands are observed on about day 16, developing thymic tissue (thymus anlagen) is present on day 21, already on day 22 the developing spleen is detected. A phenotypic characterisation of lymphocytes is possible beginning with day 30, then $CD3^+$ lymphoid cells are detected, γ/δ T cells are present both in and outside the thymus [49,50]. The majority of T cells in the thymus are neither $CD4^+$ nor $CD8^+$ at week 6 of the intrauterine period, but the development of T cells into $CD4^+$ and $CD8^+$ lymphocyte subpopulations was detected in the porcine thymus around day 44 of gestation [51]. Especially γ/δ T cells are of interest in the immune system with respect to the non-protein antigens and to super-antigens [52]. It has not been examined yet whether the γ/δ T cell development in pigs resembles that in other species [53]. In the later intrauterine life γ/δ T cells are observed in the peripheral blood and in the intestinal wall [54]. Extra-thymic γ/δ T cells are present in pigs after birth, like in other species, but the initial prenatal organ of origin is not known [29,55]. The specific clones of γ/δ T cells observed in adult pigs may well have their origin in the embryonic and fetal period. The CDR3 spectratyping enables a more specific phenotyping of α/β and γ/δ T cells and also of B cell isotypes [56].

As there is no influence from the mother in transfer of sow immunoglobulin to the embryo/fetus, the porcine fetal B cells are a naïve population developing without idiotypic–antiidiotypic influences. Details of the B cell repertoire development – especially in the gut associated lymphoid tissue – are described in the article by Butler et al. (this issue [57]), the intrauterine repertoire development was studied in detail [58]. The first B cells (μ-$chain^+$) occur in the liver on about day 40 of gestation, and surface IgM^+ cells are found in the spleen (day 50) and bone marrow (day 60). A small amount of immunoglobulin is secreted by B cells of the spleen and liver beginning with day 50 of gestation, spontaneous isotype switching from IgM to IgG is occurring in the thymus [59]. Porcine fetal B cell areas react with various conserved molecules and antigens [51,58].

The basic structures of the organised lymphoid tissue of the gut (GALT), e.g. the Peyer's patches, are developing in pigs during the intrauterine period [60,61] as it is the case in humans. The function of GALT during the pre- and early postnatal development is described in more detail in the articles by Sinkora and Butler [50] and Butler et al. [57].

3. Thymus

The thymus is a lympho-epithelial organ, its organ reticulum is derived from the third branchial pouch/groove and thus has entodermal and ectodermal origin. As primary lymphoid organ the thymus is important for primary T cell development [50]. Because of the organ size and the surgical accessibility many studies have been performed in pigs analysing thymic lymphocyte immigration and emigration [62,63]. Resection of the thymus in neonatal pigs resulted in a reduction of about 95% of the γ/δ null T cells and in an increase of double positive T cells ($CD4^+CD8$ low positive) [55]; the small residual proportion of γ/δ T null cells may have their origin in the intestinal tract [29]. Especially the cortical T cells were reduced in pigs that had undergone cortisone therapy [64]. The complexity of thymic T cell development became clear by the fact that also B cells were observed in the porcine thymus [59] and that lymphocytes immigrate from the peripheral blood into the thymus [62].

For induction of acquired tolerance in heart/kidney transplantation experiments the host thymus is essential [65]. As the porcine thymus is easily accessible for surgery, vascularised thymic transplants were used in xenotransplant studies to understand in more detail the development of tolerance [66,67].

4. Tonsils

The palatine tonsils in the pig are lymphoid tissue in the mucosa of the oral cavity, they are located at the palate bones [68]. Further, lymphoid tissue is located close to the pharyngeal opening of the Eustachian tube as pharyngeal tonsil, covered with respiratory epithelium [69]. Lymphoid tissue at the basis of the tongue is called lingual tonsil [70]. Although often described as "lymphoepithelial" tissue; the organ reticulum of the tonsils is originating from the connective tissue of the mesoderm. The close contact of the tonsils to the oral cavity and to the nasal airways raises the question how the tonsils contribute to mucosal immunity. The epithelium is extended into deep crypts [71]. Typically, the epithelium of the tonsillar surface and crypts is containing many T and B cells [72]. These cells obviously migrate from the lamina propria of the mucosa into the epithelium. Based on this diapedesis the tonsil epithelium is often described as lympho-epithelium.