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The Swine Leukocyte Antigen (SLA) Complex Molecular Genetics and Importance in Veterinary Vaccine Research

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for the teaching subject

"Immunology"

submitted by

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TO BE A GOOD SCIENTIST, YOU NEED THE BRAIN OF A SCIENTIST AND YOU ALSO NEED TO FIND A WAY TO USE IT.

YOU HAVE TO BE SOCIALLY ADAPTED TO RUN A TEAM WELL; IN MOST DISCIPLINES, YOU CANNOT DO SCIENCE ALONE.

ORGANIZATIONAL SKILLS ARE ALSO IMPORTANT. BUT, MORE THAN THAT, YOU NEED TO BE HIGHLY PERSISTENT TO SUCCEED.

YOU CAN'T BE FIRST EVERY TIME, BUT AT LEAST YOU CAN TRY.

Prof. Dr. Christine Van Broeckhoven
Department of Molecular Genetics
University of Antwerp, Belgium

Dedicated to Alexandra

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1. Introduction – Background and Rationale

1.1 The Major Histocompatibility Complex (MHC)

The Major Histocompatibility Complex (MHC) is a cluster of genes that play an important role in various immunological processes. This genomic region encodes glycoproteins that belong to the immunoglobulin (Ig) superfamily and are exposed on the cell surface. The molecules encoded in the MHC bind foreign peptide antigens or peptide antigens specific to the organism, which are presented to the appropriate T cells, inducing immune response cascades (*Kulski et al. 2002, Murphy & Weaver 2016*, *Wieczorek et al. 2017*).

Mainly, MHC molecules are classified into two major classes, which differ not only in structure but also in function regarding their role in the immune system. The MHC class I gene products are primarily associated with intracellular pathogens. On the other hand, the MHC class II molecules are associated with antigen presentation of extracellular pathogens (*Spurgin et al. 2010*). Although they are expressed on different cell types, they are missing on red blood cells; hence, some pathogens specifically attack erythrocytes to avoid detection by e.g., cytolytic (cytotoxic) T cells (CTLs).

The MHC gene cluster in humans - so-called Human Leukocyte Antigen (HLA) - is located on chromosome 6 and contains over 200 genes. HLA genes are inherited in a Mendelian co-dominant fashion, meaning that a copy of each HLA gene (i.e., one haplotype) is inherited from each parent and expressed as antigens. These genes are associated with many different diseases. The MHC class I genes are linked to Leprosy, Type-1 autoimmune hepatitis or resistance to Malaria. On the other hand class I genes may be associated with diseases such as Kawasaki disease, Systemic Lupus, Asthma, Nephropathy or Leukaemia. Both MHC classes contribute to immunological processes in Multiple Sclerosis or Psoriasis or other immune-related diseases (e.g., *Garamszegi et al. 2014, Shrestha et al. 2016, Fiorillo et al. 2017, Lokki & Paakkanen 2019; Liu et al. 2021*).

The MHC region is polygenic, thus it carries several different genes of both MHC gene classes, so that each human individual is provided with a set of MHC molecules with different regions of peptide-binding properties. MHC genes are highly polymorphic, meaning that the type of molecule occurs in numerous different versions (so-called alleles) within the population. The MHC genes are known to be the most polymorphic genes and their polymorphism has an intrusive influence on antigen recognition by T cells (*Sommer et al. 2005*). MHC products also play other important roles, including the positive and negative selection of developing T cells described earlier, stimulation of naive and memory T cells that is necessary for their survival (homeostatic proliferation), the induction of T cell tolerance and anergy, and interaction with NK cells and other inhibitory and activating receptors (*Murphy & Weaver 2016*). The MHC is also partly involved in other processes such as shaping of mating preferences resulting in offspring MHC-heterozygosity (*Roberts et al. 2009*).

1.1.1 MHC class I molecules

The MHC class I molecules are expressed on the surface of all nucleus-containing cells of the organism. They are heterodimers comprised of an alpha heavy chain with three subunits and a beta-2 (β_2) microglobulin, whereby only the alpha component of the molecule is polymorphic (**Figure 1**). The β_2 microglobulin is a 12 kilodalton (kDa) polypeptide encoded by the *B2M* gene being located on chromosome 15 in human (*Güssow et al. 1987*).

The alpha (α) chain of the MHC class I molecule has an immunoglobulin (Ig) fold structure and is non-covalently associated with β2 microglobulin. The α_3 domain partially spans the plasma membrane and carries the CD8 binding site. The binding groove of the MHC class I molecule is formed by domains α_1 and α_2, and form a socket like opening, which can bind peptides of size 8-10 amino acids due to conserved tyrosine residues in the molecule (*Wieczorek et al. 2017*). The antigen fragments are bound with strong hydrogen bonds in the binding pocket. In the middle of the groove, there is a deep polymorphic pocket, which provides chemical and

structural complementarity for one of the anchor residues of the peptide and thus significantly contributes to the allele-specific peptide binding (*Matsumura et al. 1992, Liepe et al. 2016*). The MHC class I molecules are located on the lumen of the endoplasmic reticulum and bind cytosolic peptide fragments from the cell that originate from viral proteins or tumour proteins. After presentation on the cell surface, bound peptide fragments are selectively recognised by cytotoxic $CD8^+$ T cells. Tapasin-molecules, which catalyse the loading of peptides, provides an effective antigen presentation (*Brocke et al. 2002, Sadegh-Nasseri et al. 2008, Dong et al. 2009*). The three different MHC class I molecules (HLA-A, HLA-B and HLA-C) are coded by eight exons with most polymorphic exons 2, 3 and 4 (*Lokki & Paakkanen 2019*).

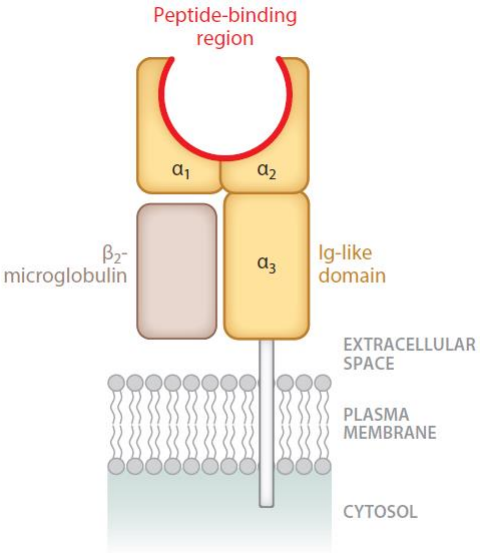

Figure 1. Model structure of MHC class I protein. Source: *Hammer et al. 2020* (IP-P5, Figure 2a).

1.1.2 MHC class II molecules

The MHC class II molecules have common features with the structure of MHC class I molecules (**Figure 2**). Like MHC class I molecules, MHC class II molecules are heterodimers. In this case, the MHC class II molecules consist of two noncovalent homogeneous peptides, alpha (e.g., DRA) and beta (e.g., DRB) chains, which have both extracellular and membrane spanning regions. The MHC encodes both chains of the MHC class II molecules. The α_1 and β_1 domains form the antigen-binding cleft of the MHC class II molecules. In both, alpha and beta chain of

class II proteins, there are Ig domains. The β_2 domain of the MHC II protein carries the CD4 binding site. The MHC class II glycoproteins are expressed on the surface of specialized antigen-presenting cells. They are found on dendritic cells (DCs), macrophages and B-lymphocytes. The antigens, which are mostly of extracellular origin and are internalised into the cell, bind to MHC II proteins and presented on the cell surface. The presented peptide is recognized by $CD4^+$ T cells. The contact of the $CD4^+$ T cells with the presented antigen triggers their activation, which subsequently causes the release of cytokines (*Murphy & Weaver 2016, Wieczorek et al. 2017, Lokki & Paakkanen 2019*).

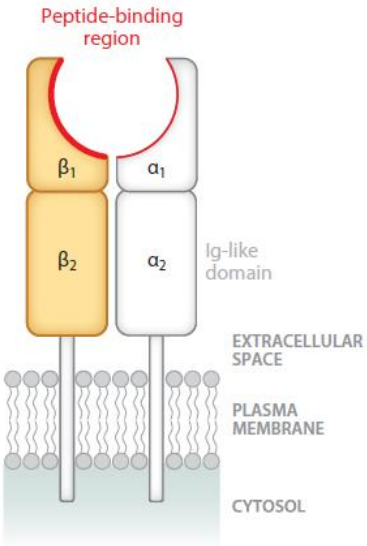

Figure 2. Model structure of MHC class II protein. Source: *Hammer et al. 2020* (IP-P5, Figure 2b).

1.1.3 Variability of the MHC

In the light of evolution, to some extent the organism's adaptation to different environmental influences can be explained by genetic modification. The high genetic variability of the MHC plays particularly an essential role in the adaptation of an organism to susceptibility to infectious or autoimmune diseases, or resistance to pathogens, thus ensuring the survival of the organism. For this evolutionary demand, the ultimate polymorphism arose. In humans, 30,039 distinct HLA alleles have been reported to the Immuno Polymorphism Database (IPD)-IMGT HLA sequence database (IPD-IMGT/HLA Release 3.44.0, 2021-04-20). The allelic

variations differ between populations, therefore determining their susceptibility to various diseases. Under the most polymorphic HLA class I molecules are *HLA-A* genes with 6,766 reported alleles, *HLA-B* genes with 7,967 different alleles and *HLA-C* with 6,621 reported alleles (**Table 1**). To HLA class II genes with high polymorphism rate belong *HLA-DRB* genes, *HLA-DQB1* genes and *HLA-DPB1* genes with 3,701, 1,997 and 1,749 reported alleles, respectively (**Table 1**). According to Lokki & Paakkanen, approximately 10,000 different HLA haplotypes have been described so far (*Lokki & Paakkanen 2019*).

Polymorphism of the MHC molecules, and thus the allelic variation has an impact on the composition of the antigen-binding region of the molecules. With particularly diverse sequences of MHC genes, binding sites of different MHC molecules are competent to bind a broad spectrum of pathogen-derived peptides and present them to an appropriate T-cell population. Several studies have discussed the advantage of heterozygosity of an individual in which the organism can bind, present and subsequently recognize by T cells a greater variety of antigen fragments, which give the organism a better reaction to peptides of pathogenic origin. The variation that originates from genetic polymorphism, by altering the amino acid sequence of the molecule subunits, is narrowed to the amino terminal domains of the molecule that form the binding groove (*Spurgin et al. 2010, Lokki & Paakkanen 2019*).

1.1.4 Interaction of the MHC with T cells

The adaptive immune system of vertebrates with two types of antigen receptors has two distinct ways of antigen detection. In contrast to B cells, T cells need antigens presented on the cell surface by MHC molecules. Different types of pathogens initiate distinct immunological responses that are initiated by binding peptide fragments of foreign origin to MHC molecules. Originates the pathogen attacking the organism intracellularly, the peptide fragments are transported from cytosol -where they have been processed- to endoplasmic reticulum (ER) and bind to MHC class I glycoproteins. The peptide fragment, stabilizing the

MHC molecule allows the molecule to be exported to the cell surface (*Murphy & Weaver 2016*).

Extracellular foreign antigens are internalised into intracellular vesicles, in which the acidification takes place that activates the degradation of foreign proteins to peptide fragments that can be subsequently bound to a newly synthesized MHC class II molecule, afterwards transported to the cell surface as a peptide-loaded MHC class II molecule. The peptide fragments presented on the cell surface are detected by appropriate T cells with their T-cell receptors (TCRs), which are constructed of two polypeptide chains bound through disulphide bonds. These TCRs interact with the peptide-loaded MHC molecules. Cross-presentation is a process by which certain antigen-presenting cells (APCs) take up, process, and present extracellular antigen on class I molecules to $CD8^+$ T cells. This mechanism is necessary for immunity against tumours and viruses that do not infect APCs. For the transduction of the signal generated by the contact with the peptide-loaded MHC glycoproteins, CD3 molecules need to be present. The signals transduced with help of these molecules then activate the T cells that, in case of MHC class I molecule interacting with $CD8^+$ T cells, initiate the response of cytotoxic T cells to eliminate the respective APCs.

Supposing the $CD4^+$ T cells are bound to peptide-loaded MHC-II molecules, the transduced signal can start for several responses, guided by the respective cytokine milieu. For instance, an Interleukin (IL) 12-producing DC is presenting the antigen; the $CD4^+$ T cell is activated and becomes an Interferon (IFN)-γ-producing effector T cell. The IFN-γ producing effector T cells activate macrophages to kill intracellular pathogens (*Murphy & Weaver 2016, Rock et al. 2016, Wieczorek et al. 2016*).

1.2 Comparative aspects of the human and porcine MHC

The human leukocyte antigen (HLA) and swine leukocyte antigen (SLA) complex are located on human chromosome 6 (HSA6) and swine chromosome 7 (SSC7), respectively. In human,

the HLA complex maps to HSA 6p21 (p, short arm), whereas in pig to SSC 7p11-7q11 (q, long arm) (**Figure 3**). The human and swine leukocyte antigens were compared in numerous studies. The results identified some differences including nonappearance of some class I-like loci and lack of HLA-DP-like loci in swine. In common human haplotypes (Hp), *DRB3*, *DRB4*, and *DRB8(ψ)* are found instead of *DRB5* and *DRB6(ψ)*, *DRB2(ψ)*, and *DRB7(ψ)* are found instead of *DRB6* (**Figure 4, Table 1**).

The SLA complex in swine corresponds to a genomic region of 2.40 (Hp-1.1) to 2.66 Megabases (Mb) (genome assembly Sscrofa11.1) that maps to SSC7, consisting of three regions. The SLA class I and class III regions map to 7p1.1 and the SLA class II region maps to 7q1.1, respectively (**Figure 3**) (*Renard et al. 2006*). In terms of genomic organisation in the light of evolution, the split of the MHC complex by the centromere in swine is unique among so far studied mammalian species (**Figure 3 and 4**) (*Renard et al. 2006; Hammer et al. 2020:IP-P5*).

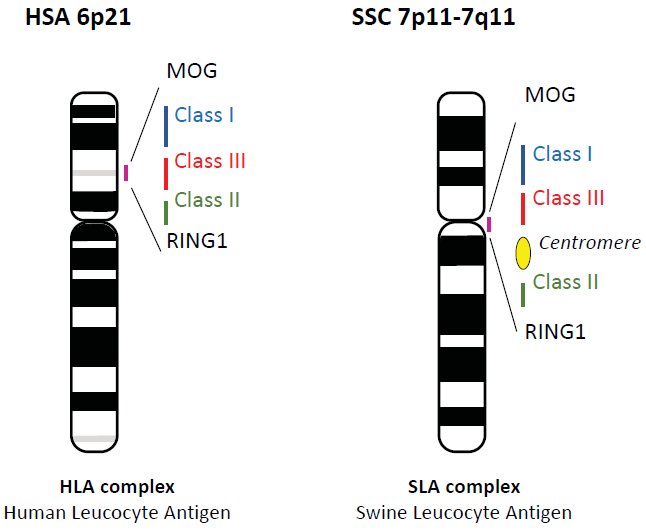

Figure 3. Schematic representation of the chromosome mapping and orientation of the HLA complex on HSA 6p21 and of the pig SLA complex on both sides of the centromere on swine chromosome 7 (SSC7). Source: *Hammer et al. 2020* (IP-P5, Supplemental Figure 1a).

In **Figure 4**, the class I and II HLA genes are indicated on top and the SLA class I and II genes at the bottom of the figure. Again, the two genomic regions are delimited by *MOG* upstream from the class I region and *RING1* downstream from the class II region. The nucleotide positions 29,657,002 to 33,212,722 refer to the human genome assembly GRCh38.p12

(Ensembl Release 96), whereas the nucleotide positions 22,595,564 to 25,255,204 refer to the swine genome assembly Sscrofa11.1 (Ensembl Release 96).

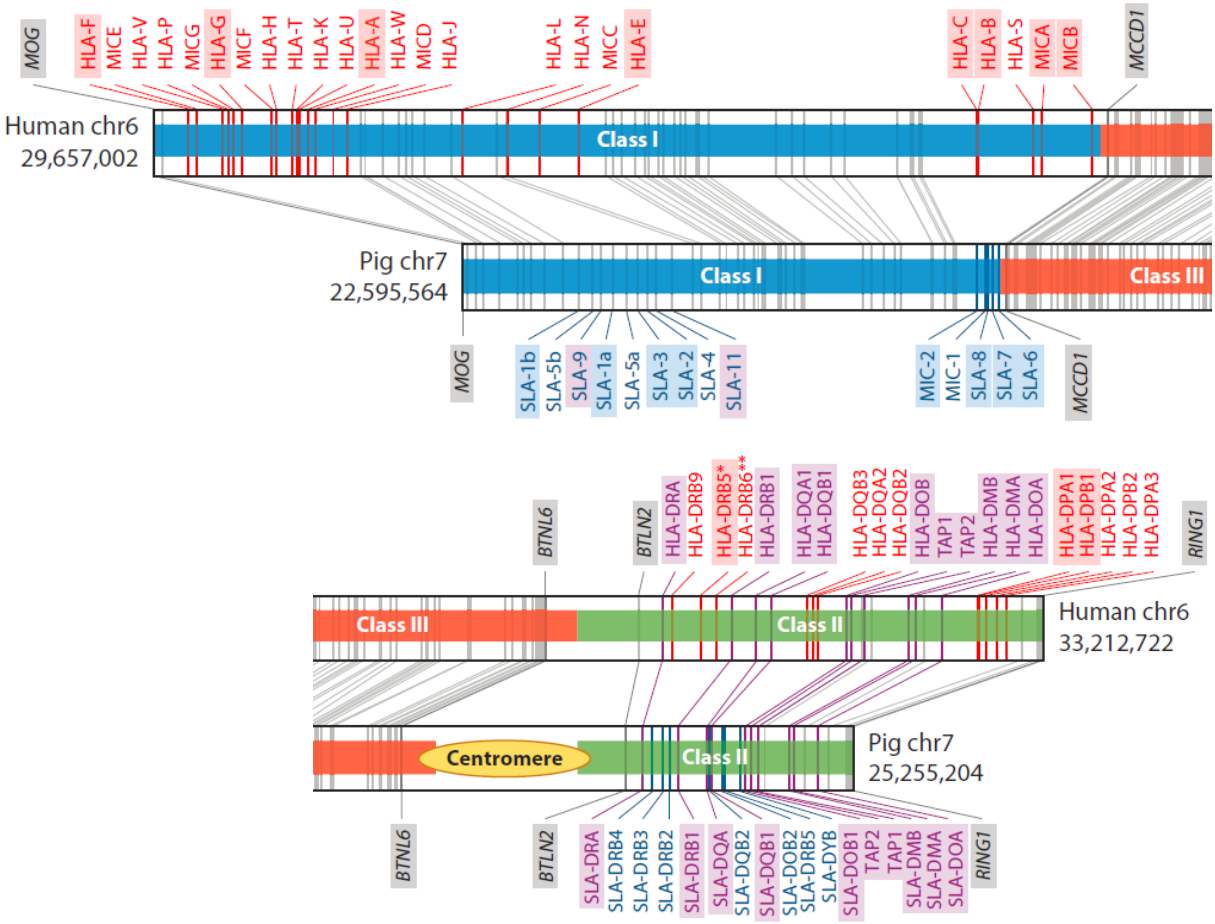

Figure 4. Comparative maps between human leukocyte antigen (HLA) and swine leukocyte antigen (SLA) complex. Orthologies between human and pig genes are indicated by gray lines (anchor genes) and purple lines (MHC genes). Functional MHC genes predicted to encode proteins are highlighted. No orthologies are identified between the HLA and SLA class I genes that are organized in three clusters in human and two clusters in swine. In the class II region, orthologous functional MHC class II genes are in purple, other loci correspond to species-specific genes and pseudogenes. Source: *Hammer et al. 2020* (IP-P5, Figure 1a).

The IPD-IMGT/HLA (Release 3.44.0, 2021-04-20) and IPD-MHC (Release 3.6.0.1, 2021-05-14, build 148) Databases provide a centralised repository for sequences of the MHC from a number of different species (*Maccari et al. 2017, Ballingall et al. 2018, Maccari et al. 2018, Robinson et al. 2020*). Below **Table 1** provides a summarizing overview about the numbers of confirmed human (HLA) and swine leukocyte antigen (SLA) alleles and proteins that are current content of the IPD data repository.

Table 1. Numbers of confirmed human (HLA) and swine leukocyte antigen (SLA) alleles and proteins.

	Human			Swine		
	locus	alleles	proteins	locus	alleles	proteins
Classical MHC class I (Ia)	HLA-A	6,766	4,064	SLA-1	90	88
	HLA-B	7,967	4,962	SLA-2	96	94
	HLA-C	6,621	3,831	SLA-3	43	39
Non-Classical MHC class I (Ib)	HLA-E	271	110	SLA-6	10	10
	HLA-F	45	6	SLA-7	3	3
	HLA-G	82	22	SLA-8	5	5
Classical MHC class II (II α; Iiβ)	DRA	29	2	DRA	14	6
	DRB1, DRB3 to 5	3,688	2,557	DRB1	99	92
	DRB2, DRB6 to 9	*13*	*0*	*DRB2 to 5*	*19*	*0*
	DQA1, DQA2	346	154	DQA	26	24
	DQB1	1,997	1,303	DQB1	53	48
				DQB2	*1*	*0*
	DPA1	258	107			
	DPA2	*5*	*0*			
	DPB1	1,749	1,106			
	DPB2	*6*	*0*			
Non-Classical MHC class II (II α; Iiβ)	DMA	7	4	DMA	7	5
	DMB	13	7	DMB	1	1
	DOA	12	3	DOA	2	2
	DOB	13	5	DOB1	3	3
				DOB2	*1*	*0*
				DYB	*1*	*0*

Pseudogenes are given in *italics*. Source: IPD-IMGT/HLA (Release 3.44.0, 2021-04-20) and IPD-MHC (Release 3.6.0.1, 2021-05-14, build 148) Databases. The IPD-MHC Database for Suids can be accessed at https://www.ebi.ac.uk/ipd/mhc/group/SLA/.

1.3 The Swine Leukocyte Antigen (SLA)

The porcine MHC or Swine Leukocyte Antigen (SLA) complex is one of the densest regions of the porcine genome. The SLA genomic complex can be found on chromosome 7, with SLA class I and class III on the short arm, and SLA class II on the long arm of the chromosome (**Figure 3**). SLA class I spans 1.06 Mb, SLA class III 0.67 Mb, the centromeric region 0.47 Mb and SLA class II 0.46 Mb of the entire 2.66 Mb of the SLA region (**Figure 4**) (*Vaiman et al. 1998*, *Chardon et al. 1999, Chardon et al. 2000, Lunney et al. 2009*).

The genes coded by SLA are of the highest importance in concept of immunological responses to infectious agents and vaccines (*Lunney at al. 2009, Hammer et al. 2020: IP-P5, section 2*). Alongside their fundamental function of protecting the organism against pathogens, the SLA

showed linkage to growth rate and fat accumulation in various complex studies (*Renard et al. 2006, Ho et al. 2009b*). SLA molecules are expressed co-dominantly, meaning that both inherited alleles are producing the resulting molecule (*Maroilley et al. 2017*). Like HLA glycoproteins, the SLA molecules operate in inspecting and presenting processed antigens to T cells (*Murphy & Weaver 2016*).

1.3.1 Structure of the SLA class I region

The SLA class I region includes three classical genes, namely *SLA-1*, *SLA-2* and *SLA-3*, that besides encoded regular functions carry promoter coding regions. Further, the gene cluster contains several pseudogenes such as *SLA-4(ψ)* or *SLA-9(ψ)*, and three non-classical genes, denoted *SLA-6*, *SLA-7*, *SLA-8* (*Renard et al. 2001, Chardon et al. 2000, Tennant et al. 2007, Ho et al. 2009b*). The classical SLA class I genes are eminently polymorphic, of which the *SLA-1* and *SLA-2* genes exhibit the highest expression level (*Zhang et al. 2011, Pedersen et al. 2014, Gao et al. 2017*).

The SLA class I genes are composed of eight exons, of which the exons 2, 3 and 4 encode the three α domains (α_1, α_2 and α_3) building the variable component of the SLA class I molecule (**Figure 5**). The molecules transmembrane domain is encoded by the exon 5 and the cytosolic domain is encrypted in exons 6, 7 and 8 (*Lunney et al. 2009, Hammer et al. 2020: IP-P5, section 2*). The highest level of polymorphism in the SLA class I molecule is concentrated in the antigen binding site that is created by the α_1 and α_2 domains. All three classical class I loci encode functional genes that are pertinent for the activation of $CD8^+$ T lymphocytes (*Lunney et al. 2009, Sørensen et al. 2017*).

Like human MHC class I glycoproteins, the SLA class I molecules are composed of two membrane-distal domains α_1 and α_2 that assemble a peptide-binding cleft, one membrane-proximal domain α_3 and a transmembrane section and a cytosolic tail. The α_3 domain represents a binding site for the CD8 co-receptor. The complete α chain is non-covalently

associated with a β_2 microglobulin. In pig, the β_2 microglobulin is encoded by the *B2M* located on chromosome 1 (*Milland et al. 1993*).

These molecules are expressed on the surface of all cells containing a nucleus. Peptide fragments originated from cytosol during a viral infection or tumorous cell transformation are presented to and recognized by $CD8^+$ T cells that develop to cytotoxic T cells, which are able to kill cells presenting the respective antigens (*Murphy & Weaver 2016, Wieczorek et al. 2017*).

1.3.2 Structure of the SLA class II region

SLA class II are genes encoded by porcine MHC are primarily expressed on the surface of antigen presenting cells (APC) and are composed of two non-covalently bound chains, an α and a β chain. After binding of mostly exogenous antigen fragments in their peptide-binding cleft consisting of α1 and β1 domains, they present the peptides derived from exogenous antigens to $CD4^+$ T cells. The SLA class II region is separated from region III by the centromere (*Lunney et al. 2009, Hammer et al. 2020: IP-P5, section 2*). There are two groups of genes encoded in the SLA class II region: *SLA-DR* and *SLA-DQ*, whereas *DP* is missing in swine (**Figure 4, Table 1**). With the leader sequence encoded by exon 1, the SLA class II genes encoding for the α1 and α2 domains are encrypted in exon 2 and 3, respectively (**Figure 5**). Both, *DRA* and *DQA* genes consist also of a fourth exon encoding the transmembrane and cytoplasmatic domains. Most of the SLA class II genes accountable for the structure of the β-chain have a similar molecular constitution, except the classical *DRB1* and *DQB1* genes. As other β-chain genes, they have a leader sequence encoded in exon 1, extracellular domains in exons 2 and 3, but their transmembrane domain is encrypted by exon 4 and a supplementary exon 5 of *DQB1* and exons 5 and 6 of *DRB1* encode for their cytoplasmic domains. These genes show high homology with their orthologues in humans (*Piriou-Guzylack & Salmon 2008, Lunney et al. 2009, Ho et al. 2009a, Hammer et al. 2020: IP-P5, section 2*). *SLA-DRB1* and *SLA-DQB1* represent highly polymorphic loci, whereas *SLA-DQA* shows only an average degree of

polymorphism, and the polymorphism of *SLA-DRA* is rather restricted (*Lunney et al. 2009, Hammer et al. 2020: IP-P5, section 2*).

The genetic variability of porcine MHC as an adaptation element to environmental influences indicates the pig's susceptibility to infectious agents, thus its survival. The allelic variation in pig in comparison to humans is relatively low. Despite the low number of known SLA alleles, the amount of reported alleles is still increasing (IPD-MHC Release 3.6.0.1, 2021-05-14, build 148). The most polymorphic SLA class I molecules include *SLA-1* genes with 90 reported alleles, *SLA-2* genes with 96 reported alleles and *SLA-3* with 43 different alleles (**Table 1**). With respect to SLA class II, *DRB1*, *DQB1* and *DQA* genes belong to the most important polymorphic class II genes with 99, 53 and 26 reported alleles (**Table 1**) *(Maccari et al. 2017, Ballingall et al. 2018*). According to *Hammer et al.*, 73 independent class I (*SLA-1, -2, -3*) and 51 class II (*-DRB1, -DQB1*) haplotypes have been described so far (*Hammer et al. 2020: IP-P5, section 2*).

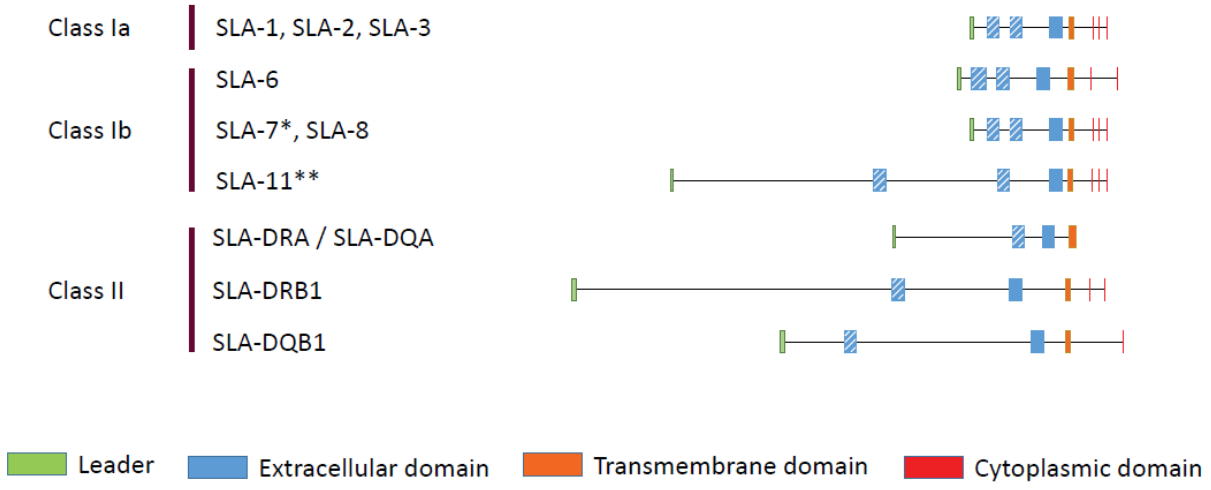

Figure 5. Gene structure of the SLA class Ia, class Ib and class II genes. The exons are represented by boxes and the introns by lines proportional to their size and connecting the exons. The exons corresponding to the peptide leader are represented in green; the exons corresponding to the extracellular domains (α1, α2 and α3 for class I genes and α1 and α2 for class II genes) are represented in blue; the exons corresponding to the transmembrane domain are represented in orange; the exons corresponding to cytoplasmic tail are represented in red. The most polymorphic exons (exons 2 and 3 for class I genes and exon 2 for class II genes) are represented by striped boxes. * On this figure, the *SLA-7* gene comprises eight exons as annotated from Sscrofa11.1 assembly and as reported by *Hu et al. 2011*; a gene structure with seven exons has also been described by *Crew et al. 2004* but has not been presented on this scheme. **The classification of SLA-11 as a class Ib protein coding gene is still provisional and needs to be confirmed. Source: *Hammer et al. 2020* (IP-P5, Supplemental Figure 3).

1.4 Assessment of SLA diversity

The SLA system is among the most well characterized MHC systems in nonhuman animal species. A systematic nomenclature for the SLA genes, alleles, and haplotypes is of high importance to the swine research community. An essential prerequisite for the establishment of an operative SLA nomenclature system focuses on the assignment of alleles at each locus into groups based on identification of group-specific polymorphic sequence motifs (*Ho et al. 2009b*). These sequence motifs correspond to the polymorphisms in the exon 2 and 3 sequences for class I alleles and exon 2 sequences for class II alleles, which encode the peptide-binding domains and interact directly with the immune cell receptors and are therefore considered to be functionally important (*Lunney et al. 2009, Hammer et al. 2020: IP-P5, section 3*).

In 2002, the SLA Nomenclature Committee[1] was established as a joint committee of the International Society for Animal Genetics (ISAG)[2] and the Veterinary Immunology Committee (VIC) of the International Union of Immunological Societies (IUIS) (*Ballingall et al. 2018*). The committee has to validate newly identified SLA sequences, according to the guidelines established for maintaining high-quality standards of the accepted sequences and to assign appropriate nomenclatures for new alleles as they are validated. Most importantly, the SLA Nomenclature Committee curates and maintains the IPD-MHC SLA sequence database (https://www.ebi.ac.uk/ipd/mhc/group/SLA, Release 3.6.0.1, 2021-05-14, build 148), a centralized platform to access the most recent information in the field of SLA research, such as the nomenclature reports and lists of SLA genes, alleles, and haplotype assignments (*Ho et al. 2009b*). It is now standard procedure for authors to submit the sequences directly to the IPD-MHC Database for curation and assignment of an official name prior to publication - this

[1]S.E. Hammer member since 2010 (vice-chair)
[2]S.E. Hammer member since 2008 (executive committee)

avoids the problems associated with renaming published sequences and the confusion of multiple names for the same sequence (*Ballingall et al. 2018, Maccari et al. 2018*).

SLA typing methods are relevant for improvement of breeding strategies, and by additional identification of T-cell epitopes for an enhancement in the development of new vaccines, thus leading to an effective prophylaxis (*Lunney et al. 2009, Hammer et al. 2020: IP-P5, section 3*).

A very time-efficient molecular approach of SLA diversity assessment with low costs are low-resolution typing methods such as PCR with sequence-specific primers (PCR-SSP). SLA Typing using the PCR-SSP assay relies on genomic DNA and therefore supports a rapid evaluation of numerous animals in large-scale studies, e.g., in outbred pig herds. On the other hand, sequence-based typing (SBT) by traditional Sanger methods and/or next-generation sequencing (NGS) would be the method of choice for the SLA type assessment of parental or founder breeding animals of pedigreed pig populations (*Ho et al. 2010b, Gimsa et al. 2017, Petersdorf & O'hUigin 2019*). Although, SBT approaches resemble more time-consuming high-resolution strategies they enable much more accurate results (*Lunney et al. 2009, Ho et al. 2009a, Essler (Hammer) et al. 2013, Gimsa et al. 2017*). Since there is a high interest in tumour, virus infection and transplantation studies and strong involvement in enhancing of vaccines, thus investigation of immunological responses at the peptide level, the relevance of precise molecular characterization of the porcine MHC is increasing (*Ladowski et al. 2019, Hammer et al. 2020: IP-P5, section 3*). High-resolution typing, by making use of SBT strategies, resolves this strong demand at a very accurate degree. SBT methods allow the determination of expressed SLA class I and class II alleles, thus an assignment of haplotypes, along with uncovering potential novel haplotypes in various pig breeds (*Luetkemeier et al. 2009, Le et al. 2012, Sørensen et al. 2017*).

1.5 Objectives of presented projects in this thesis

The MHC class I system is designed to sample cytosolic proteins to detect tumours or intracellular pathogens, such as virus and intracellular bacteria. MHC Class I molecules are recognized by $CD8^+$ T cells and thus provide a surveillance mechanism to target infected or malignant cells for destruction by CTLs. The MHC class II system is designed to sample extracellular proteins that have been taken up and processed by APCs. MHC Class II molecules are recognized by $CD4^+$ T helper cells and allow for the generation of immune responses to invading pathogens that are phagocytosed by APCs. MHC class I and II genes are highly polymorphic in the regions that encode the peptide-binding groove. These polymorphisms help to ensure survival of the species by increasing the variety of peptides that can be presented to T cells.

The publications collected in this thesis contribute to the molecular characterisation of MHC (SLA) class I and II gene diversity in swine and gain insights whether or not the CD4/CD8 T cell responses to particular immunogenic peptides (or vaccines) could be attributed to a certain MHC (SLA) haplotypes. They address the following main topics:

- Assessment of the SLA gene diversity in different pig lines (IP^3-P1, IP-P2, IP-P3).
- Investigation of the immunodominance in the CD4/CD8 T cell response with antigen-specificity dependent on certain SLA haplotypes. Economical important porcine viruses under investigation are:
    - Classical Swine Fever Virus (CSFV) (CP^4-P1)
    - Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) (CP-P2, CP-P3, CP-P4)
    - H1N2 Swine Influenza A Virus (FLUAVsw) (CP-P5, CP-P6)
    - Foot-and-Mouth Disease Virus (FMDV) (CP-P7, IP-P4)
- Reviewing the importance of the MHC (SLA) in swine health and biomedical research (CP-P8, IP-P5).

[3]Independent projects: IP-P1 to IP-P5 (first and senior authorships)
[4]Collaborative projects: CP-P1 to CP-P8 (joint author contributions)

2. Achievements – Publications contributing to the thesis

Publications are listed in order of appearance in the discussion section (page 255 *et seqq.*). They are composed of independent projects (*IP-P1 to P5*) representing first and senior authorship papers together with collaborative projects (*CP-P1 to P8*) with essential joint author contributions.

Detailed information can be found in the publication section of the Research Information System 'VetDoc' at the University of Veterinary Medicine Vienna (Vetmeduni Vienna). Publication records can also accessed at the National Library of Medicine (National Center for Biotechnology Information – NCBI): PubMed 2012-2014 (Sabina E. Essler[5] and co-workers) and PubMed 2005-2021 (Sabina E. Hammer[6] and co-workers).

IP-P1 **Essler (Hammer), S.E.**, Ertl, W., Deutsch, J., Rütgen, B.C., Groiss, S., Stadler, M., Wysoudil, B., Gerner, W., Ho, C.-S., Saalmüller, A. 2013. Molecular characterization of swine leukocyte antigen gene diversity in purebred Pietrain pigs. Animal Genetics 2013 Apr;44(2):202-5. Epub 2012 May 16. [J26, Q1] https://doi.org/10.1111/j.1365-2052.2012.02375.x

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IP-P2 Gimsa, U., Ho, C.-S., **Hammer, S.E**. 2017. Preferred SLA class I/class II haplotype combinations in German Landrace pigs. Immunogenetics 2017 Jan;69(1):39-47. [J49, Q3] https://doi.org/10.1007/s00251-016-0946-6

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IP-P3 **Hammer, S.E.**, Duckova, T., Groiss, S., Stadler, M., Jensen-Waern, M., Golde, W.T., Gimsa, U., Saalmüller, A. 2021. Comparative analysis of swine leukocyte antigen gene diversity in European farmed pigs. Animal Genetics doi: 10.1111/age.13090 [J66, Q1]

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CP-P1 Franzoni, G., Kurkure, N.V., **Essler (Hammer), S.E.**, Everett, H.E., Bodman-Smith, K., Crooke, H.R., Graham, S.P. 2013. Proteome-wide screening reveals immunodominance in the CD8 T cell response against classical swine fever virus with antigen-specificity dependant on MHC class I haplotype. PLoS One 2013 Dec 23;8(12):e84246.
Contribution S.E. Hammer: Molecular characterization of swine leukocyte antigen (SLA) gene diversity by applying a PCR-based low-resolution SLA typing assay. (Results: Table 3) [J33, Q1] https://doi.org/10.1371/journal.pone.0084246

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CP-P2 Burgara-Estrella, A., Díaz, I., Rodríguez-Gómez, I.M., **Essler (Hammer), S.E.**, Hernández, J., Mateu, E. 2013. Predicted peptides from non-structural proteins of porcine

[5]Name change due to marriage in 2011.
[6]Name change back to name of birth in 2014.

reproductive and respiratory syndrome virus are able to induce INF-γ and IL-10. Viruses 2013; 5(2):663-677.

Contribution S.E. Hammer: Molecular characterization of swine leukocyte antigen (SLA) gene diversity by applying a PCR-based low-resolution SLA typing assay. (Results: Table 3) [J28, Q2] https://doi.org/10.3390/v5020663

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CP-P3 Mokhtar, H., Eck, M., Morgan, S.B., **Essler (Hammer), S.E.**, Frossard, J.P., Ruggli, N., Graham, S.P. 2014. Proteome-wide screening of the European porcine reproductive and respiratory syndrome virus reveals a broad range of T cell antigen reactivity. Vaccine 2014 Nov 28;32(50):6828-37. Epub 2014 May 17.

Contribution S.E. Hammer: Molecular characterization of swine leukocyte antigen (SLA) gene diversity by applying a PCR-based low-resolution SLA typing assay. (Results and Discussion section, p. 6831 ff.) [J34, Q2] https://doi.org/10.1016/j.vaccine.2014.04.054

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CP-P4 Mokhtar, H., Pedrera, M., Frossard, J.P., Biffar, L., Choudhury, B., **Hammer, S.E.**, Kvisgaard, L.K., Larsen, L.E., Stewart, G.R., Somaravarapu, S., Steinbach, F., Graham, S.P. 2016. The non-structural protein 5 and matrix protein are major antigenic targets of T cell immunity to porcine reproductive and respiratory syndrome virus. Frontiers in Immunology 2016 Feb 16;7:40. eCollection 2016.

Contribution S.E. Hammer: Molecular characterization of swine leukocyte antigen (SLA) gene diversity by applying a PCR-based low-resolution SLA typing assay. (Results: Table 1) [J43, Q1] https://doi.org/10.3389/fimmu.2016.00040

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CP-P5 Talker, S.C., Koinig, H., Stadler, M., Graage, R., Klingler, E., Ladinig, A., Mair, K.H., **Hammer, S.E.**, Dürrwald, R., Ritzmann, M., Saalmüller, A., Gerner, W. 2015. Magnitude and kinetics of multifunctional $CD4^+$ and $CD8^+$ T cells in pigs infected with swine influenza A virus. Veterinary Research 2015 May 14;46(1):52.

Contribution S.E. Hammer: Molecular characterization of swine leukocyte antigen (SLA) gene diversity by applying a PCR-based low-resolution SLA typing assay. (Results: Table 2) [J41, Q1] https://doi.org/10.1186/s13567-015-0182-3

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CP-P6 Talker, S.C., Stadler, M., Koinig, H.C., Mair, K.H., Rodriguez-Gomez, I.M., Graage, R., Zell, R., Dürrwald, R., Starick, E., Harder, T., Weissenböck, H., Lamp, B., **Hammer, S.E.**, Ladinig, A., Saalmüller, A., Gerner, W. 2016. Influenza A virus infection in pigs attracts multifunctional and cross-reactive T cells to the lung. Journal of Virology 2016 Sep 29;90(20):9364-9382.

Contribution S.E. Hammer: Molecular characterization of swine leukocyte antigen (SLA) gene diversity by applying a PCR-based low-resolution SLA typing assay. (Results: Table 1) [J48, Q1] https://doi.org/10.1128/JVI.01211-16

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CP-P7 Gerner, W., **Hammer, S.E.**, Wiesmüller, K.-H., Saalmüller, A. 2009. Identification of MHC restriction and anchor residues of Foot-and-Mouth Disease Virus derived bovine T cell epitopes. Journal of Virology 2009 May;83(9):4039-50. Epub 2009 Feb 11.

Contribution S.E. Hammer: Molecular characterization of bovine leukocyte antigen (BoLA) gene diversity by applying sequence-based high-resolution typing of MHC class II DRB3, DQA, and DQB alleles in cattle. (Results: Tables 1 and 3, Figure 6) [J14, Q1] https://doi.org/10.1128/JVI.01534-08

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IP-P4 De León, P., Cañas-Arranz, R., Saez, Y., Forner, M., Defaus, S., Bustos, M.J., Torres, E., Rodríguez-Pulido, M., Andreu, D., Blanco, E., Sobrino, F., **Hammer, S.E**. 2020. Association of porcine Swine Leukocyte Antigen (SLA) haplotypes with B- and T-cell immune response to foot-and-mouth disease virus (FMDV) peptides. Vaccines (Basel) 2020 Sep 8;8(3):E513. [J63, Q1] https://doi.org/10.3390/vaccines8030513

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CP-P8 Schwartz, J.C., Hemmink, J.D., Graham, S.P., Tchilian, E., Charleston, B., **Hammer, S.E.**, Ho, C.-S., Hammond, J.A. 2018. The MHC homozygous inbred Babraham pig as a resource for veterinary and translational medicine. HLA 2018 Apr 23;92(1):40-43. Epub ahead of print.

Contribution S.E. Hammer: Molecular characterization of swine leukocyte antigen (SLA) gene diversity by applying a PCR-based low-resolution SLA typing assay. (Results: Table 2) [J57, Q2] https://doi.org/10.1111/tan.13281

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IP-P5 **Hammer, S.E.**, Ho, C.-S., Ando, A., Rogel-Gaillard, C., Charles, M., Tector, M., Tector, A.J., Lunney, J.K. 2020. Importance of the MHC (SLA) in swine health and biomedical research. Annual Reviews of Animal Biosciences 2020 Feb 15;8:171-198. Epub 2019 Dec 17. [J62, Q1] https://doi.org/10.1146/annurev-animal-020518-115014

3. Discussion

3.1. Assessment of SLA gene diversity in different pig lines (IP-P1 to IP-P3)

The highly polymorphic swine leukocyte antigen (SLA) is associated with different levels of immunologic responses to infectious diseases, vaccines, and transplantation. Pig breeds with known SLA haplotypes are important genetic resources for veterinary vaccine research.

3.1.1 Austrian purebred Pietrain pigs (IP-P1)

As already stated in the introduction section, the overall knowledge on the SLA background of domesticated pig lines around the globe is still scarce. Local pig breeds are maintained as pure breeds, and their cross breeding is an acknowledged strategy to produce fattening pigs with high growth rates and good meat quality throughout the world. In the case of Austria, most of the farmed pigs are F2 descendants of F1 Large White/Landrace crossbreds paired with Pietrain boars. We can therefore argue that the repertoire of SLA alleles and haplotypes present in Pietrain pigs has an important influence on that of their descendants.

After successfully establishing PCR-based low-resolution (Lr) typing in our lab (PCR-SSP assay), we characterized the SLA class I (*SLA-1*, *SLA-2*, *SLA-3*) and class II (*SLA-DRB1*, *SLA-DQB1*, *SLA-DQA*) genes of 27 purebred Pietrain pigs using a combination of PCR-SSP and the high-resolution sequence-based typing method (*Essler (Hammer) et al. 2012: IP-P1*).

Surprisingly, among the 27 animals, 15 out of the known 73 class I (SLA-I) and 13 out of the described 51 class II (SLA-II) low-resolution haplotypes were identified (*IP-P1, Tables 1 and 2*) (*Hammer et al. 2020: IP-P5, section 3*). Nevertheless, 11 pigs exhibited the most common SLA-I haplotype Lr-43.0 (SLA-1*11XX-SLA-3*04XX-SLA-2*04XX) corresponding to a frequency of 20% (*IP-P1, Table 1*). For SLA-II, the most prevalent haplotype, Lr-0.14 (SLA-DRB1*09:01-SLA-DQB1*08:01-SLA-DQA*03XX), was found in 14 animals with a frequency of 26% (*IP-P1, Table 2*). In the light of this initial SLA background assessment in Pietrain pigs, we were not surprised to detect two potential novel SLA class II haplotypes, tentatively designated as Lr-Pie-0.1 (SLA-

DRB1*01:01-SLA-DQB1*05:01-SLA-DQA*blank) and Lr-Pie-0.2 (SLA-DRB1*06:02-SLA-DQB1*03:03-SLA-DQA*03XX) (*IP-P1, Table 2*). To date, these two SLA-II haplotypes can be considered as being novel, as they are not been reported in other pig populations so far.

With respect to allele group frequencies, *SLA-1* was more polymorphic than *SLA-2* followed by *SLA-3* (*IP-P1, Suppl. Table S2*). For *SLA-1*, we found 10 allele groups and SLA-1*11XX explained 28% of the diversity. For *SLA-2*, 2 out of 9 detected allele groups were responsible for 54% of the diversity; SLA-2*10XX and SLA-2*04XX showed frequencies of 33 and 20%. The lesser polymorphic locus, *SLA-3*, was characterized by five allele groups and among them, SLA-3*04XX (50%) and SLA-3*05XX (35%) explained 85% of the diversity (*IP-P1, Suppl. Table S2*).

As expected, regarding SLA-II allele group frequencies, *DRB1* was more polymorphic than *DQB1* followed by *DQA* (*IP-P1, Suppl. Table S2*). For DRB1, we found eight allele groups, and two of them explained 56% of the diversity. Specifically, DRB1*04XX and DRB1*09XX represented frequencies of 30 and 26%, respectively. For *DQB1*, 2 out of 8 detected allele groups were responsible for 52% of the diversity with DQB1*08XX and DQB1*07XX showing frequencies of 28 and 24%. The lesser polymorphic locus, *DQA*, was characterized by five allele groups and among them, DQA*03XX (44%) and DQA*01XX (28%) explained 72% of the diversity (*IP-P1, Suppl. Table S2*).

Shared SLA-I and SLA-II haplotypes of Austrian purebred Pietrain pigs with other pig populations (*IP-P1, Suppl. Table S5*) are summarized in **Table 2** and are discussed together with the molecular characterization of SLA-I/II haplotype combinations in purebred German Landrace pigs (*IP-P2, Suppl. Table S3*).

We conclude that this initial characterization of SLA diversity in purebred Pietrain pigs provides the first data on the MHC haplotype repertoire of Austrian commercial pigs. However, future studies have to pursue the characterization of both parental origins together with the high-resolution signature of abundant allele groups.

3.1.2 German purebred Landrace pigs (IP-P2)

Cross breeding of German Landrace and Large White pigs is an approved strategy for the production of fattening pigs with high growth rates and good meat quality throughout Europe and America. Since the 1900s, various Landrace pig lines have been developed in Denmark by crossing Large White pigs from England with local domestic pigs. German Landrace dams are often used for breeding F1 hybrids because they show early and high fertility, high milk production and good mothering abilities, resulting in a high number of vital piglets (*Gimsa … Hammer et al. 2017: IP-P2*). To gain insight in the SLA background of a research-relevant resource population of purebred German Landrace pigs, we subjected a cohort of 155 animals to low-resolution haplotyping. Among the studied pigs, 90 piglets were generated by mating seven boars to ten sows, whereas the remaining 65 pigs (including the seven boars and ten sows) are referred to as founders. At the three SLA-I loci (*SLA-1*, *SLA-2*, *SLA-3*), we identified thirty allele groups (*IP-P2, Figure 1*), comprising 22 haplotypes (*IP-P2, Table 1*). The most frequent SLA-I haplotype was Lr-62.0 (SLA-1*14:XX-SLA-3*04:XX-SLA-2*06:XX), which was observed in 21 pigs at a frequency of 16 % in the cohort of founders (*IP-P2, Table 1*). The three predominant *SLA-1* allele groups SLA-1*08:XX, SLA-1*14:XX, and SLA-1*11:XX explained 48.5 % of the diversity (*IP-P2, Suppl. Table S3*). For *SLA-3*, SLA-3*04:XX occurred at the highest frequency (40.8 %), and at the *SLA-2* locus, SLA-2*05:XX (29.2%) and SLA-2*06:XX (22.3%) were the most abundant allele groups (*IP-P2, Suppl. Table S3*).

In the founder cohort of 65 pigs, we identified the three most frequent SLA-II allele groups, DRB1*06:XX (40.8 %), DQB1*07:XX (48.5 %), and DQA*01:XX (51.5 %) (*IP-P2, Suppl. Table S3*). Among the found 11 SLA-II haplotypes, the allele group combination Lr-0.12 (DRB1*06:XX-DQB1*07:XX-DQA*01:XX) occurred at a frequency of 40.8% (*IP-P2, Table 2*).

In this study, ten litters of pigs (90 piglets) and their parents (ten sows, seven boars) were genotyped, and we were therefore able to assign offspring haplotypes to the parents. Based

on these results and the knowledge about the sows' and boars' ancestry, along with information on their SLA, we performed a pedigree analysis, showing that SLA-I and SLA-II haplotypes in our cohort had always been inherited together without crossovers (*IP-P2, Figure 2*). Already three SLA-I/II haplotype combinations, namely Lr-62.0/Lr-0.12, Lr-34.0/Lr-0.21, and Lr-6.0/Lr-0.12 explained 39% of the diversity (*IP-P2, Table 3*). Interestingly, in Austrian purebred Pietrain and three-way crossbred (25% Large White, 25% Landrace, and 50% Pietrain) pigs, haplotypes occurred that could result in combinations we found in the German Landrace pigs (*IP-P1, IP-P3*). As another example, in Korean native pigs, Cho et al. found different combinations e.g., Lr-56.0/Lr-0.23, Lr-56.0/Lr-0.30, Lr-59.0/Lr-0.01, and Lr-07.0/Lr-0.23 (*Cho et al. 2010*).

Table 2 illustrates noticeable examples of a comparative analysis of **purebred Austrian Pietrain and German Landrace pigs** (*IP-P1, Suppl. Table S5; IP-P2, Suppl. Table S4*) with previously published SLA-typing studies including the following pig populations. Animals of unknown origin, housed at the Kansas State University and being used to study PRRSV (KSU), pigs of unknown origin with susceptibility to subgroups of PCV type 2 (PCV), Large White/Landrace crosses (Big Pig) together with pathogen-free Yorkshire (YS) and Landrace (LR) pigs of Canadian origin (*Ho et al. 2009a, Ho et al. 2010a, Gao et al. 2017*).

The SLA-I haplotype Lr-01.0 was shared by Austrian Pietrain, German Landrace, KSU, PCV, Large White/Landrace crossbred and Yorkshire pigs. Interestingly, Austrian Pietrain, German Landrace, KSU, PCV and Large White/Landrace crossbred pigs shared each of SLA-I and SLA-II haplotypes, namely Lr-25.0 and Lr-0.19a. Together with KSU, Yorkshire and Landrace, Austrian Pietrain and German Landrace pigs shared Lr-26.0, whereas Lr-28.0 was found in the latter two as well as in PCV, Large White/Landrace crossbred and Landrace pigs. Lastly, Lr-35.0 occurred in Austrian Pietrain, Yorkshire, German and Canadian Landrace pigs. The SLA-II haplotype Lr-0.12 was detected in Austrian Pietrain, PCV, German and Canadian Landrace pigs

and Large White/Landrace crossbreds. In contrast, Austrian Pietrain, Yorkshire, German and Canadian Landrace pigs and Large White/Landrace crossbreds, shared Lr-0.23.

Taking together, our findings strongly suggest that the combination of particular but different haplotypes in different geographical regions may have developed under the evolutionary pressure of regionally endemic pathogens. This proposed mechanism ensures an efficient immune response despite low recombination rates.

Table 2. Shared SLA class I and class II haplotypes of purebred Austrian Pietrain and German Landrace pigs with other pig populations.

		Lr-1.0	Lr-2.0	Lr-4.0	Lr-6.0	Lr-7.0	Lr-11.0	Lr-25.0	Lr-26.0	Lr-28.0	Lr-29.0	Lr-29.0	Lr-34.0	Lr-35.0	Lr-39.0	Lr-43.0	Lr-59.0	Lr-61.0	Lr-62.0	Lr-64.0
KSU[a]	Pietrain	Lr-1.0						Lr-25.0	Lr-26.0							Lr-43.0				
	GER LR	Lr-1.0		Lr-4.0				Lr-25.0	Lr-26.0							Lr-43.0				
PCV[b]	Pietrain	Lr-1.0					Lr-11.0	Lr-25.0		Lr-28.0										
	GER LR	Lr-1.0	Lr-2.0			Lr-7.0		Lr-25.0		Lr-28.0							Lr-59.0	Lr-61.0		
Big Pig[c]	Pietrain	Lr-1.0						Lr-25.0		Lr-28.0	Lr-29.0	Lr-32.0			Lr-39.0					
	GER LR	Lr-1.0		Lr-4.0	Lr-6.0			Lr-25.0		Lr-28.0	Lr-32.0		Lr-34.0		Lr-39.0					
YS[d]	Pietrain	Lr-1.0							Lr-26.0					Lr-35.0						
	GER LR	Lr-1.0		Lr-4.0					Lr-26.0					Lr-35.0						
LR[e]	Pietrain								Lr-26.0	Lr-28.0		Lr-29.0		Lr-35.0						Lr-64.0
	GER LR			Lr-4.0	Lr-6.0	Lr-7.0			Lr-26.0	Lr-28.0		Lr-32.0		Lr-35.0					Lr-62.0	

		Lr-0.1	Lr-0.8b	Lr-0.12	Lr-0.13	Lr-0.14	Lr-0.15	Lr-0.19a	Lr-0.20	Lr-0.21	Lr-0.23	Lr-0.24	Lr-0.25	Lr-0.26
KSU[a]	Pietrain	Lr-0.1				Lr-0.14		Lr-0.19a	Lr-0.20					
	GER LR					Lr-0.14		Lr-0.19a		Lr-0.21				
PCV[b]	Pietrain	Lr-0.1		Lr-0.12	Lr-0.13			Lr-0.19a						
	GER LR		Lr-0.8b	Lr-0.12				Lr-0.19a		Lr-0.21				
Big Pig[c]	Pietrain	Lr-0.1		Lr-0.12	Lr-0.13			Lr-0.19a			Lr-0.23	Lr-0.24	Lr-0.25	
	GER LR			Lr-0.12				Lr-0.19a			Lr-0.23		Lr-0.25	Lr-0.26
YS[d]	Pietrain						Lr-0.15				Lr-0.23	Lr-0.24		
	GER LR		Lr-0.8b								Lr-0.23			
LR[e]	Pietrain			Lr-0.12							Lr-0.23			
	GER LR			Lr-0.12							Lr-0.23			

SLA, swine leukocyte antigen; Lr, Low-resolution SLA haplotypes identified by PCR-SSP; GER LR, German Landrace.

[a]Kansas State University (PRRSV study, unknown origin; *Ho et al. 2009a, Ho et al. 2010a*)

[b]Porcine Circo Virus (pigs with susceptibility to subgroups of PCV type 2, unknown origin; *Ho et al. 2009a, Ho et al. 2010a*)

[c]Large White/Landrace crosses (*Ho et al. 2009a, Ho et al. 2010a*)

[d]Yorkshire (Canadian origin, *Gao et al. 2017*)

[e]Landrace (Canadian origin; *Gao et al. 2017*)

3.1.3 European farmed pigs (IP-P3)

In the introduction section, we brought forward the argument that for reasons of the extensive polymorphic nature of SLA genes, accurate and sensitive typing methods are crucial for studying their influences, particularly in outbred pigs with diverse genetic backgrounds. Again, we have to recall that pigs represent economically important farm animals and have become a preferred preclinical large animal model for biomedical studies. We believe that the

expanded use of pigs as models for e.g., organ-transplantation experiments, and their use in infection studies for veterinary vaccine design warrants the increasing need for SLA haplotyping and its pivotal importance.

In a comprehensive study, we assessed the SLA-I (*SLA-1*, *SLA-2*, *SLA-3*) and SLA-II (*DRB1*, *DQB1*, *DQA*) gene diversity of 549 European farmed pigs representing nine commercial pig lines by low-resolution SLA haplotyping (*Hammer et al. 2021: IP-P3*). The studied cohort comprised three-way crossbred pigs (25% Landrace, 25% Yorkshire, 50% Duroc; 25% Large White, 25% Landrace, 50% Pietrain), Large White/Landrace, Yorkshire/Dutch Landrace as well as Yorkshire/Hampshire crosses, together with purebred German Landrace, Austrian Pietrain, and Large White pigs (*IP-P3, Figure 1, Suppl. Table S1*). The studied cohort of 549 animals, comprised 50 out of the described 73 SLA-I and 37 out of the 51 known SLA-II haplotypes, including three and seven potential novel allele-group combinations, respectively (*IP-P3, Table 1, Suppl. Figure S2*) (*Hammer et al. 2020: IP-P5, section 3*).

Eight haplotypes (Lr-04.0, Lr-32.0, Lr-22.0, Lr-01.0, Lr-59.0, Lr-24.0, Lr-37.0, and Lr-43.0) explained 51.4% of the found SLA-I diversity (*IP-P3, Suppl. Figures S2a and S3a*). Among these, Lr-04.0 (SLA-1*04XX-SLA-3*04:04-SLA-2*04XX) and Lr-32.0 (SLA-1*07XX-SLA-3*04:04-SLA-2*02XX) occurred at frequencies of 11 and 8% (*IP-P3, Suppl. Figures S2a and S3a*). The pig populations KSU, PCV, Large White/Landrace crossbred, Yorkshire and Landrace pigs have already been introduced in the previous sections, when discussing the contributions IP-P1 (*Essler (Hammer) et al. 2013*) and IP-P2 (*Gimsa … Hammer et al. 2017*). SLA-I haplotype Lr-04.0 was also found in the KSU, PCV, Large White/Landrace crosses and Yorkshire pigs. In contrast, Lr-32.0 was observed only in the White/Landrace crosses and Landrace pigs (*Ho et al. 2009a, Gao et al. 2017*).

For the *SLA-1* gene, we found 23 allele groups, and three of them explained 46.3% of the diversity, with approximately equal frequencies of SLA-1*08XX (16.6%), SLA-1*07XX (15%) and

SLA-*blank (14.7%; Note: *blank indicates an untyped SLA-I locus) (*IP-P3, Figure 2*). For *SLA-2*, 3 out of 22 detected allele groups were responsible for 47.5% of the diversity (SLA-2*04XX: 15.2%, SLA-2*05XX:11.8%, SLA-2*02XX: 10.7%) (*IP-P3, Figure 2*). Lastly, the lesser polymorphic locus, *SLA-3*, was characterized by 10 allele groups and among them, SLA-3*04XX (39.8%) and SLA-3*05XX (22.8%) explained 62.6% of the diversity (*IP-P3, Figure 2*).

For SLA-II, the three most prevalent haplotypes Lr-0.15b (DRB1*04XX(04:05/04:06)-DQB1*02XX(02:02)-DQA*02XX), Lr-0.12 (DRB1*06XX-DQB1*07XX-DQA*01XX) and Lr-0.23 (DRB1*10:06-DQB1*06:03-DQA*01XX) occurred at frequencies of 14.4, 12.5, and 11.6%, respectively (*IP-P3, Suppl. Figures S2b and S3b*). Lr-0.15b was also found in the KSU, PCV, Large White/Landrace crosses and Yorkshire pigs. Additionally, Lr-0.12 was shared with Large White/Landrace crosses as well as PCV and Landrace pigs (*Ho et al. 2010a, Gao et al. 2017*).

For *DRB1*, we found 13 allele groups, and two of them explained 41.1% of the observed diversity. Specifically, DRB1*04XX and DRB1*06XX represented frequencies of 23.2 and 17.6%, respectively (*IP-P3, Figure 2*). Regarding *DQB1*, 2 out of 12 detected allele groups were responsible for 46% of the diversity with DQB1*02XX (28%) and DQB1*07XX (18%) (*IP-P3, Figure 2*). The lesser polymorphic locus, *DQA*, was characterized by seven allele groups and among them, DQA*02XX (33.3%) and DQA*07XX (30.8%) explained 64.4% of the diversity (*IP-P3, Figure 2*).

Among the studied Pietrain (*IP-P1*), German Landrace (*IP-P2*) and European farmed pigs (*IP-P3*) we detected untyped SLA-I loci, so-called 'blank' alleles, preferably for *SLA-1* (frequency: 14.8%). For the remaining loci, *SLA-2* and *SLA-3*, 'blank' alleles occurred at much lower frequencies, namely 1.9 and 0.5%, respectively (*IP-P1, Suppl. Table S2; IP-P2, Suppl. Table S3; IP-P3, Figure 2*). Consequently, we modified the PCR primer panel for genotyping SLA-I (*IP-P3, Suppl. Table S2a*) to enhance specificity of the PCR-SSP assay for typing SLA-1*15XX, SLA-1*15:01, and SLA-1*16:02 by exchanging primers in panel positions B2 and H3, respectively.

For typing SLA-2*15XX, SLA-2*15:01, and SLA-2*16:02 plus SLA-2*17:01 by replacing primers in panel positions D6 and B6. Lastly, for typing SLA-3*08XX and SLA-3*08:01, we added a novel primer pair to panel position D6 (*IP-P3, Suppl. Table S2a*).

Since 2013, our lab contributed to several initial veterinary vaccine correlation studies (e.g., PRRSV, CSFV, FLUAVsw, and FMDV) pioneering this field of research by elucidating the immunodominance in the T-cell response with antigen-specificity dependent on certain SLA-I and SLA-II haplotypes. In the next section, we will demonstrate that the found SLA-immune response correlations could facilitate tailored vaccine development, as the SLA-I haplotypes Lr-01.0, Lr-04.0, Lr-22.0, and Lr-59.0 as well as the SLA-II haplotypes Lr-0.15b, Lr-0.23 and Lr-0.27 are abundant in European farmed pigs (**Table 3**; *IP-P3, Table 1 and 2*).

3.2. SLA-restricted CD4/CD8 T cell response against porcine viruses (IP-P4, CP-P1 to CP-P7)

In the previous section, we demonstrated that SLA genotyping using PCR-SSP-based assays represents a rapid and cost-effective way to study SLA diversity in outbred commercial pigs and may facilitate the development of more effective vaccines or identification of disease-resistant pigs in the context of SLA antigens to improve overall swine health. In the light of veterinary vaccine design, the characterization of the peptide-binding specificity of SLA-I and SLA-II molecules is pivotal to understand adaptive immune responses of swine toward infectious pathogens [*Lunney et al. 2009 and Hammer et al. 2020 (IP-P5, section 4*)]. We therefore proposed two basics concepts for SLA haplotyping-assisted animal trials in vaccine and transplantation research (*IP-P3, Suppl. Figure S1*). One approach addresses SLA typing of the resource population to enable directed mating of founder animals based on their SLA-background (*IP-P3, Suppl. Figure S1*). On the other hand, the designation of SLA-defined study groups achieves an experimental advantage of pre-selecting animals expressing certain SLA phenotypes and thus enhancing the understanding of experimental outcomes (*IP-P3, Suppl. Figure S1*).

In this section, we briefly discuss key findings on the correlation of SLA haplotypes and immune responses for the animals enrolled in seven infection studies addressing four different viruses being of relevance in veterinary medicine and veterinary immunology.

3.2.1 Classical Swine Fever Virus (CSFV) (CP-P1)

In previous studies, infection of target cells with various vaccinia virus/CSFV recombinants led to the identification of a major antigenic site for CSFV-specific CTLs near the cleavage site between the non-structural proteins p80 (NS3) and p10 (NS4a). Pauly et al. used synthetic overlapping nonapeptides, which covered the sequence ENALLVALF of this protein region to identify this sequence as very first MHC-I-restricted T cell epitope recognized by CSFV-specific CTLs (*Pauly et al. 1995*). In 2002, Armengol et al. identified the pentadecapeptide 290 as being mainly MHC-II and partially MHC-I restricted. CSFV-specific CTLs were able to lyse peptide 290-loaded target cells. These findings indicate the existence of a CSFV-specific helper T-cell epitope and a CTL epitope in this peptide (*Armengol et al. 2002*). Vaccination with live attenuated CSFV induced solid protection after only 5 days, which has been associated with virus-specific IFN-γ responses of $CD3^{+}CD4^{-}CD8^{high}$ T cells (*Franzoni et al. 2013a*). Next, vaccinated animals were challenged with virulent CSFV after 5 days and after a further 28 days. Virus-specific CD4 T cell ($CD3^{+}CD4^{+}CD8\alpha^{+}$) IFN-γ responses were confirmed, and the dominant response was again deriving from the CD8 T cell population further characterized as $CD44^{high}CD62L^{-}$ and they expressed variable levels of CD25 and CD27 indicating a mixed effector and effector memory phenotype (*Franzoni et al. 2013a*). In a subsequent study, Franzoni et al. asked if the different specificity of CD8 T-cell responses between pigs was due to them bearing different MHC-I haplotypes (*Franzoni et al. 2013b: CP-P1*). To assess whether recognition of the two antigenic peptides was associated with the previously identified SLA-I haplotypes, C-strain vaccinated and challenged pigs were typed for SLA-I using the PCR-SSP-based assay (*CP-P1, Table 3*). No haplotypes were found to be shared between these pigs and

the animals used to define the antigenic peptides, but all pigs that reacted against NS2 8mer STVTGIFL displayed the haplotype Lr-22.0 (SLA-1*08XX-SLA-3*06:01-SLA-2*12XX) (*CP-P1, Figures 2 to 5, Figure 7*). Pigs that reacted against the NS3 11mer VEYSFIFLDEY carried the Lr-01.0 haplotype (SLA-1*01XX-SLA-3*01XX-SLA-2*01XX) (*CP-P1, Figures 2 to 5, Figure 7*). Interestingly, three NS3 reactor animals also expressed Lr-22.0 but no response was detected against NS2 (*CP-P1, Figures 2 to 5, Figure 7*). Hence, the variability in the antigen-specificity of these immunodominant CD8 T-cell responses was confirmed to be associated with expression of distinct SLA-I haplotypes. Moreover, recognition of $NS2_{1223–1230}$ STVTGIFL (Lr-22.0) and $NS3_{1902–1912}$ VEYSFIFLDEY (Lr-01.0) by a larger group of C-strain vaccinated animals showed that these peptides could be restricted by additional haplotypes (*CP-P1, Table 3, Figures 2 to 5, Figure 7*). This proteome-wide screening revealed immunodominance in the CD8 T-cell response against CSFV with antigen-specificity dependent on SLA-I haplotypes (*CP-P1*).

3.2.2 Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) (CP-P2 to P4)

Immunity against PRRSV is not well understood, although there is evidence suggesting that virus-specific T-cell IFN-γ responses play an important role (*Burgara-Estrella et al. 2013: CP-P2*). It could be demonstrated that PRRSV vaccinated and challenged pigs carrying SLA-I haplotype Lr-01.0/04.0 (SLA-1*04XX-SLA-3*01XX-SLA-2*01XX) or Lr-59.0 (SLA-1*11:03-SLA-3*05XX-SLA-2*16:02) and SLA-II haplotype Lr-0.27 (DRB1*09:06-DQB1*-09XX-DQA*04XX+05XX) showed significant IFN-γ responses, pointing towards a positive correlation of SLA haplotype and T-cell response (*CP-P2, Tables 2 to 4*). In more detail, in pigs carrying Lr-01.0/04.0, Lr-59.0 and/ or Lr-0.27, the antigenic region of the non-structural proteins $NSP2_{1141-1149}$ (WLFAGVVLL) induced significant IFN-γ responses (*CP-P2, Table 2*). On the other hand, in the same animals, the antigenic regions $NSP5_{1929-1937}$ (LLNEILPAV) and $NSP9_{258-266}$ (VLPGVLRLV) also induced remarkable IL-10 responses (*CP-P2, Table 4*). Another study suggested the antigenic region $NSP5_{156–167}$ (DGSFSSAFFLRY) to be restricted by the SLA-I haplotype Lr-22.0

(SLA-1*08XX-SLA-3*06:01-SLA-2*12XX) (*Mokhtar et al. 2014: CP-P3, Figures 4 and 5; Mokhtar et al. 2016: CP-P4, Table 1, Figures 2 to 6*). Additionally, pigs exhibiting $CD4^+$ T cell responses to the antigenic peptide $M_{29–43}$ (MIYALKVSRGRLLGL) were haploidentical, sharing both SLA-II haplotypes Lr-0.01 (DRB1*01XX-DQB1*01XX-DQA*01XX) and Lr-0.15b (DRB1*04:05(04:06)-DQB1*02:02-DQA*02XX). This combination appearing exclusively in these animals suggests restriction by one of these two haplotypes (*CP-P4, Table 1, Figures 2 to 6*).

In 2016, Lunney et al. reviewed important issues of PRRSV infection, immunity, pathogenesis, and control. They addressed the gaps in our knowledge and emphasized the need for advanced molecular and immunological tools to stimulate PRRS research and field applications (*Lunney et al. 2016*). Following *in silico* prediction of SLA-I-specific CD8 and SLA-II-specific CD4 T cell epitopes of PRRSV-2 NADC20, Tian et al. developed subunit PRRSV-2 vaccines by expressing SLA-I and SLA-II allele-specific epitope antigens in a robust adenovirus vector. Subsequent poly-T cell epitope peptide design focused on 39 SLA-I epitopes, consisting of eight top-ranked epitopes specific to each of five SLA-I alleles, and fusion to five SLA-II epitopes specific to SLA-II alleles. Unfortunately, these vaccines provided little or no protection, further corroborating the tremendous challenges for developing an effective PRRSV-2 subunit vaccine (*Tian et al. 2020*). Wu et al. highlighted the mechanism of SLA-II-mediated antigen presentation via promoting SLA-DR expression to present immunopeptides from PRRSV NSPs, which contributes to the induction of non-neutralizing antibody *in vivo* (*Wu et al. 2020*). Further attempts aimed to validate a certain PRRSV-specific SLA-1*15:02-restricted CTL epitope by crystallography, biochemistry, and pathogen-free swine experiments. Non-structural protein NSP9-TMP9 was determined as a PRRSV CTL epitope with strong immunogenicity by flow cytometry and IFN-γ expression (*Pan et al. 2020*).

As demonstrated for different porcine tissues and several SLA genes (e.g., *SLA-1*, *SLA-6*, *SLA-DQA*, and *SLA-DQB1*), certain variants of the 3'-untranslated region microRNA target sites are

linked to antigen processing and presentation functions. Meanwhile, this is known to be associated with disease traits as reported for altered microRNA binding to MHC genes in human (*Endale Ahanda et al. 2012*). In mock- versus PRRSV-infected pig endometrial epithelial cells, differentially expressed microRNAs targeting *SLA-DQB1* were found. These genes are known to participate in the apoptosis signal, an indication of increased susceptibility to PRRSV infection (*Zhang et al. 2019*).

Antibody disrupting the interaction between the macrophage CD163 receptor and the structural proteins GP2, GP3, and GP4 heterotrimer that protrudes from the surface of the virion could represent alternative potential vaccine targets. Recently, escape mutation of virus from neutralizing antibody were mapped to the ectodomain regions of structural proteins GP5 and M. However, GP5 has a conserved epitope flanked by N-glycosylation sites and hypervariable regions, a pattern of conserved epitopes shared by other viruses. This issue needs to be resolved to advance PRRSV vaccine development (*Stoian & Rowland 2019*). Nevertheless, *CD163* was identified as the striking receptor in PRRSV entry and its knockout from the genome by gene editing has led to the production of pigs that were completely resistant to PRRSV – a milestone in modern pig breeding. Eventually, the importance of *CD163* for homeostasis, defense and immunity demands for more insight before its complete or partial silencing can be answered (*Reiner 2016, Whitworth & Prather 2017*).

3.2.3 Swine Influenza A Virus (FLUAVsw) (CP-P5 and P6)

Influenza A viruses (FLUAVsw) cause acute respiratory infections in swine that result in significant economic losses for the global pig production. The following three subtypes of FLUAVsw are found worldwide: H1N1, H3N2, and H1N2 (*Mancera Gracia et al. 2020*). Monoclonal antibodies, recombinant cytokines and chemokines, gene probes, tetramers, and inbred pigs allow refined analysis of immune responses against FLUAVsw (*Holzer et al. 2019*). Detailed specificity and mechanisms of cross-protective T-cell immunity and the repertoire of

pig antibody response to FLUAVsw have yet to be fully described, although it is known that the local immune response is of importance (*Holzer et al. 2019*). To date, inactivated whole-virus vaccines are applied that should induce specific antibodies against hemagglutinin for clearing the infection. These vaccines are safe and protect against antigenically identical or very similar strains in the absence of maternally derived antibodies (*Mancera Gracia et al. 2020*). However, pigs vaccinated with inactivated whole-virus and challenged with heterologous strains, developed vaccine-associated enhanced respiratory disease. Apparently, together with the increasingly complex epidemiology of FLUAVsw, novel vaccination strategies are still needed (*Mancera Gracia et al. 2020*).

In the analysis of FLUAVsw, the porcine T-cell response has been poorly characterized to date. In a cohort of 40 outbred pigs, animals with a strong expansion of Ki-67$^+$CD8β$^+$ T cells and the highest frequencies of FLUAVsw-specific cytokine-producing CD4$^+$ T cells were homozygous for the SLA-I haplotype Lr-01.0 (SLA-1*01XX-SLA-3*01XX-SLA-2*01XX) and for the SLA-DQA allele group (DQA*02XX) (*Talker et al. 2015: CP-P5, Table 2, Figure 2A and 2B*). After restimulation with homologous H1N2 and heterologous H3N2, frequencies of IFN-γ-defined subsets within lung-derived CD4$^+$ or CD8β$^+$ T cells were highest in animals with Lr-43.0 (SLA-1*11XX-SLA-3*04XX-SLA-2*04XX), Lr-0.11 (DRB1*09XX-DQB1*04XX-DQA*03XX) and/ or Lr-0.23 (DRB1*10XX-DQB1*06XX-DQA*01XX) background (*Talker et al. 2016: CP-P6, Table 1, Figure 12*).

In 2018, Schwartz et al. fully characterized the SLA background of the inbred Babraham pigs on high-resolution level and formalise their SLA haplotype as Hp-55.6 (*Schwartz et al. 2018: CP-P8*). Based on this SLA-defined pig model, it was then possible to develop a toolset that included the identification of novel immune-dominant FLUAVsw-derived T-cell epitopes (*Schwartz et al. 2018: CP-P8, Tungatt et al. 2018*). Inbred Babraham and commercial outbred pigs were used to characterize TCR-αβ, TCR-γδ T cell and antibody immune responses to the

2009 pandemic H1N1 (H1N1pdm09) virus infection (*Edmans et al. 2021*). TCR-γδ T cells produced cytokines *ex vivo* at day 2 post infection, while virus reactive IFN-γ producing TCR-γδ T cells were detected from day 7 post infection. Broncho-alveolar lavage contained the most highly activated CD8, CD4, and TCR-γδ T cells producing large amounts of cytokines, which likely contribute to elimination of virus. Edmans et al. demonstrated that the immune response in Babraham pigs following H1N1pdm09 infection was comparable to that of outbred animals, hence, the concurrent use of these two swine models will provide unparalleled power to analyse immune responses to influenza (*Edmans et al. 2021*).

3.2.4 Foot-and-Mouth Disease Virus (FMDV) (CP-P7, IP-P4)

FMDV-specific T cell lines and clones have been obtained from SLA inbred miniature pigs [SLA (c/c) phenotype, Hp-3.3 and SLA (d/d) phenotype, Hp-4a.4] vaccinated with chemically inactivated virus (*Sachs et al. 1976, Rodríguez et al. 1996*). One of the obtained clones (CE3) exhibited a specific and heterotypic proliferation against infectious but not inactivated FMDV in the presence of syngeneic peripheral blood mononuclear cells. Consistent with the CD8 phenotype, the proliferative response of CE3 was inhibited by anti-SLA-I and anti-CD8 monoclonal antibodies. Consequently, these results indicate that CE3 is a porcine SLA-I-restricted $CD8^+$ T cell clone that recognizes a heterotypic FMDV antigen. Unfortunately, no common epitope was found, but the overlapping peptide may prove useful for FMD vaccine design (*Rodríguez et al. 1996, Gerner et al. 2006*).

In cattle, lymphocytes from two experimentally FMDV serotype A24-vaccinated and homologous challenged cattle were tested for recognition of FMDV-derived penta-decapeptides in proliferation assays (*Gerner et al. 2009: CP-P7*). One epitope was found that was recognized by two animals, pointing towards common MHC class II (BoLA-II) alleles. Molecular analyses of BoLA-II genes showed that both animals carried BoLA-DQA*022:02:01 and BoLA-DQB*013:01 but had no common BoLA-DRB3 allele (*CP-P7, Tables 1 and 3*).

Investigation of amino acid residues involved in MHC presentation by peptides with alanine substitutions showed that the amino acid residues in positions 5 and 9 within the pentadecapeptide representing the 1A epitope were important for MHC binding in both cattle. These data demonstrated that the epitope located on FMDV protein 1A can be presented by BoLA-II DQ molecules encoded by the alleles BoLA-DQA*022:02:01 and BoLA-DQB*013:01 and present the first evidence of the binding motif of this particular DQ molecule (*CP-P7, Figure 6*).

Previous studies already showed the promising potential of dendrimer peptides as vaccine candidates against FMDV in swine (reviewed in *Forner et al. 2021*). Several B-cell epitope dendrimers, harboring a major FMDV antigenic B-cell site in VP1 protein that are covalently linked to heterotypic T-cell epitopes from 3A and/or 3D proteins elicited consistent levels of neutralizing antibodies and IFN-γ-producing cells in pigs (*De León … Hammer et al. 2020: IP-P4*). Correlations of SLA-I haplotypes Lr-22.0, and Lr-59.0 as well as SLA-II haplotypes Lr-0.15b, Lr-0.24, and Lr-0.27 with antibody titers and IFN-γ-producing cells were found (*IP-P4, Tables 4 and 5, Suppl. Figures S1 and S2*). These findings support the contribution of SLA-II-restricted T cells to the magnitude of the T-cell response and to the antibody response evoked by the B_2T dendrimers, being of potential value for peptide vaccine design against FMDV (*IP-P4, Tables 4 and 5, Suppl. Figures S1 and S2*). In addition, Patch and colleagues used inbred Yucatan minipigs (4b.0/4b.0) to show that FMDV infection results in induction of cytotoxic T cell responses that are classically antigen specific and MHC restricted (*Ho et al. 2009b, Patch et al. 2011*). Following on, these investigators used SLA-1*04:01 and SLA-2*04:01 class I tetramers to show, upon vaccination with replication defective adenovirus 5 vectors expressing the FMDV P1 protein, T-cell specificities expand with each vaccine boost (*Pedersen et al. 2013, Pedersen et al. 2016*).

Table 3 summarizes the SLA class I and class II haplotypes that showed positive SLA-immune response correlations after challenge and/ or restimulation with antigenic peptides deriving from CSFV, PRRSV, FLUAVsw, and FMDV.

Table 3. SLA class I and class II Lr-Hp for which positive SLA-immune response correlations after challenge and/ or restimulation with antigenic peptides of CSFV, PRRSV, FLUAVsw, and FMDV were found.

Lr-Hp	**Allele Specificity[a]**			**Source of antigenic peptides**				**European farmed pigs[b]**
SLA-I	***SLA-1***	***SLA-3***	***SLA-2***	**CSFV**	**PRRSV**	**FLUAVsw**	**FMDV**	
01.0	01XX	01XX	01XX					6.28%
01.0/04.0[c]	04XX	01XX	01XX					0.82%
04.0	04XX	04XX	04XX					11.02%
22.0	08XX	06:01	12XX					6.74%
43.0	11XX	04:04	04XX					3.83%
59.0	11:03	05XX	16:02					6.19%
55.0	15XX	04:04	11:04					1.73%
SLA-II	***DRB1***	***DQB1***	***DQA***	**CSFV**	**PRRSV**	**FLUAVsw**	**FMDV**	**EU pigs[b]**
0.01	01XX	01XX	01XX					4.55%
0.04	02XX	04XX	02XX					0.88%
0.15b[d]	04:05~06	02:02	02XX					14.37%
0.06	05XX	08XX	01XX					1.17%
0.24	07XX	02XX	02XX					2.49%
0.11	09:06	04XX	03XX					3.67%
0.27	09:06	09XX	04XX+05XX[e]					5.72%
0.23	10:06	06:03	01XX					11.58%

SLA, Swine leukocyte antigen; Lr-Hp, Low-resolution haplotype identified by PCR-SSP; CSFV, Classical Swine Fever Virus (CP-P1); PRRSV, Porcine Reproductive and Respiratory Syndrome Virus (CP-P2 to P4); FLUAVsw, Swine Influenza A Virus (CP-P5 and P6); FMDV, Foot-and-Mouth Disease Virus (CP-P7, IP-P4); Lr, Low-resolution SLA haplotypes identified by PCR-SSP; n.a., not analysed.

[a] Allele designation(s) in parentheses indicates medium- or high-resolution specificities.

[b] Haplotype frequencies (%) among European farmed pigs (*IP-P3, Tables 1 and 2*).

[c] Not yet confirmed haplotype (*IP-P3, Table 1*).

[d] The alphabetical suffix in haplotype designations was used to differentiate between closely related haplotypes (i.e. haplotypes with identical low-resolution group specificities, but different allele specificities).

[e] Positive with both *DQA**04XX primer sets in lanes D12 and C12 (*IP-P3, Suppl. Table S2b*).

Consequently, the found correlations could facilitate tailored vaccine development, as the SLA-I haplotypes Lr-01.0, Lr-04.0, Lr-22.0, and Lr-59.0 as well as the SLA-II haplotypes Lr-0.15b, Lr-0.23 and Lr-0.27 are abundant in European farmed pigs (*IP-P3, Table 1 and 2*). Furthermore, e.g., MHC-cluster analyses of the functional diversities of identified frequent SLA-I and SLA-II molecules could be helpful to confirm the potential existence of supertypes (*Thomsen et al. 2013*). However, future association analyses of SLA-immune response correlations to

individual SLA locus alleles and not just haplotypes will need to be carried out using detailed SLA-DNA typing methods, including, if possible, NGS techniques and, importantly, testing in multiple breeds and broader pig populations [*Lunney et al. 2009, Hammer et al. 2020 (IP-P5, section 7*)]. Taking together, these results will be useful for studying the adaptive immune response and immunological phenotypic differences in pigs, screening potential T-cell epitopes, and further developing the more effective vaccines.

3.3 Importance of the SLA in swine health and biomedical research (CP-P8, IP-P5)

The SLA complex plays a key role for swine models in biomedical research. In pigs, production traits are influenced by SLA polymorphism and diversity. Pathogen effects on SLA gene expression drive the regulation of swine immune responses. SLA-typed pigs are used in vaccine design, disease models, and allogeneic and xenogeneic transplantation [*Lunney et al. 2007, Schook et al. 2015, Hammer et al. 2020 (IP-P5, section 2*)]. Using pigs from the same litter or transgenic pigs, facilitates comparative analyses and genetic mapping. The availability of well-defined cell lines, representing a broad range of tissues, further facilitates testing of e.g., gene expression and drug susceptibility (*Lunney 2007*, *Käser 2021*). Advances in genomics have put forward our understanding of the complexity of the immune system and the genes that influence disease and vaccine responses, with the most important being the SLA complex. Gene sequencing data have advanced efforts to define the polymorphisms of SLA class I and class II genes, setting the stage for probing the role of these genes in swine health and disease. Owing to their physiological similarity to humans, pigs are an important biomedical model, particularly in the areas of heart and gut function, toxicology, and drug screening as well as transplantation research (*Hammer et al. 2020: IP-P5, sections 4 to 6*).

3.3.1 Babraham pigs in genetic variation controlled studies (CP-P8)

Despite strong selection for production traits and inbreeding, domesticated pigs have maintained a significant level of genetic diversity, both within and between breeds (*Yang et*

al. 2017, Yang et al. 2020). Although beneficial for an individual's fitness, this diversity is problematic for infectious disease research and quantitative trait mapping studies in which complex and uncontrolled genetic variation may confound results and reduce statistical power (*Schwartz et al. 2018: CP-P8*). While there have been developed several MHC (SLA) inbred miniature pig breeds (e.g., NIH and Yucatan pigs), they resemble the genetic background of commercial breeds to a lesser extent (*Sachs et al. 1976, Choi et al. 2016*). For this reason, large inbred pig models are fundamentally important scientific resources. Originally, Babraham pigs were derived from a Large White commercial background during the 1970s but confirmation of homozygosity at the specific SLA loci was still lacking (*Signer et al. 1999*). By means of PCR-SSP and SBT, we confirm that Babraham pigs are essentially homozygous. The SLA-I and SLA-II high-resolution haplotypes are the following: SLA-1*14:02-SLA-3*04:03~02-SLA-2*11:04 (Hp-55.0) and DRB1*05:01-DQB1*08:01~02-DQA*01:03 (Hp-0.06) (*Schwartz et al. 2018: CP-P8, Table 2*). This emphasizes the importance of the Babraham pig as a useful biomedical model for studies in which controlling for genetic variation is important (*Nicholls et al. 2016, Tungatt et al. 2018*, *Edmans et al. 2021, Ladowski et al. 2021*). Based on this SLA-defined pig model, Tungatt et al. developed a toolset that included the identification of novel immune-dominant FLUAVsw-derived T-cell epitopes (*Tungatt et al. 2018*). Subsequently, inbred Babraham and commercial outbred pigs were used to characterize TCR-αβ, TCR-γδ T cell and antibody immune responses to the H1N1pdm09 virus infection (*Edmans et al. 2021*).

Immunogenetic variation also presents significant problems for preclinical studies with the pig as a model and future efforts to enable xenotransplantation (*Ladowski et al. 2019, Ladowski et al. 2021*). Furthermore, having a defined SLA allows to predict tolerance and associated clinical interventions. As an example, the power of the inbred Babraham pig model has recently been demonstrated in a genome-wide analysis, which identified MHC antigens involved in corneal transplant rejection (*Nicholls et al. 2016*). Similar studies are only feasible

using pigs with a defined MHC region, thus facilitating matching and mismatching of histocompatibility loci (*Wakeman et al.* 2006, *Ladowski et al. 2019, Ladowski et al. 2021*).

These findings are in line of argument for our proposition of SLA haplotyping-assisted animal trials in vaccine and transplantation research (*IP-P3, Suppl. Figure S1*).

3.3.2 Pig models in biomedical research (IP-P5)

Because of the Babraham's Large White background, their SLA-I haplotype Hp-55.0 was found in a cohort of outbred pigs (Large White mother lines) being used in vascularized composite allograft (VCA) transplantation. For purposes of VCA research, and for end-stage renal disease, we SLA typed 100 of these animals together with 80 Yorkshire and Yorkshire/Hampshire crosses, to establish porcine transplantation models with SLA-mismatched outbred large pigs (*I. Arenas Hoyos, M. Jensen-Waern, unpublished data*). To defuse the shortage of human donor organs or tissues, miniature pigs are considered suitable donor animals. As organs from conventional pigs are usually too large, those from minipigs are more appropriate. Hence, outbred miniature pig breeds (e.g., Göttingen Minipigs and Mini-LEWE) are also of key interest in biomedical research.

The Göttingen Minipig (GMP) developed at the University of Göttingen (Germany) is a synthetic breed that combines the high fertility of the Vietnamese potbellied pig, the low body weight of the Minnesota Minipig and the white coat colour of the German Landrace pig (*Gärke et al. 2014, Reimer et al. 2020*). In a series of collaborative SLA-mismatched transplantation studies (e.g. lung, pancreatic islet cells), we genotyped 130 GMP and found only 8 SLA-I and four SLA-II low-resolution haplotypes (*C. Figueiredo, S.E. Hammer & T. Duckova, unpublished data*). The most abundant SLA-I haplotype Lr-24.0 (SLA-1*05XX/15XX-SLA-3*04XX-SLA-2*06XX) occurred at a frequency of 37.7%. The SLA-II haplotype Lr-0.21 (DRB1*01XX-DQB1*05XX-DQA*04XX) was found at a frequency of 51.8%. Based upon a low variety of SLA haplotypes and their similarity, and considering the genetically background of these animals,

GMP seem to already have a restricted gene pool. However, among the studied GMP cohort, a couple of potential private haplotypes were found (*C. Figueiredo, S.E. Hammer & T. Duckova, unpublished data*).

Likewise, Mini-LEWE was bred as an animal model for experimental research (e.g., allogeneic kidney transplantation) in the former Democratic Republic of Germany and resembles the genetic background of the Minnesota Minipig, the Vietnamese Pot-bellied pig, and the German Landrace pig (*Schachler et al. 2019*). The SLA-defined study group of 60 Mini-LEWE comprised seven SLA-I and nine SLA-II haplotypes. The most abundant SLA-I haplotype Lr-59.0 (SLA-1*11:03-SLA-3*05XX-SLA-2*16:02) occurred at a frequency of 27.1% and the SLA-II haplotype Lr-0.26 (DRB1*11XX-DQB1*04XX-DQA*02XX) was found at a frequency of 41.4% (*N. Wenzel, S.E. Hammer & T. Duckova, unpublished data*).

GMP and Mini-LEWE share with European farmed pigs only Lr-49.0, Lr-55.0, but no SLA-II haplotypes, whereas GMP and farmed pigs have Lr-24.0, Lr-67.0, and Lr-0.21 in common. Mini-LEWE and farmed pigs share five SLA-I (Lr-04.0, Lr-28.0, Lr-29.0, Lr-34.0, Lr-59.0) and 8 SLA-II (Lr-0.01, Lr-0.04, Lr-0.11, Lr-0.15, Lr-0.23, Lr-0.26, Lr-0.27, Lr-0.33) haplotypes. To our knowledge, this is the first characterization of SLA gene diversity in Göttingen Minipigs[7] and Mini-LEWE by low-resolution PCR-SSP typing (*C. Figueiredo, N. Wenzel, S.E. Hammer & T. Duckova, unpublished data*).

SLA-defined pigs have served as a significant transplantation model and, with gene editing, a potential source of xeno-organs (*Hammer et al. 2020: IP-P5, sections 5 and 6*). As an example, SLA expression was silenced in porcine vascular endothelium during normothermic *ex vivo* lung perfusion to make use of them to be transferred to humans (*Figueiredo et al. 2019*). Furthermore, low immunogenic porcine pancreatic islet cell clusters have been generated for xenotransplantation (*Carvalho-Oliveira et al. 2020*). In line with these previous studies,

[7]Minipig-derived SLA typing data are confidential because of a non-disclosure agreement.

porcine kidneys were genetically engineered to permanently silence SLA transcripts during *ex vivo* organ perfusion (*Yuzefovych et al. 2020*). Advancement was made to generate SLA knockout pigs to prevent humoral and cellular xenogeneic immune responses. Fischer et al. reported the generation of viable SLA class I knockout pigs in combination with extra genetic modifications (GTKO, CMAH, B4GALNT2) by targeting *B2M* or the SLA-I heavy α-chain to decrease activation of human $CD8^+$ T cells when exposed to *B2M* knockout pig cells (*Fischer et al. 2020, Hein et al. 2020*). Nevertheless, Sake et al. have alerted for potential negative effects of *B2M* knockout pigs generation in terms of animals viability (*Sake et al. 2019*).

Recently, a report described the generation of pigs with a combined knockout for *B2M* and *CIITA* genes for the generation of SLA-I and SLA-II-deficient pigs (*Fu et al. 2020*). Both genetic discrepancies such as the absence of α-Gal epitopes in humans and gene similarities like between HLA and SLA that support the recognition by the human T-cell receptor contribute to the histoincompatibility between humans and pigs pose significant hurdles to the success of xenotransplantation.

However, recent advances in biotechnological and gene-editing tools build a robust and efficient platform to create bioengineered xenotransplants with improved histocompatibility. Hence, the identification of the ideal combination of genetic modifications on the organ-source pig is a crucial step to move forward into first-in-human clinical studies (*Ladowski et al. 2019, Carvalho-Oliveira et al. 2021, Ladowski et al. 2021*).

4. Summary and future perspectives

This thesis comprises 13 studies (IP P1 to P5 and CP P1 to P8) that were published between 2009 and 2021 and addressed topics related to the molecular genetics and importance of the Swine Leukocyte Antigen (SLA) complex in veterinary vaccine and biomedical research.

The last decade has seen major progress in swine immunology and genetics, and particularly in understanding of the SLA complex, its genetic loci, and the role of SLA in normal immunity and in infectious disease and vaccine responses (*IP-P5, Figure 4*). The stage is now set for deeper probing of the role of SLA alleles and haplotypes in controlling these responses, for determining specific antigenic epitopes that stimulate immune and vaccine responses, and for identifying critical immune cell subsets and the exact SLA loci that facilitate cellular interactions for effective immune responses. As biosecurity improves and consumers expect pork products free of antibiotics and swine pathogens, the need for vaccines will change. The relevance of disease-resistant breeding stock will become more important, along with pigs with improved vaccination responses (*IP-P5*).

Research using improved swine genome sequence and updated genomic and proteomic tools may reveal novel immune pathways regulated by SLA genes. It will help to verify the effects of specific SLA alleles on QTL and disease responses and to identify exactly which genes enable pigs to resist infection by specific pathogens. Detailed swine genomics, particularly of the SLA complex alleles and their diversity, will amplify the importance of the pig for allotransplantation and biomedical research. As appropriate genetically modified pigs are developed, the stage is now set for determining the critical role of SLA genes and proteins in cell and tissue xenotransplantation. The role of individual SLA antigens in swine biomedical models and for overall pig health and productivity will continue to be clarified (*IP-P5*).

4.1. Summary points (*IP-P5*)

(1) The SLA system is among the most well characterized MHC systems in nonhuman animal species. A systematic nomenclature for the genes, alleles, and haplotypes of the SLA complex is critical to research in swine genetic diversity, immunology, health, and vaccinology, as well as organ and cell transplantation.

(2) Based on our new, detailed annotation of the Sscrofa11.1 genome assembly, the SLA complex encodes approximately 150 loci, with at least 120 genes predicted to be functional.

(3) Despite the ongoing domestication process, involving selection for favourable traits, pigs have still maintained a high degree of SLA diversity, as demonstrated by the presence of the 266 and 227 class I and class II alleles, respectively.

(4) Pig disease models provide better understanding of host-pathogen interactions. Pathogen effects on SLA gene expression drive the regulation of swine immune responses. Novel trait association data indicate that SLA alleles or haplotypes may be useful genetic markers for use in improving pig-breeding programs.

(5) Swine have become the preferred preclinical large animal model for biomedical studies, transplantation, xenotransplantation, and regenerative medicine research. Allogeneic transplantation research in pigs has improved understanding of rejection mechanisms of both host-versus-graft and graft-versus-host disease.

(6) Improved cross-matched genetically engineered pigs could reduce antibody-mediated rejection of pig xenografts in highly HLA-sensitized patients. Modifying SLA genes could improve pigs as donors for xenotransplantation.

4.2. Future issues (*IP-P5*)

(1) The impact of SLA genes on swine production and health traits needs to be attributed to individual SLA locus alleles and not just haplotypes.

(2) Renewed typing methods, from PCR SSP to NGS, will enable reliable typing of outbred pigs. To truly explore diversity, data based on large cohorts of pigs are necessary.

(3) Functional studies on MHC effects on cell interactions and on microbiota diversification are needed to understand the impact of SLA genes on the education of the porcine immune system.

(4) In-depth analysis of peptide presentation via major SLA genes will identify the broad range of functionally relevant vaccine targets.

(5) Identification and maintenance of important SLA-defined pig lines (e.g., NIH/MGH, Yucatan, or Babraham pigs) are essential as resources for pig biomedical models.

(6) Future tool development is needed for the swine biomedical model; this includes SLA class I and first SLA class II tetramers, T-cell receptor profiling, SLA-informed SNP chips, and panels of monoclonal antibody reagents to swine immune proteins.

(7) Availability of well-characterized, genetically engineered pigs for human disease models will lead to development and validation of novel therapeutics and improvements in xenotransplantation research.

(8) Human cross matching with SLA class I and II will facilitate xenotransplantation. Histocompatibility testing of pigs needs to be improved in analogy to human allogeneic transplantation.

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Zhang, N., Qi, J., Feng, S., Gao, F., Liu, J., Pan, X., Gao, G. F. (2011) Crystal structure of swine major histocompatibility complex class I SLA-1 0401 and identification of 2009 pandemic swine-origin influenza A H1N1 virus cytotoxic T lymphocyte epitope peptides. Journal of Virology, 85: 11709–11724.

Zhang, K., Ge, L., Dong, S., Liu, Y., Wang, D., Zhou, C., Ma, C., Wang Y, Su, F., Jiang, Y. (2019) Global miRNA, lncRNA, and mRNA transcriptome profiling of endometrial epithelial cells reveals genes related to porcine reproductive failure caused by porcine reproductive and respiratory syndrome virus. Frontiers in Immunology, 10: 1221.

6. Curriculum vitae

I. Personal Information

May 14, 1968	Born in Vienna, Austria
Nationality	Austrian
Current address	Institute of Immunology Department of Pathobiology University of Veterinary Medicine Vienna Veterinaerplatz 1, 1210 Vienna, Austria E-mail: sabine.hammer@phylo-dat.net http://www.vetmeduni.ac.at/immunologie/

II. University Education and Scientific Career

10/1986 – 09/1989	Vienna University (Austria) - Biochemistry
09/1989 – 04/1996	Vienna University (Austria) - Molecular Genetics
03/1996	Graduation, MSc - Master of Natural Sciences (Mag.rer.nat)
07/1996 – 01/1997	Research scientist at the Research Institute of Wildlife Ecology, University of Veterinary Medicine Vienna (Austria)
03/1997 – 11/2001	PhD Thesis at the Institute of Zoology, Department of Systematic Zoology and Developmental History, Vienna University (Austria). Supervisor: Prof. Mag. Dr. Luitfried Salvini-Plawen
03/1997 – 02/2000	Research assistant at the Department of Systematic Zoology and Developmental History, Institute of Zoology, Vienna University (Austria)
06/2000 – 01/2001	Employed at Sigma-Aldrich Austria in the Life Sciences division (Technical Service Support and Product Management)
11/2001	Graduation, PhD (Dr.rer.nat.)
04/2001 – 06/2002	Postdoctoral Research Fellow at the Research Institute of Wildlife Ecology, University of Veterinary Medicine Vienna (Austria)
11/2002 – 04/2005	Postdoctoral Research Fellow at the Laboratories of Genome Dynamics, Centre of Anatomy and Cell Biology, Medical University of Vienna (Austria)
01/2006 – present	Senior Scientist at the Institute of Immunology, Department of Pathobiology - Department II, University of Veterinary Medicine Vienna (Austria)

III. Publications and Grants

Publications	Total number of publications in peer reviewed journals: 66 Total number of conference abstracts in peer-reviewed journals: 34 Book chapters: 6
Presentations	Total number of talks: 103 Total number of posters: 107
Conferences	Total number of participations: 72
Grants	Total number of grants: 3

IV. Awards, Promotions and Scientific Activities

Scientific Awards

- 2019: Scientific award (VÖK Award) given by the Austrian Society of Small Animal Medical practitioners (Vereinigung Österreichischer Kleintiermediziner – VÖK) for the publication: **Hammer SE**[8], Groiss S, Fuchs-Baumgartinger A, Nedorost N, Luckschander-Zeller N, Saalmüller A, Schwendenwein I, Rütgen BC. 2017. Characterization of a PCR-based lymphocyte clonality assay as a complementary tool for the diagnosis of feline lymphoma. Vet Comp Oncol. 2017 Dec;15(4):1354-1369. Epub 2016 Nov 4. https://doi.org/10.1111/vco.12277
- 2019: IUIS VIC Travel Award for the participation at the 12th International Symposium of Veterinary Immunology (IVIS 2019) (August 13-16, 2019, Seattle, WA, USA)
- 2018: 1st place poster award given in the "Science" category by the University of Veterinary Medicine Vienna: **Hammer SE**, Leopold M, Mair KH, Prawits L-M, Dolezal M, Endler L, Ravens S, Gerner W,

[8]Name change back to name of birth in 2014.

Saalmüller A. Development of a RACE-based RNA-Seq approach to characterize the T cell receptor repertoire of porcine γδ T cells.

- Inventor of the year 2014 of the University of Veterinary Medicine Vienna (Soft-IP for the production of monoclonal antibodies)

Reviewing activities and Board memberships

Peer-reviewed journals	Total number: 41
Research grants	Total number: 3
Editorial Board Membership	Frontiers in Immunology (Comparative Immunology section)
Biosafety Committee	Member of the Biosafety Committee for the Biosafety Level 2 Laboratory at the Institute of Medical Genetics, Center of Pathobiochemistry and Genetics, Medical University of Vienna, Währinger Straße 10, 1090 Vienna, Austria

IATA Dangerous Goods Regulations – Personnel category 1 | UN1845, UN3373, UN3245"
(University of Veterinary Medicine Vienna, Austria – since 2016)

Society memberships

- European Society for Veterinary Oncology (since 2015)
- SLA Nomenclature Committee member (since 2010)
- European Canine Lymphoma Network (since 2009, founding member)
- Women in Science – an interdisciplinary association – WISIA (since 2009, founding member)
- International Society of Animal Genetics – ISAG (since 2008, member)
  - since 2016 member of the Genetics of Immune Response Committee
  - since 2019 member of the Executive Committee and member of the Comparative MHC Steering Committee
- German Society of Mammalogy, DGS (since 2002)

Academic distinctions and professional development (selection)

- Webinars (University of Veterinary Medicine Vienna, Austria – 2020-2021)
  - LeadingVet: "Basic leadership concepts" and "Bringing ideas for leadership"
  - Home-Office: "Data protection" and "Resilience"
  - LeadingVet: "Governance und self-governance" and "On Air - professional online communication"
  - Didactics & teaching: "Building self-tests for interim evaluations", "Implementation of multimedia learning tools", "Learning outcome-oriented and interactive teaching - effective use of Audio-Response-Systems", "Follow-up Poll everywhere", "Thesis supervision" and "Vetucation for lecturers – Poll everywhere"; "Online didactics – Hands on" (FH Joanneum Graz, Austria, two Online-Workshops)
- Didactics & teaching (University of Veterinary Medicine Vienna, Austria – 2015-2019)
  - "Interactive learning and teaching – The use of the 'Klicker' system in academic teaching"
  - "Tools for condensed and focused academic teaching"
  - "Tools for motivation and excitement in academic teaching"
  - "Using professional visualization tools for academic teaching"
  - "Lost in too many details – Balancing personal effectivity and perfection"
  - "The role of university teachers as student coaches"
  - "Voice and speech training"
  - "The two wings of success - yes and no"
- Training course "Methods for analysing professional competence in veterinary studies" as part of the KELDAT project (University of Veterinary Medicine Vienna, Austria – 2013-2014)
- "Career development training for Women in Technology and Science" (fFORTE Coaching program of the Austrian Federal Ministry of Science and Research – 2008-2009)
- "Application training for state doctorate candidates" (University of Graz, Austria – 2008)

V. Teaching Activities

Lectures and practical training courses

- University of Veterinary Medicine Vienna (Austria): Teaching assignments in the Diploma Programme in Veterinary Medicine, the Bachelor's Programme in Biomedicine and Biotechnology, the Master's Programme Comparative Biomedicine, Doctoral Programme in Veterinary Medicine, PhD Programme (since 2006).

- Medical University of Vienna (Austria): Teaching assignment in the courses "MCW-Module 8: Disease, aetiology and clinical picture" and "MCW-Part 6 Chapter II, Theme 8: The evolution of humans and their diseases" (since 2005).
- Guest lecturer at the Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad (Serbia and Montenegro) for the one-week course "On trees and networks: Basic approaches in phylogenetic reconstruction" (Brain Gain Programme, WUS Austria) (2004)

Supervision of theses (selection since 2013, in total 22)

- Julie Welter (2021): Revisiting lymphocyte clonality testing in feline B cell lymphoma (Diploma MMV thesis, University of Veterinary Medicine Vienna, Austria)
- Lena Kasper (2021): Molecular characterization of swine leukocyte antigen gene diversity in pigs (Bachelor thesis, University of Mainz, Germany)
- Sophia Unterkreuter (2021): Lymphocyte clonality testing as an adjunct tool in the characterisation of lymphoma in big cats (Diploma MMV thesis, University of Veterinary Medicine Vienna, Austria)
- Julia Huber (2021) Lymphocyte Clonality Testing in Feline Intestinal Lymphoplasmacytic Infiltration: Friend or Foe? (Diploma MMV thesis, University of Veterinary Medicine Vienna, Austria)
- Tereza Duckova (2019): Molecular characterization of swine leukocyte antigen gene diversity in Göttingen Minipigs (Bachelor thesis, University of Veterinary Medicine Vienna, Austria)
- Lisa-Maria Prawits (2017) A novel 5' RACE RNA-like approach to characterize the molecular variability of the T-cell receptor in porcine γδ T cells (Bachelor thesis, Vienna University, Austria)
- Melanie Leopold (2017) A novel 5' RACE RNA-like approach to characterize the molecular variability of the T-cell receptor in porcine αβ T cells (Bachelor thesis, Vienna University, Austria)
- Amina Paquay (2017): c-Kit mutations in the canine cutaneous mast cell tumour: Establishment of a PCR-based screening assay for routine diagnostics (Bachelor thesis, University of Applied Sciences, FH Campus Vienna, Austria)
- Angela Schlerka (2016): Correlation of expressed T cell receptor repertoire to organ-specific frequencies and phenotypes of porcine γδ T-cell subsets (Bachelor thesis, Vienna University, Austria)
- Verena Gress (2015): PCR for Antigen receptor gene rearrangement (PARR) as an adjunct tool in the characterisation of intestinal lymphomas in cats (Bachelor thesis, University of Veterinary Medicine Vienna, Austria)
- Jennifer Lück (2015): Characterisation of the Interferon-γ production in porcine $CD4^+$ T cells by simultaneous detection of mRNA and protein expression. (Bachelor thesis, University of Applied Sciences, FH Campus Vienna, Austria)
- Philipp Kroiss (2014): Age-related changes of CDR3 spectratyping profiles in porcine γδ T-cells (Bachelor thesis, University of Veterinary Medicine Vienna, Austria)
- Isabella Humer (2013): CDR3 spectratyping profiles of porcine γδ T-cell subpopulations (Bachelor thesis, University of Veterinary Medicine Vienna, Austria)

Examination Committees

- Mei-Hua Hwang (2017): Characterization of the canine immunoglobulin heavy chain repertoire and design of an improved molecular clonality assay for canine B cell proliferations. Member of the MSc examination committee at the Ontario Veterinary College, University of Guelph, Ontario, Canada.
- Irene M. Rodríguez Gómez (2012): Role of immunregulatory cells during Porcine Reproductive and Respiratory Syndrome. Member of the PhD examination committee at the Veterinary Faculty, University of Cordoba, Spain.

VI. List of Publications

Original papers in peer-reviewed journals (selection since 2013, in total 66)

(1) **Hammer SE**, Duckova T, Groiss S, Stadler M, Jensen-Waern M, Golde WT, Gimsa U, Saalmüller A. 2021. Comparative analysis of swine leukocyte antigen gene diversity in European farmed pigs. Anim Genet. doi: 10.1111/age.13090

(2) Rütgen BC, Baszler E, Weingand N, Wolfesberger B, Baumgartner D, **Hammer SE**, Groiss S, Fuchs-Baumgartinger A, Saalmüller A, Schwendenwein I. 2021. Composition of lymphocyte subpopulations in normal and mildly reactive peripheral lymph nodes in cats. J Feline Med Surg. 2021 Feb 27. doi: 10.1177/1098612X211005310.

(3) Unterkreuter S, Posautz A, Rütgen BC, Groiss S, Kübber-Heiss A, **Hammer SE**. 2020. Lymphocyte clonality testing as an adjunct tool in the characterisation of lymphoma in non-domestic felines. Res Vet Sci. Volume 135, March 2021, Pages 511-516. doi: 10.1016/j.rvsc.2020.11.011. Epub 2020 Nov 19.

(4) De León P, Cañas-Arranz R, Saez Y, Forner M, Defaus S, Bustos MJ, Torres E, Rodríguez-Pulido M, Andreu D, Blanco E, Sobrino F, **Hammer SE**. 2020. Association of porcine Swine Leukocyte Antigen (SLA) haplotypes with B- and T-cell immune response to foot-and-mouth disease virus (FMDV) peptides. Special issue: Evaluation of Vaccine Immunogenicity, Vaccines (Basel). 2020 Sep 8;8(3):E513. doi: 10.3390/vaccines8030513

(5) **Hammer SE**, Ho C-S, Ando A, Rogel-Gaillard C, Charles M, Tector M, Tector AJ, Lunney JK. 2020. Importance of the MHC (SLA) in swine health and biomedical research. Annu Rev Anim Biosci. 2020 Feb 15;8:171-198. doi: 10.1146/annurev-animal-020518-115014. Epub 2019 Dec 17.

(6) **Hammer SE**, Leopold M, Mair KH, Prawits L-M, Schwartz JC, Hammond JA, Ravens S, Gerner W, Saalmüller A. 2020. Development of a RACE-based RNA-Seq approach to characterize the T-cell receptor repertoire of porcine γδ T cells. Dev Comp Immunol. 2020 Apr;105:103575. doi: 10.1016/j.dci.2019.103575. Epub 2019 Dec 14.

(7) Lagler J, Mitra T, Schmidt S, Pierron A, Vatzia E, Stadler M, **Hammer SE**, Mair KH, Grafl B, Wernsdorf P, Rauw F, Lambrecht B, Liebhart D, Gerner W. 2019. Cytokine production and phenotype of Histomonas meleagridis-specific T cells in the chicken. Vet Res. 2019 Dec 5;50(1):107. doi: 10.1186/s13567-019-0726-z.

(8) Luckschander-Zeller N, **Hammer SE**, Ruetgen BC, Thalhammer JG, Haas E, Richter B, Welle M, Burgener IA. Clonality testing as complementary tool in the assessment of different patient groups with canine chronic enteropathy. Vet Immunol Immunopathol. 2019 Aug;214:109893. doi: 10.1016/j.vetimm.2019.109893. Epub 2019 Jul 10.

(9) Rodríguez-Gómez IM, Talker SC, Käser T, Stadler M, Reiter L, Ladinig A, Milburn JV, **Hammer SE**, Mair KH, Saalmüller A, Gerner W. 2019. Expression of T-bet, Eomesodermin and GATA-3 correlates with distinct phenotypes and functional properties in porcine γδ T cells. Front. Immunol. 10:396. doi: 10.3389/fimmu.2019.00396.

(10) Schwartz JC, Hemmink JD, Graham SP, Tchilian E, **Hammer SE**, Ho C-S, Hammond JA. The MHC homozygous inbred Babraham pig as a resource for veterinary and translational medicine. HLA. 2018 Apr 23;92(1):40-43. doi: 10.1111/tan.13281. [Epub ahead of print]

(11) Taher L, Beck J, Liu W, Roolf C, Chodisetti M, Soller JT, Rütgen BC, **Hammer SE**, Sender S, Sterenczak KA, Fuellen G, Junghanss C, Brenig B, Nolte I, Schütz E, Murua Escobar H. 2018. Comparative High-Resolution Transcriptome Sequencing of Lymphoma Cell Lines and de novo Lymphomas Reveals Cell-Line-Specific Pathway Dysregulation. Sci Rep. 2018 Apr 19;8(1):6279. doi: 10.1038/s41598-018-23207-7.

(12) **Hammer SE**, Tautscher B, Pucher E, Kowarik K, Reschreiter H, Kern A, Haring E. 2018. Bronze Age meat industry: Ancient mitochondrial DNA analyses of pig bones from the prehistoric salt mines of Hallstatt (Austria). BMC Res Notes. 2018 Apr 13;11(1):243. doi: 10.1186/s13104-018-3340-7.

(13) Wolfesberger B, Fuchs-Baumgartinger A, Greß V, **Hammer SE**, Knödl K, Tichy A, Rütgen BC, Beham-Schmid C. 2018. WHO classification of lymphoid neoplasms in veterinary and human medicine: A comparative evaluation of gastrointestinal lymphomas in 61 cats. J Comp Pathol. 2018 Feb;159:1-10. doi: 10.1016/j.jcpa.2017.12.006. Epub 2018 Feb 3.

(14) Wolfesberger B, Skor O, **Hammer SE**, Flickinger I, Kleiter M, Rütgen BC, Schwendenwein I, Tichy A, Hittmaier K, Degasperi B, Fuchs-Baumgartinger A. 2017. Does categorisation of lymphoma subtypes according to the World Health Organization classification predict clinical outcome in cats? J Feline Med Surg. 2017 Aug;19(8):897-906. doi: 10.1177/1098612X16666119. Epub 2016 Aug 30.

(15) Skor O, Guija de Arespacochaga A, Ruetgen BC, **Hammer SE**, Springler G, Loerincz B, Kleiter M. 2017. Non-epitheliotropic cutaneous T-cell lymphoma - The great impostor. Wiener Tierärztliche Monatsschrift – Veterinary Medicine Austria 104:63-73.

(16) Pérez T, Fernández M, **Hammer SE**, Domínguez A. 2017. Multilocus intron trees reveal extensive male-biased homogenization of ancient populations of chamois (*Rupicapra* spp.) across Europe during Late Pleistocene. PLoS One. 2017 Feb 1;12(2):e0170392. doi: 10.1371/journal.pone.0170392. eCollection 2017.

(17) Gimsa U, Ho C-S, **Hammer SE**. 2017. Preferred SLA class I / class II haplotype combinations in German Landrace pigs. Immunogenetics. 2017 Jan;69(1):39-47. doi: 10.1007/s00251-016-0946-6. Epub 2016 Aug 2.

(18) **Hammer SE**, Groiss S, Fuchs-Baumgartinger A, Nedorost N, Luckschander-Zeller N, Saalmüller A, Schwendenwein I, Rütgen BC. 2017. Characterization of a PCR-based lymphocyte clonality assay as a complementary tool for the diagnosis of feline lymphoma. Vet Comp Oncol. 2017 Dec;15(4):1354-1369. doi: 10.1111/vco.12277. Epub 2016 Nov 4.

(19) Talker SC, Stadler M, Koinig HC, Mair KH, Rodriguez-Gomez IM, Graage R, Zell R, Dürrwald R, Starick E, Harder T, Weissenböck H, Lamp B, **Hammer SE**, Ladinig A, Saalmüller A, Gerner W. 2016. Influenza A virus infection in pigs attracts multifunctional and cross-reactive T cells to the lung. J Virol. 2016 Sep 29;90(20):9364-82. doi: 10.1128/JVI.01211-16. Print 2016 Oct 15.

(20) Greß V, Wolfesberger B, Fuchs-Baumgartinger A, Nedorost N, Saalmüller A, Schwendenwein I, Rütgen BC, **Hammer SE**. 2016. Characterization of the T-cell receptor gamma chain gene rearrangements as an adjunct

tool in the diagnosis of alimentary T-cell lymphomas in the gastrointestinal tract of cats. Res Vet Sci. 2016 Aug; 107:261-6. doi: 10.1016/j.rvsc.2016.07.004. Epub 2016 Jul 4.

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