The pig as a medical model for acquired respiratory diseases and dysfunctions: An immunological perspective

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Abstract

By definition no model is perfect, and this also holds for biology and health sciences. In medicine, murine models are, and will be indispensable for long, thanks to their reasonable cost and huge choice of transgenic strains and molecular tools. On the other side, non-human primates remain the best animal models although their use is limited because of financial and obvious ethical reasons. In the field of respiratory diseases, specific clinical models such as sheep and cotton rat for bronchiolitis, or ferret and Syrian hamster for influenza and Covid-19, have been successfully developed, however, in these species, the toolbox for biological analysis remains scarce. In this view the porcine medical model is appearing as the third, intermediate, choice, between murine and primate. Herein we would like to present the pros and cons of pig as a model for acquired respiratory conditions, through an immunological point of view. Indeed, important progresses have been made in pig immunology during the last decade that allowed the precise description of immune molecules and cell phenotypes and functions. These progresses might allow the use of pig as clinical model of human respiratory diseases but also as a species of interest to perform basic research explorations.

Generalities

In its 'Global Impact of Respiratory Disease', the World Health Organization (WHO) identifies 'the big five' respiratory diseases as chronical obstructive pulmonary disease (COPD), asthma, acute lower respiratory tract infections, tuberculosis and lung cancer. Moreover, according to Eurostat report, diseases of the respiratory system accounted for 7.5 % of all deaths in the European Union in 2016 (Eurostat, 2020), and these statistics do not take into account the current SARS-CoV-2 pandemic which will probably become the infectious disease associated to the highest number of fatalities in 2020. In the pathophysiology of all these diseases, the immune system has definitely a central role and we need to better understand how it operates in order to prevent and treat the respiratory diseases.

Current Food and Drug Administration (FDA) guidelines require testing of new pharmaceutical agents in both small and large animal models when proving a therapeutic concept (FDA, 2015). We advocate herein to consider pig as a model of choice when investigating the immunological side of respiratory diseases.

The *Suidae* family (swine, wild boar, red river hog, and warthog to cite the best known) is evolutionary more distant from the *Hominidae* family (including human being and all the great apes) than the *Muridae* family (mouse, rat, hamster and naked mole rat). Indeed, men and mice belong to the same *Euarchontoglires* superorder whereas pigs belong to the *Laurasiatheria* superorder. Despite this, and probably as a consequence of the faster genetic evolutionary rate of mouse, the pig genome has remained more similar to the human one both in terms of DNA sequences than in term of genes' synteny (Humphray et al., 2007; Wernersson et al., 2005). Because of this genetic proximity and for other reasons listed below, the pig has been largely used as a biomedical model and notably as a model for the study of human infectious diseases and for vaccine development (Gerdts et al., 2015; Käser et al., 2018; Lunney, 2007; Meurens et al., 2012). In this review we focused especially on the pig as a model to study acquired respiratory diseases except cancer, at the light of the accumulating knowledge concerning the swine respiratory immune system.

Porcine Respiratory System Structure

The size and the weight of the pig is age-, sex- and breed-dependent. Some minipig breeds like the Yucatan or the Göttingen present adult pigs with size and weight similar to human adults making the breeds attractive in biomedical research (Meurens et al., 2012; Swindle et al., 2012). Overall, the porcine anatomy and physiology are quite similar to human anatomy and physiology, both species are all-eating for instance (Käser et al., 2018; Meurens et al., 2012; Swindle et al., 2012). Moreover, pigs are widely available with usually manageable size allowing for smooth handling and easy experimental interventions.

Regarding specifically the porcine respiratory tract, the pig has been extensively characterized and compared with the human one (Judge et al., 2014; Krejci et al., 2013) as well as in exhaustive reviews that were assessing its potential as a model for cystic fibrosis (Rogers et al., 2008) and asthma (Kirschvink and Reinhold, 2008). The respiratory tract can be divided into the upper and lower respiratory tracts. The upper respiratory tract is including the nasal cavities, the sinuses, the nasopharynx, the larynx and the trachea while the lower respiratory tract includes overall the lung with the bronchi, the bronchioles, the terminal and respiratory bronchioles, and alveoli. Air enters the porcine respiratory tract through round nostrils and a pair of nasal cavities situated in the snout (*rostrum*). In the nasal cavities, the epithelium is typically pseudostratified with ciliated columnar cells. The epithelium contains loose lymphocytes, lympho-reticular tissue aggregates and tubo-alveolar glands. Regarding submucosal glands in nasal turbinates and trachea, some differences have been shown between pigs and humans using the porcine cystic fibrosis model (Cho et al., 2011). Similarly, to humans, pigs have ethmoid and maxillary sinuses at birth and develop frontal and sphenoid sinuses (Sisson et al., 1975). Then, after its circulation in the nasal cavities, the air moves through the nasopharyngeal tube into the pharynx with the possibility to gain access to the esophagus. The respiratory part of the pharynx, called nasopharynx, also presents a pseudostratified ciliated columnar epithelium with goblet cells. In the upper respiratory tract, its nasal-associated lymphoid tissue (NALT) resembles the human's Waldeyer ring and pigs, like humans, possess several tonsils (*veli palatine*, *pharyngea*, *tubaria*, *paraepiglotica* and *lingualis*) (Horter et al., 2003; Liebler-Tenorio and Pabst, 2006; Swindle et al., 2012). This lymphoid tissue is comprised of the *Lamina propria mucosae* as well as fine glandules. Then, the larynx links the nasopharynx and the lower respiratory tract, including epiglotis and vocal cords. In the larynx the mucosa showed again a pseudostratified columnar epithelium with isolated goblet cells. In the submucosa of epiglottis, *plicae aryepiglotticae* and *vestibulum larynges*, an accumulation of lymphoid tissue is observed. The trachea consists of 32-36 C-shaped rings of hyaline cartilage with strong fibro-elastic membranes observed between the rings. The trachea shows a ciliated pseudostratified columnar epithelium and isolated goblet cells. Below, in the mucosa, small islets of lympho-reticular tissue and combined tubo-alveolar glands are observed. Regarding the gross anatomy of the porcine lung, two lobes on the left sides and four on the right have been described (Rogers et al., 2008; Swindle et al., 2012). On the left side, the lobes are designated as left cranial and caudal and on the right as right cranial, right middle, right caudal and right accessory. Interestingly, the right cranial lobe is directly linked to the trachea. Looking at subgross anatomy, pig like human lung, and in contrast to murine lung shows extensive intralobular and interlobular connective tissue. This tissue joins the bronchi and the main blood vessels to the pleural surface (Tyler, 1983). However, the interlobular collagenous network is incomplete in humans whereas in the pig the interlobular septa are "complete" blocking the collateral ventilation (Rogers et al., 2008; Woolcock and Macklem, 1971). The porcine pleura like its human counterpart is relatively thick and collagenous and has a vascular supply originating from the bronchial arteries (McLaughlin et al., 1961; Tyler, 1983). Regarding the lymph nodes (LN) in the thoracic cavity, they are concentrated into four lymphoid centres, two parietal (*thoracicum dorsal*

and *ventral*) and two visceral (*mediastional* and *bronchale)*. Moreover, there are the LN adjacent to the bronchi (*tracheobronchales dextri*, *sinistri* and *medii*). While the vascular circulation of the pig follows a common pattern of organization and development with human vascular circulation there is an inverted structure of swine LN compared to their human and murine counterparts. Indeed, in most of the mammalians, including mouse and man, afferent lymph percolates in a centripetal manner from the periphery to the center (hilum) of the node. In swine, the afferent lymph diffuses from the center to the periphery in a centrifugal manner (Mcfarlin et al., 1973). In addition, in pig, lymphocytes exit the LN through the high-endothelial venules whereas in mouse and man they exit through the efferent lymphatics (Sasaki et al., 1994). The functional implications of these oddities have not been explored yet. Overall, even if similar to human airways, the porcine airways show small differences (Haworth and Hislop, 1981; McLaughlin et al., 1961; Rendas et al., 1978): i) the cartilage around porcine airways is more developed than in humans; ii) a lower number of bronchi generations, after the last cartilage plate, has been reported in pigs compared to what is reported in humans; and iii) longer terminal bronchioles and overall less-defined respiratory bronchioles are described. At the microscopic level, porcine lungs show similar cell lineages to human lungs (Haworth and Hislop, 1981; Jones et al., 1975; Mills et al., 1986; Rogers et al., 2008; Winkler and Cheville, 1984).

General systemic immunology

Before addressing specifically the porcine lung immunology, a brief overview of the systemic porcine immune system is needed. Comparably to the whole genome comparisons, the immunome analysis demonstrated a greater similarity between human and pig than between human and mouse. However, a peculiarity of swine immune system that can be highlighted is the expansion of the type I IFN gene families, especially the IFNδ and the IFNω (Dawson et al., 2013). IFNδ clearly distinguishes pig from human and mouse since pig has 11 functional IFNδ genes and mouse and man have none. IFNδ bind to the same type 1 receptor as IFNα, it has high antiviral and anti-proliferative activities on porcine cells, but not on human cells (Lefèvre et al., 1998). Conversely IFNω separates mouse from the two other species since mouse has no functional IFNω gene, man 1, and pig 7. IFNω presented highly cross-species antiviral (but little anti-proliferative) activity (Jennings and Sang, 2019; Shields et al., 2019). A recent review summarized the different porcine cytokines, chemokines and growth factors, and described the tools available to study them (Dawson et al., 2020).

Looking more precisely at the different immune cell types, three relatively recent reviews have been published on the porcine innate immune response (Mair et al., 2014), mononuclear phagocytes (Fairbairn et al., 2011) and the B and T lymphocytes development (Sinkora and Butler, 2016). However, we would like here to recall some important swine specific features:

Myeloid cells: **Neutrophil** serine proteases (NSP) have critical roles in neutrophil-associated tissue-destructive diseases. Human and mouse NSP present different peptide substrate specificities (Kalupov et al., 2009) whereas porcine NSP, present on the surface of triggered neutrophils and neutrophil extracellular traps (NETs) are efficiently inhibited by human NSP inhibitors (Bréa et al., 2012). In addition to neutrophils, porcine basophils and eosinophils can be identified by flow cytometry (Blanc et al., 2020; Haverson et al., 1994).

Peripheral blood monocytes present in pig a similar division in CCR2+/CX3CR1-/CD14+/CD163-/MHC-II- and CX3CR1+ CCR2-/CD14-/CD163+/MHC-II+ monocytes (Moreno et al., 2010). When human, mouse and porcine monocytes were compared by a transcriptomic study, porcine CD14+ and CD14- blood monocytes resembled more to their human counterparts than mouse monocytes (Fairbairn et al., 2013).

Dendritic cells (DC) subpopulations corresponding to cDC1, cDC2, pDC and moDC (Guilliams et al., 2014) can be readily identified in blood (Auray et al., 2016), skin (Marquet et al., 2014), spleen (Soledevila, personal communication), tonsils (Soldevila et al., 2018), tracheal epithelium and lung (Maisonnasse et al., 2016), with minimal species-specific features. The main swine DC peculiarity is the differential expression of TLR3, which is almost exclusively expressed on pDC in swine (Auray et al., 2016; Soldevila et al., 2018) but on cDC1 in human (Poulin et al., 2010) and mouse (Desch et al., 2011). Despite this difference, the calculation of a similarity score between swine, human and mouse cDC1, cDC2 and pDC transcriptomic signatures highlighted that the three human's blood DC populations resembled more to their porcine than to their murine counterparts, especially cDC2, which appears to be the most similar between pig and man (Auray et al., 2016).

Lymphoid cells: It has long been thought that porcine B cells were generated in ileal Peyer's patches, however it is now clear that porcine B lymphopoïesis is located in the bone marrow as for other mammals (Sinkora and Sinkorova, 2014). To note, the porcine B cell development studies are still impaired because of the absence of antibodies recognizing pan-B cells markers such as CD19 and CD20 (Dawson and Lunney, 2018) and the absence of antibodies discriminating porcine IgG isotypes. Despite these difficulties, the porcine peripheral B lymphocyte differentiation steps have been described: IgM+CD2+CD21+ B cells are composed of naive B cells, IgM+CD2-CD21+ are activated or primed B cells and CD79α+CD2+CD21- or CD2-CD21- represent antibody producing B cells (Sinkora et al., 2013), memory and effector antibody-forming B cells remaining CD79α+CD2+CD21- while CD79α+CD2-CD21- B cells are probably composed of resting plasma cells. In a recent paper (Bordet et al., 2019), we investigated the structure of the trachea-bronchial inverted porcine LN and, thanks to the conservation of the B cell affinity maturation master-genes expressions (BCL6, Pax5, IRF4, XBP1 and Blimp1), we were able to distinguish the centroblasts, centrocytes, plasmablasts and plasma cells in their respective follicular and extrafollicular LN sublocalisations, as observed in mouse and human (for review see (Klein and Dalla-Favera, 2008)). Interestingly, we also identified a specific feature of swine inverted LN which is a centroblasts-like B cell type decorated with surface CD169 proteins (Bordet et al., 2019), that interact probably with perifollicular macrophages in order to capture antigens and transport them to follicular DC (fDC). This feature might be a consequence of LN inversion in pig. In the regular murine LN, naïve B cells have been shown to play this antigen-transporting role (Phan et al., 2009). Follicular DC have not been described so far in pig, however they have been identified in an evolutionary close species, the sheep, and their phenotype appeared similar to murine and human fDC (Melzi et al., 2016).

Development of thymic αβT cells follows mice and humans T-cell maturation model (Sinkora et al., 2013). However, it is important to note that swine possess extrathymic CD4+/CD8α+ T cells, that appear to be regular memory/activated peripheral CD4 T cells (Gerner et al., 2009). The peripheral blood re-expression of CD8α has been endowed with no specific role. Indeed, so far the same CD4 T cell subtypes have been described in pig, namely Th1, Th2, Th17, Treg expressing the same transcription factors (respectively T-bet, GATA-3, RORγT and FoxP3) than human and murine CD4 T cells (for review see (Gerner et al., 2015)). To note, in swine some clues are in agreement with a B cell activation/Th2 pathways relying more on IL13 than on IL4 (Murtaugh et al., 2009). Finally peripheral CD8 T cells expressing Eomesodermin (Eomes), a transcription factor involved in the differentiation and long-term survival of CD8 memory T cells have been described in pig like in mouse and human blood, although these cells are less numerous in swine than in human (Rodríguez-Gómez et al., 2016). Finally, by using CD27 staining, Talker *et al* were able to distinguish *bona fide* effector memory cells ($CD27^{neg}$) from naïve and early activated lymphocytes (Reutner et al., 2013).

As in other *Laurasiatheria*, pigs present a high proportion of **γδ T cells** (Binns et al., 1992), paralleled with more D-segments in the TCR delta gene than in human and mouse (Dawson et al., 2013). Thus porcine γδ TCR have a strong diversity potential, with no tissue-specificity (Holtmeier et

al., 2004) or subset restrictions (Stepanova and Sinkora, 2013) unlike in humans and mice. Moreover, conversely to murine and human ones, a consistent proportion of porcine γδ T cells retained a strong expression of GATA3 (Rodríguez-Gómez et al., 2019).

Invariant NK T cells expressed a semi-invariant TCR which recognizes glycolipids presented on the non-polymorphic CD1d molecules and corresponding either to stress-related self-ligand or to microorganism surface-antigens. Interestingly, iNKT cells frequency (Artiaga et al., 2014) as well as specific CD1d/TCR molecular interactions (Yang et al., 2019) are closer between swine and human than with mouse.

Conversely to the majority of mammals, swine possess an **NK cell subset** that does not express NKp46 (Mair et al., 2012). This might be relevant here since NKp46 recognizes hemagglutinins (HA) of influenza, parainfluenza, and Sendai virus and that its ligation leads to lysis of infected cells (Arnon et al., 2004). In swine, NKP46 expression has also been observed on CD3+ cells expressing αβ or γδ TCR but presenting the main functions of NK cells (Mair et al., 2016). Cells with a similar phenotype have been described in mice (Arnon et al., 2004) humans (Arnon et al., 2004) and cattle (Connelley et al., 2014).

Specific Lung immunology

Macrophages

In all mammalian species, lung macrophages are prominently composed of alveolar macrophages (AM). AM main roles are the phagocytosis of inhaled particles and the clearance of surfactant (for review see (Hussell and Bell, 2014)). AM have been shown in mouse to originate from embryonic monocytes that settled before birth in the alveoli and self-renew independently of peripheral blood adult monocytes (Guilliams et al., 2013). This scheme can be modified upon events that trigger both strong inflammation and partial or complete AM depletion. In this case, as documented in mice, recruited inflammatory monocytes will differentiate in pro-inflammatory monocyte-derived macrophages (moMθ) before entering AM 'niche' (for review see (Guilliams and Scott, 2017)) and differentiating finally in 'true' AM, undistinguishable from the original AM population (Aegerter et al., 2020). Although not yet formally demonstrated, the belonging of human AM to the local-self-renewable macrophage type is also postulated. By analyzing AM from lung grafted patients, several teams tried to clarify the importance of AM self-renewal *versus* blood monocytes replacement in human, leading to contradictory conclusions (Bittmann et al., 2001; Eguíluz-Gracia et al., 2016; Nayak et al., 2016). However a recent work using up to date single cell technology (Byrne et al., 2020) arbitrated toward a strong contribution of blood monocytes for long term AM maintenance, the authors arguing that human beings, conversely to lab's mice, are constantly challenged by inflammatory stimuli, in a much longer time frame, and that in human everyday life, the AM pool might be replaced by moMθ within a year. In porcine lung, we observed that AM did not express blood-related genes such as cKit, CSF1R CCR2 or CX3CR1 but did expressed HDAC10, PU.1 (Bordet et al., 2018; Maisonnasse et al., 2016) whose expressions are related to embryonically-derived macrophage precursors in mouse (Guilliams et al., 2013; Schulz et al., 2012), in agreement with a similar local-self-renewable capacity of porcine and mouse AM. However, according to the 'niche theory', these local-self-renewable AM could well be former moMθ that transdifferentiated in true AM upon AM-niche occupancy as postulated in human.

To note, similarly to mouse and man (Keller et al., 2015), porcine AM expressed the immunoproteasome subunits LMP2, LMP7, and MECL-1 upon respiratory viral infection (Liu et al., 2018, 2017).

One intriguing feature of pulmonary macrophage network in swine, and the majority of the *Laurasiatheria*, is the presence of pulmonary intravascular macrophages (PIM, for review (Schneberger et al., 2012)) that are not present, at least at steady state, in mouse, rat and human

(Brain et al., 1999). PIM are resident lung intravascular macrophages intimately tight with endothelial cells, what differentiates them from marginated blood monocytes (for review see (Kuebler and Goetz, 2002)). We recently isolated porcine PIM and demonstrated that they were very similar to AM (Bordet et al., 2018; Crisci et al., 2020), indeed PIM and AM overexpressed genes such as PU.1 and HDAC10, leading us to suggest than PIM were, like AM, self-renewable embryonically derived macrophages, and/or that PIM may directly originate from AM (Bordet et al., 2018). PIM have been involved in acute lung inflammation in *Laurasiatheria* species (Cantu et al., 2007; Singh et al., 2004). Interestingly PIM can be induced in rat model of systemic induced inflammation triggered by biliary duct ligature (Gill et al., 2008) and some clues of PIM induction in humans suffering of liver dysfunctions (Klingensmith et al., 1978, 1976) have been reported. In a recent report using mice grafted with human bone-marrow ('humanized' mice), the authors detected PIM which were, in this model, monocyte-derived human macrophages in tight contact with murine endothelial cells (Evren et al., 2021).

Interstitial macrophages

Interstitial lung moMθ identities and functions have been recently revisited in mouse and man by the identification of two distinct populations (Chakarov et al., 2019), one Lyve1^{low}/MHC-II^{high}/CD163^{low}, and the other Lyve1^{high}/MHC-II^{low}/CD163^{high} respectively residing adjacent to nerve bundles and to blood vessels. The later Lyve1^{high}/MHC-II^{low} moMθ secreted a high basal level of IL10, supported blood vessel integrity, and decreased inflammatory cells infiltrations upon pulmonary inflammation in mice. This degree of precision has not been reached in pig, although we identified an MHC-II^{high}/CD163^{low}/CD169^{high} moMθ population (Maisonnasse et al., 2016) as well as an MHC-II^{low}/CD163^{high}/CD169^{low} (unpublished data), whose differential roles and locations remain to be explored.

Dendritic cells

Swine DC have been identified in tonsils (Soldevila et al., 2018), tracheal epithelium and pulmonary parenchyma (Maisonnasse et al., 2016). They can be divided in cDC1, cDC2 and moDC whose RNAseq signatures cluster with their mouse spleen and/or lung counterparts (Crisci et al., 2020). Plasmacytoid DC have also been identified in swine tonsil, they are E2.2 and IRF7 positive as observed in mouse and man. The porcine lung cDC and moDC populations present the functional characteristics of *bona fide* DC, migrating toward LN chemokine upon maturation, whereas only cDC, but not moDC significantly trigger naïve CD8 and CD4 T cells proliferation. Conventional DC2 are better in activating CD4 T cells than cDC1, and this in lung as well as in tonsil (Maisonnasse et al., 2016; Soldevila et al., 2018), as previously described in mouse and human (Schlitzer and Ginhoux, 2014). Again consistent in tonsil and in lung, both porcine cDC1 and cDC2 activate CD8 T cells, conversely to what is observed in mouse (Ng et al., 2018). Interestingly, in humanized mice, human cDC1 and cDC2 expend similarly influenza specific CD8 T cells, however only cDC2 induced mucosal effector CD8 T cells (Yu et al., 2013). This property of cDC2 has not been explored yet in swine lung.

Another intriguing transpecies feature of DC is the expression of Langerin on cDC2 in pig (Maisonnasse et al., 2016) and human (Bigley et al., 2015) but on cDC1 in mouse (Sung et al., 2006). It has been shown in human that Langerin is rapidly induced in blood cDC2 upon TGFβ exposure (Bigley et al., 2015). Interestingly, this might be interpreted in conjunction with the expression of CD103 (αEβ7 integrin) on lung porcine cDC2, as well as with the sub-epithelial location of cDC2 in man (Yu et al., 2013) and pig (Maisonnasse et al., 2016). Indeed, TGFβ is largely produced by respiratory epithelial cells and Langerin, like CD103, is induced upon TGFβ exposure (Parker et al., 1992; Picarda et al., 2016). The sub-epithelial location of cDC2 in humans and pigs might explain both the Langerin and CD103 expression phenotype and the higher involvement of cDC2 in the activation

of CD8 response compared with their mouse counterparts (Yu et al., 2013). FcεRIα is expressed in pig (Maisonnasse et al., 2016) and human (Greer et al., 2014) but not in mouse cDC2. Indeed in mouse inflammatory moDC expressed the FcεRIα (for review see (Lambrecht and Hammad, 2012)). This difference might be of great importance in the feedback of allergic response since IgE/FcεRIα signaling in human cDC2 trigger an inhibition of the inflammatory response (Platzer et al., 2015) whereas it is thought that FcεRIα expression on mouse moDC would allow an IgE-mediated allergen presentation in a positive amplification loop. Interestingly, a recent work in mice complicated the cDC1/cDC2/moDC picture by demonstrating that upon inflammation lung cDC2 can arbor cDC1 and moDC features such as IRF8, CD64 and FcεRIα expressions (Bosteels et al., 2020). It would be interesting to investigate if these modifications stand true for human and porcine cDC2. Finally, in pig, monocyte-derived DC have been identified upon influenza infection and their phenotype and functions are compatible with inflammatory DC, harboring low migratory capacities and strong production of IL1β and IL8 upon stimulation (Maisonnasse et al., 2016).

Innate lymphocytes

NK cells were shown to decrease in peripheral blood and to preferentially localize close to infected area in porcine influenza infected lung (Forberg et al., 2014), in agreement with an important role in the control of influenza infection as observed in mice (Gazit et al., 2006). The CD3+NKp46+ lymphocyte population described by Mair et al. (Mair et al., 2016) was minimally detected in spleen, blood and mediastinal LN (< 2%) but presented a higher frequency, associated to a large variability according to the animal, in lung (1% to 10%), what may suggest a role of this population in lung immune surveillance in swine. Invariant NKT (iNKT) cells binding human CD1d tetramers loaded with an α-GalCer analog have been described in the porcine lung. They are CD3 and CD44 positive but do not express Nkp46 and CD11b, and can be subdivided in three main populations expressing no, low or high levels of CD8α (Yang et al., 2017a). Interestingly, it has been shown recently that porcine iNKT cells were able to respond to influenza-exposed myeloid cells *in vitro* (Schäfer et al., 2019).

To our knowledge, an interesting observation has been done only in pig lung, which is the demonstration that γδ T cells were the main alveolar population able to migrate to bronchial LN (Pabst and Binns, 1995).

T Lymphocytes

As expected from mouse and man data, it has been observed in pig that parasitic *Ascaris suum* infection bias the lung immune response toward a Th2 response (GATA3, IL4, IL5 upregulation in the whole lung tissue) (Steenhard et al., 2009) and that *Actinobacillus pleuropneumoniae* bacterial infection triggered a Th17 immune response, as validated by the presence of antigen-specific IL17A secreting CD4 T cells (Sassu et al., 2017). Intranasal influenza vaccination triggered resident memory T cells (Holzer et al., 2018). Moreover, proliferating poly-functional Th1 (IFNγ, TNFα and IL2-secreting) and CD8 (IFNγ, TNFα-secreting) lymphocytes can be detected in the trachea-bronchial LN and the lung of influenza infected animals, from 6 to 44 days post-infection (Talker et al., 2016). Interestingly flu-specific IFNγ-secreting CD8 T cells expressed perforine and were 30 times overrepresented in the lung than in the peripheral blood. Cytokine production was dominated by T cells with an early effector phenotype or central memory phenotype ($CD27^{pos}$) as observed in mice (Ballesteros-Tato et al., 2010) in agreement with the existence in porcine lung of tissue-resident memory T cells.

B lymphocytes

To our knowledge the B cell response in the porcine respiratory tract has only been addressed through nasal swabs and broncho-alveolar lavages antibody monitoring (among others (Bernelin-Cottet et al., 2019; Heinen et al., 2000)).

In conclusion, considering the respiratory immune system, mice and swine present both similarities and differences compared with human beings (Table 1), which must be consciously considered before choosing one species as an animal model for the pathogen of interest.

Model

We will consider here the porcine models developed to tackle the main respiratory diseases identified by the WHO, at the exception of lung cancer. *Id est,* respiratory infections and vaccination, allergy and asthma, acute and chronic inflammations, to which we will add two short chapters on microbiota and lung graft (Table 2).

Respiratory infections

Swine has been frequently used to study human respiratory pathogens naturally infecting the pigs, including, amongst others , *Mycobacterium* sp (Bolin et al., 1997; Ramos et al., 2017), *Pseudomonas aeruginosa* (Dehring et al., 1983; Luna et al., 2009), *Staphylococcus aureus* (Martínez-Olondris et al., 2010) and *Alphainfluenzavirus* (Rajao and Vincent, 2015). Alternatively, pigs can be susceptible to experimental infections of otherwise strictly human pathogens such as *Bordetella pertussis* (Foreman-Wykert and Miller, 2005). One of the main gap of naturally occurring respiratory infections in swine is the absence of a porcine equivalent of the human respiratory syncytial virus (RSV), a very significant pathogen in human, although an orthopneumovirus close to RSV has been recently detected in pigs (Hause et al., 2016) and might be present in France too (Richard et al., 2018). In this chapter we will expose the interest of the pig model for *P. aeruginosa*, influenza A and coronavirus infections.

-The bacterium ***P. aeruginosa*** rarely infects human lungs unless the host immune system has been impaired because of cystic fibrosis (CF), chronic obstructive disease (COPD) or ventilator-associated pneumonia. An infectious model has been developed in pig that recapitulates all the main features of this human infection, including bronchial contraction, transient increase in pro-inflammatory cytokines (IL8, IL6 and TNFα), neutrophilia, neutrophil extracellular trap (NET)osis, and the secretion of massive amounts of neutrophil serine proteases leading to lung damages (Chevaleyre et al., 2016).

-***Orthomyxoviridae*** and more specifically *Alphainfluenzavirus* (IAV) are pathogens of major importance in animal and human medicines. As recently reviewed, pig is more and more considered as an alternative model to consider with ferret and mouse, for the study of human influenza infection (Starbæk et al., 2018). Pigs are natural hosts of different strains of IAV (Kuntz-Simon and Madec, 2009), presenting frequent interspecies transmissions from pig to man and the reverse (Chastagner et al., 2019a, 2019b), what underlines the proximity of swine and human IAV as well as the similarity of the respiratory systems in both species. The most frequently encountered subtypes in pigs, H1N1, H1N2 and H3N2, are the same than in humans. Besides that, the interest for the pig model is strengthened by the fact that pig is considered as a mixing vessel of human, porcine and avian influenza virus for the generation of new influenza virus reassortants (Ma et al., 2009). The susceptibility of pigs to infections with both avian and mammalian influenza virus was recently explained to some extent in a study showing that the porcine host factor ANP32A, contrary to porcine ANP32B and other mammalian ANP32, had stronger supporting activity to the avian viral RNA polymerase (Zhang et al., 2020). In pigs, IAV are responsible of mild diseases (for review (Crisci et al., 2013)) with low fever (40.5°C *versus* 39.5°C for normal body temperature), low inflammatory signs of the upper respiratory tract (nasal/ ocular discharge and conjunctivitis), dyspnea and

coughing (Talker et al., 2016) very similar to human common, mild influenza infections. In mice, which are not the natural hosts for IAV, adapted strains trigger whole lung infection and inflammation, leading to death, more similar to highly pathogenic avian influenza infections in human (Gao et al., 2013). Ferrets are recognized among the best model for testing pathogenicity and transmission of human respiratory viruses including IAV (Enkirch and von Messling, 2015), although the evolutionary reasons for this convergence are still a mystery. Interestingly, a recent comparison of influenza heterosubtypic vaccination in pigs and ferrets, showed different outcome between species and underlined the interest of using different preclinical models to assess new vaccines against flu (Holzer et al., 2018). Which animal model, the pig or the ferret, reflects best the situation in human remains however to be determined.

By keeping the immune response under control, AM are recognized as the 'peace-keepers' of the lung (Schneider et al., 2014). In mouse (Schneider et al., 2014) like in pig (Kim et al., 2008), AM depletion aggravate the IAV-mediated disease. In pigs, similarly to humans, disease severity has been associated with increased local pro-inflammatory cytokines (Barbé et al., 2011; Khatri et al., 2010). This cytokine storm has been associated in mice to the arrival of inflammatory moDC in the lung, indeed, pharmacological or genetic downregulation of moDC trafficking moderates the early inflammation, reduces morbidity and mortality without impacting the mounting of the adaptive immune response (Aldridge et al., 2009). This model might also stand for IAV infection in swine, since we observed the arrival of similar pro-inflammatory moDC in the porcine lung as soon as 2 days post-IAV inoculation (Maisonnasse et al., 2016). As in mouse (Westerhof et al., 2019) and human (Bonduelle et al., 2014), influenza virus infections in pigs trigger multifunctional blood (Talker et al., 2015) and lung (Talker et al., 2016) antigen-specific CD8 and CD4 T cells. Interestingly a strain of inbred animals, the Babrahama pigs, allowed the use of swine major histocompatibility class I (MHC-I) tetramers to analyze the anti-NP CD8 T cells raised upon influenza intranasal vaccination (Tungatt et al., 2018), and influenza infection (Edmans et al., 2021). This last study also detected a strong influx of γδ T cells in the alveolar space during influenza infection. Similarly, and in agreement with a role in respiratory immunity, the CD3+NKp46+ lymphocyte population described in swine by Mair et al. (Mair et al., 2016) presented a strong increase in the lung of influenza infected animal, most probably due to local proliferation. SCID human beings and pigs presenting an autologous deficiency in Artemis gene (DCLRE1C) (Moshous et al., 2001) do not develop adaptive immune T and B lymphocytes but harbor functional NK cells (Powell et al., 2016). Human affected by this mutation present recurrent respiratory infections but not recorded influenza infections (Volk et al., 2015), and survived early childhood thanks to intravenous Ig therapy. Interestingly SCID/Artemis pigs presented higher virus excretion and delay in virus clearance, but milder lung lesions and clinical signs compared with their heterologous counterparts (Rajao et al., 2017), in agreement with a role of the adaptive immune response both in the control of the viremia and in the inflammatory pathology. In short, pigs can be an interesting model of human influenza infection using endemic porcine or human influenza viruses. The height of the influenza porcine model being to use pig-originated pdmH1N1 (Mena et al., 2016) human pandemic virus in porcine infectious assay as a model of the human infection (Schwaiger et al., 2019).

-**Coronavirus**: With the recent COVID-19 crisis there is new need for the development of relevant animal models to study coronavirus infections. In pig, the only respiratory coronavirus (Porcine Respiratory Coronavirus, PrCoV) is an *Alphacoronavirus* which is a variant of the Transmissible Gastroenteritis Virus (TGEV) (Wang et al., 2019). Indeed, a large 5' deletion in the Spike gene of TGEV altered the tropism and the virulence of PrCoV. PrCoV receptor is the aminopeptidase N (APN also named CD13), mainly expressed on respiratory and intestinal epithelial cells, whereas human SARS-CoV and SARS-CoV-2 bind to ACE2, a protein similarly expressed on respiratory and intestinal

epithelial cells but also on endothelial cells, which may have strong implication in virus pathogeny (Saponaro et al., 2020). Indeed, engineering transgenic mice expressing the human ACE2 suffice to trigger the full clinical picture of SARS-CoV-2 in mice (Bao et al., 2020; Israelow et al., 2020; Sun et al., 2020). Regarding the first SARS-CoV, the pig has been demonstrated to be susceptible (Chen et al., 2005). With the SARS-CoV-2, the situation is less clear and contradictory results have been reported (Meekins et al., 2020; Pickering et al., 2020; Sclottau et al., 2020; Shi et al., 2020). In 3 out of 4 of these reports (Meekins et al., 2020; Sclottau et al., 2020; Shi et al., 2020), pigs inoculated using nasal or oral routes did not develop any lesions nor clinical signs. Moreover, authors did not detect any viral excretion. However in one of these 3 studies (Meekins et al., 2020) SARS-CoV-2 replicated in porcine epithelial cell lines. In the fourth study (Pickering et al., 2020), mild clinical signs were observed in some inoculated pigs and viral RNA was detected in nasal lavage and oral fluid. Furthermore, infectious virus was isolated from submandibular LN in one pig 13 days post-inoculation and a serological response was evidenced in two animals 11 days post-inoculation (Meekins et al., 2020). Thus, at this early stage, it is very difficult to definitely conclude about the relevance of the pig model for the study of SARS-CoV-2.

The SARS-Cov-2 emergence revealed the lack of knowledge on seasonal human coronavirus. The prototype viruses from the two main HCoV lineages are 229E (*Alphacoronavirus*, like the porcine PrCOV) and OC43 (*Betacoronavirus*). They cause 15–29% of all common colds, and are the best characterized HCoV, although 2 other human coronavirus are also considered as endemic (NL63 and HKU1) (Su et al., 2016). In addition, HCOV-229E uses the same receptor as PrCOV. Thus swine might be a good model of seasonal coronavirus infections allowing, in addition, the investigation of coronavirus and IAV co-infections.

Regarding respiratory infections, it is worth to report here that pig lungs are suitable for the generation of precision cut lung slices (PCLS) permitting the analysis, using multiple *in vitro* conditions, of monoinfections (Delgado-Ortega et al., 2014) as well as of coinfecting viruses' complex interactions on primary tissue-samples (Dobrescu et al., 2014; Saade et al., 2020).

Vaccination

Reviews arguing for the use of large animal models, including swine, have been published in 2015 that inventoried pig usage for vaccination and challenge tests against *Bordetella pertussis*, *Chlamydia trachomatis*, human norovirus, rotavirus and IAV (Gerdts et al., 2015; Rajao and Vincent, 2015). In addition to allow the evaluation of vaccines directly through protection against an infectious challenge, animal models can permit to evaluate vaccines indirectly through immune responses measurement without the final step of the infectious challenge. In this case, the animal model is not anymore needed to be susceptible to the pathogen. For instance, swine are commonly used to assess the potency of delivery devices and adjuvants.

-Delivery: The place and mode of delivery determine the localization of memory and effector cells upon challenge. For instance it has been shown in mice that epicutaneous (EP) vaccination allows mucosal immune response in mice (Belyakov et al., 2004). Pig skin is recognized as a good model of human skin because of structure (hairiness, epidermis and dermis thickness, subcutaneous fat) and lipid composition (Hammond et al., 2000) similarities, which makes pig skin permeability similar to the human one, as well as because of dendritic cells and macrophages resemblance (Marquet et al., 2014; Summerfield et al., 2015). Thus pig is a model of choice to test EP, transcutaneous (TC) and intradermic (ID) vaccination protocols for the induction of respiratory immune protection. Direct intramuscular (IM) DNA vaccination presented early encouraging success in mouse (Ulmer et al., 1993) that were not reproduced in large animals including humans and pigs. Although not further investigated, this discrepancy might rely on tissue stiffness differences according to the animal size. Vaccination against respiratory diseases can also favor a pulmonary localized immune response by

directly exposing the target tissue to the antigens using aerosol (AS), intranasal (IN) or intratracheal (IT) deliveries. For that purpose, the size of swine upper respiratory tract, more similar to the human ones than small animals such as mouse or ferret, might guarantee a better compliance with clinical situations (Kirschvink and Reinhold, 2008; Rogers et al., 2008). As an illustration, herein some technics used in vaccination (mainly against influenza) in swine: prime-boost DNA/whole virus vaccine delivered ID/IM (Hewitt et al., 2019), single ID Aujeszky's Disease Virus glycoproteins vaccine (Le Luduec et al., 2016), influenza-proteins, DC-targeted ID or IM vaccination (Bernelin-Cottet et al., 2016), protein, epicutaneous anti-RSV vaccination (Hervé et al., 2016), single-cycle influenza virus delivered AS (Holzer et al., 2018), influenza virus-derived-replicon delivered IM (Ricklin et al., 2017), nanoparticules whole influenza virus encapsulation delivered IN (Dhakal et al., 2017) and aerosol intranasal delivery (Martini et al., 2020).

- Adjuvant: In addition to antigen and delivery, an essential part of a vaccine is the adjuvant used to increase the immunogenicity of dead vaccines. One of the main developing arm of adjuvant is the use of TLR-ligands, that trigger inflammation and antigen presenting cells activation. In that instance, swine present a restrictive expression of TLR3 on pDC (Auray et al., 2016; Soldevila et al., 2018) compared to the TLR3 expression on cDC1 in human (Poulin et al., 2010) and mouse (Desch et al., 2011). Conversely, pig and human, but not mouse, expressed an active TLR8 receptor that can detect RNA specifically from live bacteria (Ugolini et al., 2018), leading to a better activation of follicular DC and the differentiation of high affinity antibodies. Finally, it has been proposed to use αGalSer as an adjuvant in human vaccination that would harness iNKT cells (Speir et al., 2017) and help for CD8 T cells activation. This strategy has been prospected in pig (for review (Yang et al., 2017b)). A first assay using αGalSer as immunostimulant before IAV infection did not allow protection (Gu et al., 2021), although this result does not preclude the use of αGalSer as true vaccine adjuvant.

Microbiota

In the last years the importance of the microbiota and especially the gut microbiota in the preservation of homeostasis and health has been extensively studied. The gut microbiota can influence the lung health and the susceptibility of porcine host to respiratory infections (Bassaganya-Riera et al., 2003; Niederwerder, 2017). It is also known that all the mucosa in the body have some connections forming the common mucosal immune system in man (Dang and Marsland, 2019) as in pig (Wilson and Obradovic, 2020). Because of the use of pigs in biomedical research, some comparisons between human and pig gut microbiota have been carried out. By deep metagenome sequencing of faecal DNA from 287 pigs, Xiao and collaborators showed that among functional pathways found in humans, 96% were present in pigs (Xiao et al., 2016). However, in other studies comparing gut microbiota (Xiao et al., 2016) and fecal microbiota (Kobayashi et al., 2020) in different species, it was shown that pig was less close to humans than marmosets and three shrews. Moreover the pig, even if omnivorous like humans, had fecal microbiota showing some common features with herbivores as the presence of *Fibrobacter*, a cellulolytic bacterium (Kobayashi et al., 2020). Recently a further demonstration of the gut lung axis has been demonstrated in swine with an impact of a porcine herpesvirus (Aujeszky's Disease Virus) on microbial community and immune status in the ileum and colon of piglets (Zhang et al., 2019). Finally, the last decade firmly established the existence and precisely described a symbiotic, stable respiratory microbiota in mouse and man (for review see (Man et al., 2017). These investigations remain to be done in pig.

Respiratory Allergy and Asthma

Respiratory allergy corresponds to the improper activation of a Th2 response, leading to the establishment of an IgE mediated anamnestic immune response that will trigger eosinophils

degranulation and asthma. Although food allergy is well-established in swine (for review see (Rupa et al., 2009)), allergic asthma does not occur spontaneously in the animal world, albeit guinea pigs, ferrets and pigs can be artificially sensitized. The establishment of a stable chronic porcine asthma model appears to be difficult because the sensitivity to the antigen declines after repeated allergen exposure (Szelenyi, 2000). However, upon *Ascaris suum* antigen sensitization consisting of an *A. suum* extract in $Al(OH)_3$ followed by two booster doses and a challenge with nebulized allergen, pigs developed airway inflammation associated with eosinophils and neutrophils infiltration (Fornhem et al., 1996). On the induction side, cDC2 have been demonstrated to be the main trigger of the Th2 bias involved in the allergic response. As specified in the dendritic cells chapter above, porcine cDC2 resemble more to human than to mice cDC2 on several aspects in blood (Auray et al., 2016) as well as in the respiratory tract (Crisci et al., 2020). For instance, the two main features important to recall here are the expression by cDC2 of the FcεRIα, a receptor of IgE, and the intra-epithelial and sub-epithelial cDC2 location in the trachea and the bronchia respectively (Maisonnasse et al., 2016; Yu et al., 2013). Invariant NKT cells have been strongly involved in the Th2 bias of allergic responses (for review see (Matangkasombut et al., 2009)), interestingly Renukaradhya *et al*. demonstrated that intra-tracheal instillation of α-GalCer analog in pigs triggered iNKT cells activation and acute airway hyperactivity associated with a Th2 cytokine profile (Renukaradhya et al., 2011). On the effector side, dendrograms indicate that porcine IgE is more similar to IgE of carnivores, horses and humans than to other artiodactyls IgE (Butler et al., 2009). In swine as in human, mast cell tryptase inhibitor administrated prior to allergen challenge prevented acute bronchoconstrictive response and decreased histamine release (Ploeg et al., 2002), while corticoid administration decreased airways inflammatory cells infiltration (Fornhem et al., 1996). Still on the clinical side, porcine lung smooth muscles reactions to inflammatory stimuli are a current model of asthma-muscle contraction (Ram-Mohan et al., 2020; Sieck et al., 2019). Interestingly, airway contractions upon *in vivo* acid treatment in piglets (Reznikov et al., 2019) recapitulated the airway hyper-responsiveness (AHR) gender bias observed in infants (Van Merode et al., 2007).

Several intravenously administered compound such as nanomedicines, radiologic contrast agents and other pharmaceutical products may trigger in human rare events of pseudoallergic infusion reactions which are hypersensitivity reactions. Thanks to their high sensitivity, pigs have been used for decades to detect such reactions, using the “complement activation-related pseudoallergy” (CARPA) model (for review (Szebeni and Bawa, 2020)). Interestingly, pig high sensitivity seems strongly related to the presence of constitutive PIM (Csukás et al., 2015), what might be a clue of the presence of induced PIM in humans susceptible to such reactions.

Acute and Chronic inflammations

Acute lung injury (ALI) and its severe form acute respiratory distress syndrome (ARDS) are responsible for more than 10% of intensive care unit admissions. ARDS conditions can be artificially reproduced in swine by using lung repeated lavages, and oleic acid or endotoxin instillations (for review see (Ballard-Croft et al., 2012)). Porcine models more related to human medical conditions have been developed recently such as hemorrhagic shock (Morris et al., 2020), hyperoxia (Katalan et al., 2017) or ricin induced-ARDS (Katalan et al., 2017). To our knowledge, no porcine model of ARDS triggered upon infection-induced pneumonia has been reported. The early ARDS stage is characterized by an acute inflammatory response that includes release of IL1, TNF, IL8 and subsequent neutrophil recruitment (for review see (Ware, 2006)), that can be mimicked in pig (Morris et al., 2020).

In developed countries, chronic obstructive disease (COPD) is mostly provoked by smoking and result in the remodeling and narrowing of small airway associated with pulmonary emphysema,

likely due to chronic inflammation leading to increased numbers of neutrophils, macrophages, DC, T and B lymphocytes. Chronic inflammation is maintained by macrophages and neutrophils infiltration although autoimmunity related to autoantibodies and activated CD8 is suspected (for review see (Caramori et al., 2016)). The emphysema appears to be linked to metalloproteinases (MMP) catalytic activity. A mutated-pig phenotype has been described presenting emphysema associated with MMP9 and 12 hyper-expression which might be the first step toward the development of a COPD porcine model (Bruun et al., 2013). To note, pigs are already used as model for cigarette smoke short (Gilman et al., 1981) and middle (Gilman et al., 1981) term effects. The development of pig as a COPD model would allow to explore an interesting hypothesis linking COPD and non-alcoholic fatty liver diseases (Lonardo et al., 2017) that we would like to bring together with the potential pro-inflammatory PIM induction in humans suffering of liver dysfunctions (Klingensmith et al., 1978, 1976). Whether the constitutional presence of PIM in swine might favor or impaired this model remains to be established.

Lung graft

Herein we will not tackle the field of xenotransplantation, that is beyond the scope of this review and has been reviewed recently elsewhere (Burdorf et al., 2018). Regular lung transplantation is the final treatment option for patients presenting end-stage lung diseases. The 1-year survival has greatly improved in the last 40 years, reaching now 84% (Chambers et al., 2017). Pig is used as a model of lung transplantation (Mariscal et al., 2018) to study primary graft dysfunctions and test new preventive interventions to reduce or avoid these conditions (Iskender et al., 2016; Martins et al., 2004). Interestingly, pig lungs are currently used to improve and further develop the promising *ex vivo* lung perfusion (EVLP) method (for review (Tane et al., 2017)) leading to the reconditioning of lung graft rejected in first instances because of not reaching the graft quality criteria. Porcine model allowed to validate methods that decreased the expression of acute lung injury related genes (Dromparis et al., 2019) or that increased the extracorporeal surviving time (Hozain et al., 2020). Moreover, EVLP development give accessibility and time for intervention on the lung graft before grafting. The pig model allows to test extracorporeal intervention such as IL10 gene-therapy in order to act specifically on the immune tolerability of the transplant (MacHuca et al., 2017).

Conclusions and perspectives:

In conclusion, we think that the development of pig as a respiratory model of medical conditions might be encouraged following three main research fields:

Because of the resemblances between porcine and human cutaneous, respiratory and intestinal systems, associated to the immunological similarities of these species, pig appears to be a model of choice to study the interactions between the different mucosae. In this perspective, a better knowledge of porcine skin, lung and intestinal microbiota will be needed.

The second important point would be to develop pig as a model of respiratory allergies according to the high similarity of cDC2 in pig and man, as well as to the good knowledge of porcine neutrophils and iNKT cells. The main immunological hurdle for this development remains the lack of reagents to follow the B cell response (B cells markers, such as CD19 and CD20 and tools to discriminate the different IgG isotypes).

Finally, according to the large knowledge accumulated on the pig blood monocytes and respiratory macrophages, as well as to the relative long life span of pigs, the long term consequences of immune system alterations triggered by immunostimulants or primary infections, leading to trained immunity (Angulo et al., 2020; Guillon et al., 2020; Stylianou et al., 2019; Yao et al., 2018) or tolerance (Bouras et al., 2018; Didierlaurent et al., 2008) might be advantageously studied in this model.

To summarize, thanks to the knowledge and tools developed during the last years, pig appears now as the third medical model after mice and non-human primates, and might be seriously considered when looking for a respiratory experimental model, especially in the fields of mucosal interactions, allergies and trained immunity.

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Table I: Immune-related respiratory tract remarkable features of mouse, human and pig

	Mouse	*Human*	*Pig*	*Comments*
Airways	-Muzzle	-Nose	-Snout	-**hm ≠ pg ≠ ms** ≠ in higher respiratory tract size and structure
	-Small airways and lungs	-Large airways and lungs	-Large airways and lungs	-**hm & pg ≠ ms** ≠ in lower respiratory tract accessibility
Lymph nodes	-Centripetal circulation	-Centripetal circulation	-Centrifugal circulation	-**hm & ms ≠ pg** no known consequences
	-Lymphocytes exit through efferent lymphatics	-Lymphocytes exit through efferent lymphatics	-Lymphocytes exit through HEV	-**hm & ms ≠ pg** no known consequences
Granulocytes	-Neutrophil serine proteases not-sensitive to human inhibitors	-Neutrophil serine proteases sensitive to human inhibitors	-Neutrophil serine proteases sensitive to human inhibitors	-**hm & pg ≠ ms** ≠ in tissue inflammation handling
AM	-Embryonic origin	-Probably of embryonic origin	-Probably of embryonic origin	-**hm=ms=pg**
	-Self-renew	-No data	-No data	
	-Inflammation, replacement by moAM	-Large part of moAM	-No data	
PIM	-No information	-Induced PIM upon liver conditions?	-Constitutive inflammatory PIM	-**hm & ms ≠ pg** high susceptibility of pigs to infused particles
moMθ/ interstitial Mθ	-2 populations: nerve-associated blood vessel associated	-2 populations: nerve-associated blood vessel associated	-2 populations: location not explored	-**hm=ms=pg**
DC	-TLR3 expressed on cDC1	-TLR3 expressed on cDC1	-TLR3 expressed on pDC	-**hm & ms ≠ pg** ≠ dsRNA response?
	-cDC1 mainly involved in T CD8 activation	-cDC1 and cDC2 involved in T CD8 activation	-cDC1 and cDC2 involved in T CD8 activation	-**hm & pg ≠ ms** ≠ T CD8 induction?
	-cDC2 FcεRIα-, Lang-, CD103-, interstitial location	-cDC2 FcεRIα+, Lang+, CD103+, subepithelial location	-cDC2 FcεRIα+, Lang+, CD103+, subepithelial location	-**hm & pg ≠ ms** ≠ allergy induction?
NK/NKT cells	-NKp46 specifically expressed on NK cells	-NKp46 specifically expressed on NK cells	-NK subset NKP46-, T lymphocyte subset NKP46+	-**hm & ms ≠ pg** no known consequences
T Lymphocytes	-CD4 T cells devoid of CD8 expression	-CD4 T cells devoid of CD8 expression	-CD8α on memory CD4 T cells	-**hm & ms ≠ pg** no known consequences
	-Low proportion of γδ T cells	-Low proportion of γδ T cells	-High proportion of γδ T cells	-**hm & ms ≠ pg** ≠ innate immune response?
B Lymphocytes	-Naïve B cells transporting antigens to fDC	-Not described	-Centroblasts transporting antigens to fDC?	-**hm & ms ≠ pg** no known consequences

HEV: High-Endothelial Venules; AM: Alveolar Macrophage; PIM: Pulmonary Intravascular Macrophage; moAM: monocyte-derived AM; Mθ: Macrophages, moMθ: monocyte derived Macrophage; DC: Dendritic Cell; cDC: conventional Dendritic Cell; pDC: plasmacytoid Dendritic Cell; fDC: follicular Dendritic Cell; NK: Natural Killer; hm: human; ms: mouse; pg: pig. Bibliographic references are in the main text.

Table 2: Non-transmissible respiratory diseases pro and con of the porcine model

		PROS	CONS
RESPIRATORY INFECTIONS	*B. pertussis*	-naturally infected	
	Mycobacterium sp	-naturally infected	
	S. aureus	-naturally infected	
	P. aeroginosa	-naturally infected	
	Influenzavirus	-natural sensitivity to human and swine strains with mild clinical signs	-no example of highly pathogenic infections
	Coronavirus	-natural porcine *αCoronavirus* (PrCoV) similar to the human seasonal HCoV-229E	-disputed sensitivity to SARS-CoV-2
	Orthopneumovirus	-natural porcine *Orthopneumovirus*?	-not sensitive to hRSV
VACCINATION	Delivery	-skin structure -upper respiratory tract structure	
	Adjuvant	-functional TLR8 -cDC1 interstitial and cDC2 intra-subepithelial locations -αGalSer response	-TLR3 expression on pDC not on cDC1
MICROBIOTA		-omnivorous species	-intestinal microbiota similar to herbivore - data paucity on porcine respiratory microbiota
RESPIRATORY ALLERGY AND ASTHMA	Induction	-cDC2 location -cDC2 expressing IgE receptor (FcεRIα)	-no stable chronic allergic asthma porcine model
	Clinical signs	-IgE similar to human -lung smooth muscles porcine model -efficacy of tryptase inhibitors	
ACUTE AND CHRONIC INFLAMMATIONS		-model of early inflammatory ARDS responses -constitutive PIM	-no infectious model of ARDS induction -constitutive PIM
LUNG GRAFT		-size allowing similar surgical intervention -primary graft dysfunction model -*ex vivo* lung perfusion model	

SARS: Severe Acute Respiratory Syndrome; hRSV: human Respiratory Syncytial Virus; cDC: conventional Dendritic Cell; pDC: plasmacytoid Dendritic Cell; ARDS: Acute respiratory distress syndrome; PIM: Pulmonary Intravascular Lymphocyte. Bibliographic references are in the main text.