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Antibody repertoire development in swine

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Abstract

Swine belong to the Order *Artiodactyla* and like mice and humans, express IgM, IgD, IgG, IgE and IgA antibodies but a larger number of IgG subclasses. Like rabbits and chickens, expressed V_H genes belong to the ancestral V_H3 family and only 5 comprise >80% of the pre-immune repertoire. Since they use primarily two D_H segments and have a single J_H like chickens, junctional diversity plays a relatively greater role in repertoire formation than in humans and mice. Proportional light chain usage surprisingly resembles that in humans and is therefore distinctly different from the predominant kappa chain usage (>90%) of lab rodents and predominant lambda chain usage in other ungulates (>90%). The pre-immune V_κ repertoire also appears restricted since >95% of $V_\kappa J_\kappa$ rearrangements use only a few members of the IGKV2 family and only $J_\kappa 2$. Two V_λ families (IGLV3 and IGLV8) are used in forming the pre-immune repertoire. Antibodies that do not utilize light chains as in camelids, or the lengthy CDR3 regions seen in cattle that use V_H4 family genes, have not been reported in swine.

B cell lymphogenesis first occurs in the yolk sac but early VDJ rearrangements differ from mice and humans in that nearly 100% are in-frame and N-region additions are already present. Swine possess ileal Peyers patches like sheep which may be important for antigen-independent B cell repertoire diversification. The presence of pro B-like cells in interlobular areas of thymus and mature B cells in the thymic medulla that have switched to especially IgA in early gestation, is so far unique among mammals.

The offspring of swine are believed to receive no passive immunity in utero and are precosial. Thus, they are a useful model for studies on fetal–neonatal immunological development. The model has already shown that: (a) colonization of the gut is required for responsiveness to TD and TI-2 antigens, (b) responsiveness due to colonization depends on bacterial PAMPs and (c) some viral pathogens can interfere with the establishment of immune homeostasis in neonates.

Studies on swine reinforce concerns that caution be used when paradigms arising from studies in one mammal are extrapolated to other mammals, even when similarities are predicted by taxonomy and phylogeny. Swine exemplify a situation in which evolutionary diversification of the immune system is not characteristic of an entire order or even of other related systems in the same species.

Keywords: Swine; B cells; Immunoglobulins; Ontogeny; Fetal; Colonizing bacteria; TLR ligands; Thymus

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1. The porcine germline repertoire

1.1. The constant region heavy chain repertoire of swine

The heavy chain immunoglobulin locus of swine has only been partially mapped, although sequence analyses of hundreds of V(D)J rearrangements together with Southern blots and partial mapping studies have provided insight into this species' germline potential. Such information indicates that genes encoding the same isotypes found in mice and humans also occur in swine (Table 1). The most notable deviation from the mouse and human genome is the greater number of apparent IgG subclasses, a feature shared with the horse (Wagner et al., this volume). While the exact number of IgG subclasses and alleles has not been established, sequences for seven are known and the large number is consistent with Southern blots [1; Wertz & Butler, unpublished]. Multiple Cγ genes suggest recent gene duplication in the Cγ region of the locus while the single Cε and single Cα gene indicate that duplication did not involve the entire C_H locus as in humans or the Cα region as in rabbit [2]. However, the swine Cα locus does contain an interesting allelic variant that encodes an IgA lacking four amino acids of the hinge [3]. At this time, no evidence has been obtained to indicate any disfunction of this 'hingeless' variant of porcine IgA although its breed-association supports its genetic origin [4]. Noteworthy is that in heterozygotes, allelic expression of the porcine IgA variant is not equal although the basis for this non-random expression of allelic variants remains unknown [5].

While a gene encoding IgD was not initially recovered [6], a separate strategy involving the intermediate use of a bovine IgD-specific gene probe, recovered a non-conserved gene preceded by a very short switch region [7,8; Fig. 1]. In mice and humans, all expressed isotypes except IgD are preceded by 5′ switch regions and their domain exons are followed by 3′ exons encoding either for membrane expression (mIg) or extracellular secretion (sIg). The possibility that Cδ is preceded by a Sδ as in cattle [7] has been confirmed, although it is only 0.5 kb in length [8] compared to 3.4 kb for Sμ [9; Fig. 1]. The average length of all switch regions is 2–3 kb [10].

Sequence comparisons of genes encoding IgM, IgA, IgG and IgE to those from other species show highest sequence similarity with their apparent 'sequence homologs' from humans (>70%)

Table 1
The porcine C_H repertoire

Current designation	Chain& Allotype	Gene	Serological allele	Sequence allele	GenBank Acct. No.	Major features and other information
IgG1	γ1	IGHG1			U03778	Possible allele of IgG3
IgG2[a]	γ2[a]	IGHG2		G2*01	U03779	Allotypic differences confined to Cγ1 domain. Differences from IgG1 occur throughout the sequence
IgG2[b]	γ2[b]	IGHG2		G2*02	U03780	Possible allele of γ2[a]
IgG3	γ3	IGHG3			U03781	Near identity to IgG1 in Cγ1, hinge and Cγ2. Five aa differences in Cγ3
IgG4	γ4	IGHG4			U03782	Similar to IgG2 in hinge and Cγ2. Differs from IgG2 in Cγ3 domain
IgG5	γ5	IGHG5	-	-	-	Extended hinge
IgG6	γ6	IGHG6	-	-	-	Truncated hinge
IgA	α	IGHA	A[a]	A+01	U12594	Splice acceptor site mutation in IgA[b] results in a four aa reduction in hinge length
			A[b]	A+02		
IgM	μ	IGHM			U50148	
					U50149	Sequence of membrane form of porcine Cμ
IgE	ε	IGHE			U96100	
IgD	δ	IGHD			AF411239 and AF515674	μCH1 like δCH1, short hinge

and ruminant artiodactyls [1,9,11,12]. By comparison, porcine IgD shows only ≃40% homology to IgD in mice and humans [8]. In the case of IgM, the sequence similarity of Cμ4, Cμm and Cμs with human and mouse is >90% [9].

All major porcine isotypes (C_H genes) are transcribed at or before birth (Fig. 3A and B) and IgM, IgG and IgA are present in fetal serum [13]. Curiously, IgG and IgA are preferentially transcribed and synthesized in thymus at this time (Fig. 2A).

1.2. The variable heavy chain repertoire of swine

Information on the germline V_H repertoire of this species is known primarily from the V_H and D_H segments that are rearranged or transcribed in fetal and newborn piglets [14–17]. These indicate that all known porcine V_H genes belong to the V_H3 family [19]; V_H3 is considered to be the ancestral V_H family [21]. Studies of V_H gene expression in fetal and neonatal piglets consistently show that four V_H genes,

Table 2
Porcine VH repertoire

Original[a] Description	Proposed Description	Origin			CDR1[b]	CDR2[b]	GenBank Acct #	Comments
		cDNA	DNA	BAC or COSMID				
V_HA	V_HA	+	+	+	A	A	AF064686	
V_HB	V_HB	+	+	+	B	B	AF064687	
V_HC	V_HC	+	+	+	C	C	AF064688	
V_HD	V_HD	+			C	D		cDNA only[a] not submitted
V_HE	V_HE	+	+	+	E	E	AF064689	
V_HF	V_HF	+	+		F	F	AF064690	
V_HG	V_HN	+		+	N	N	AY911499	V_HN(K) full sequence
V_HH	V_HL		+		E	L	AY911500	V_HL(K) full sequence
V_HI	V_HI	+	+	+	E	I	AF064691	
V_HJ	V_HJ		+		J	F	AY911501	
V_HK	V_HK		+	+	K	K	AF064692	
V_HK	V_HH				O	H		
V_HL(Kim)	V_HL	+			E	L	AF321839	Heavily truncated
V_HM(Kim)	V_HM	+			F	X	AF321840	Heavily truncated
V_HN(Kim)	V_HN	+			N	N	AF321841	Heavily truncated
V_HO(Kim)	V_HO	+			O	O	AF321842	Heavily truncated
V_HP(Kim)	V_HP	+			P	O	AF321843	Heavily truncated
V_HQ(Kim)	V_HQ	+			C	O	AF321844	Heavily truncated
V_HR(Kim)	V_HR	+			O	I	AF321845	Heavily truncated
V_HS(Kim)	V_HS	+			S	S	AF321846	Heavily truncated
V_HT(Kim)	V_HT	+			E	F	AF321847	Heavily truncated
V_HU(Kim)	V_HU	+			C	U	AF321848	Heavily truncated
V_HV(Kim)	V_HV	+			A	B	AF321849	Heavily truncated
V_HW(Kim)	V_HW	+			A	F	AF321850	Heavily truncated
V_HL	V_HX			+	X	X	AY911502	
V_HN	$V_H$psg 2		+		Y	A	AY911503	Pseudogene
V_HM	V_HO	+	+		O	O		V_HO(K) full sequence
V_HO	V_HZZ		+	+	N	I	AY911504	
V_HPsg	$V_H$psg 1	+	+		P	A		Pseudogene
829 G	V_HG			+	G	E		Truncated DNA
	V_HZ	+		+	E	C		Truncated DNA
		+			B	A		not yet sequenced
	V_HY	+			C	A		cDNA only not submitted

[a] All genes with (K) are truncated cDNAs contributed by Kim. Lab policy is not to submit cDNA sequences to GenBank without genomic confirmation since they could be somatic mutants or PCR artifacts [20].

[b] Numerous porcine V_H genes share CDR regions with other V_H genes, e.g. V_HJ shares CDR2 with V_HF and V_HI shares CDR1 with V_HE.

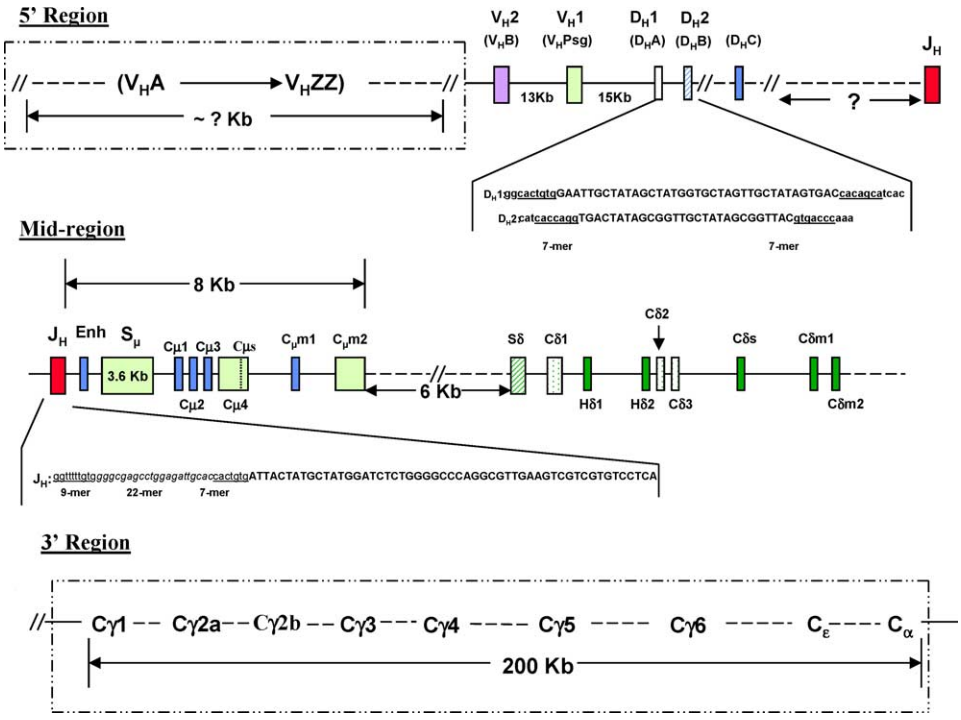

Fig. 1. Partial genomic map of the 5′ region, mid-region and presumed 3′ region of the porcine heavy chain locus. Large rectangular areas surrounded by dotted boundaries (V_H and 3′ C_H regions) are regions of the locus that have not been mapped although the V_H genes listed in Table 2 and the C_H genes listed in Table 1 are known to be present in the genome. The data are a compilation of published data [6,8,9]. D_HC has been found in a few transcripts but has not been mapped. Solid line denotes sequenced introns while dashed lines indicated non-sequenced introns. The secreted exons of IgM and IgD are designated Cμs and Cδs, respectively.

designated V_HA, V_HB, V_HC and V_HE (Table 2) account for ≃80% of total V_H gene usage in the pre-immune repertoire [14–17; Fig. 2C] with three additional genes accounting for most of the remainder [Butler, J.E., Lemke, C.A., Weber, P.A., & Wertz, N, unpublished]. The recovery of several V_H-containing cosmid clones permitted V_HB to be identified as the most 3′ functional V_H gene and to be designated V_H2 on the basis of its position in the locus (Fig. 1). V_H2 lies upstream of a V_H pseudogene that we designated V_H1 on the basis of its location (Fig. 1). In the proposed IMGT nomenclature, their encoding genes would be designated IGHV3-1, IGHV3-2, etc. Until the porcine V_H locus has been mapped, we will continue to refer to them as V_HA, V_HB, etc. (Table 2). The other heavily used porcine V_H genes have been identified in a cosmid clone that encompasses V_H2 and the region immediately upstream although they have not been mapped (Fig. 1). The use of BAC clones has also allowed certain 'hybrid V_H genes' (see below) to be identified as authentic germline genes (Butler et al., unpublished).

Initial Southern blot studies suggested ≃20 porcine V_H genes in the genome [19] although 31 different sequences have been reported (Table 2). Most have unique CDR1 and CDR2 regions or combinations that are unique. For example, V_HE has the same CDR1 sequence that is found in V_HI, V_HL and V_HT while V_HJ and V_HT have a CDR2 region that is the same as V_HF (Table 2). Table 2 provides other examples in which germline V_H appears to be evolutionary hybrids of other V_H genes. It is noteworthy that 12 of 31 sequences submitted to GenBank by Kim et al. (Table 2) are truncated transcripts, some of which could be somatic mutants or gene conversion-like products of germline V_H genes. The 31 different sequences reported may also include allelic variants so the original estimate of V_H genes in this species (≃20) [19] may eventually prove to be accurate. Sequences recovered by PCR cloning

of highly homologous genes, like the members of the porcine V_H3 family, can also be PCR artifacts. This possibility was demonstrated by mixing various plasmids containing porcine V_H genes in different ratio and then amplifying the V_H genes from the mixture. Using a one to one mixture of target VDJ rearrangements, up to 40% hybrids of the two could be generated [20]. Therefore, some V_H genes listed in Table 2 that were recovered by PCR, especially from cDNA, could be PCR hybrids. Until the porcine V_H locus has been mapped, the exact number of correct sequences for each gene or its allele, remains speculative.

Our initial report that swine have $\simeq$20 V_H genes in their repertoire [19], was novel at the time since it indicated a much smaller number of V_H genes than had been previously reported in other mammals. While this in part resulted from the original overestimation of V_H genes in mice and human (300–1000) [22], reports of only 10–20 V_H genes in cattle and sheep soon appeared [23–25]. Near to the same time, the number of mouse and human V_H genes was revised down to $<$100 [26]. As described above, the V_H genes that have so far been recovered from swine all belong to the V_H3 family, whereas most expressed V_H genes in sheep and cattle are of the V_H4 family even though these mammals belong to the same order as swine.

Rabbits and the chicken [2,27] utilize gene conversion (templated mutation) of their most 3′ V_H gene to generate their repertoire and their V_H genes belong to the same ancestral V_H3 family as in swine. However, gene conversion has not been convincingly demonstrated in swine since hybrids generated by PCR cloning render ambiguous most evidence for this mechanism [20; see above]. The limited number of V_H genes used by swine in forming their pre-immune repertoire and their near exclusive use of only two D_H segments [14,15] and a single J_H [6] substantially reduces combinatorial diversity. Thus, junctional diversity plays a proportionally greater role than in humans and mice [15]. Similar restrictions in D_H or J_H usage in other artiodactyls is discussed by other contributors to this volume. Hence, the mechanisms used by rabbits, chickens and artiodactyls in repertoire development is quite different from that in the human and mice in which V_H genes are encoded by 7 and 14 families, respectively, and gene conversion is seldom used [26]. These observations attest to the diversity among mammals in their genomic V_H repertoires and in the relative importance of the different mechanisms used to develop B cell repertoires in different species.

1.3. The light chain repertoire of swine

LeRoy Hood and colleagues reported 45 years ago that the ratio of kappa (κ) to lambda (λ) light chain usage differed greatly among higher vertebrates based on N- and C-terminal sequence analyses [28,29]. The N-termini of most lambda chains ends in the ring structure pyrrolidone carboxylic acid because of the loss of ammonia and water from glutamine or glutamic acid [30]. Kappa chains have a free α amino group at the N-terminus. Moreover, the λ-chain C terminal residues are usually G-C-S, while the κ C-terminal sequence is C-G. Using these criteria, Hood et al. [29] showed that swine like humans, display nearly equal usage of these two light chain types, whereas 95% of the light chains used by mice are kappa and cattle, sheep and horses use $>$90% lambda chains. This pioneering study has been confirmed for swine both by flow cytometry [FCM; 31] and by relative transcript expression in secondary lymphoid tissue [32]. In mice, preferential kappa usage is correlated with the number of available V_κ genes versus V_λ genes while the reciprocal seems true for sheep [26, 33]. Therefore, one might expect both humans and swine to have an equal germline potential for V_κ and V_λ. Humans have at least 76 V_κ genes in their genome and 70 V_λ genes [26]. To determine if this could explain light chain usage in swine, we undertook characterization of the porcine kappa and lambda germline and expressed repertoire. This was done by: (a) sequencing $>$100 $V_\kappa J_\kappa$ and $V_\lambda J_\lambda$ rearrangements (obtained mostly by 5′ RACE), (b) estimating the number of V_κ and V_λ genes from genomic Southern blots and sequencing BAC clones and (c) limited mapping of the kappa and lambda loci. These studies identified two families of porcine V_κ genes that shared 87% sequence similarity to human IGVK1 and IGVK2 [32,34]. Interestingly, 95% expressed V_κ rearrangements from fetal and newborn piglets used V_κ genes belonging to IGVK2 although these were confined to three of the five IGKV2 subfamilies found in the genome [32,34;

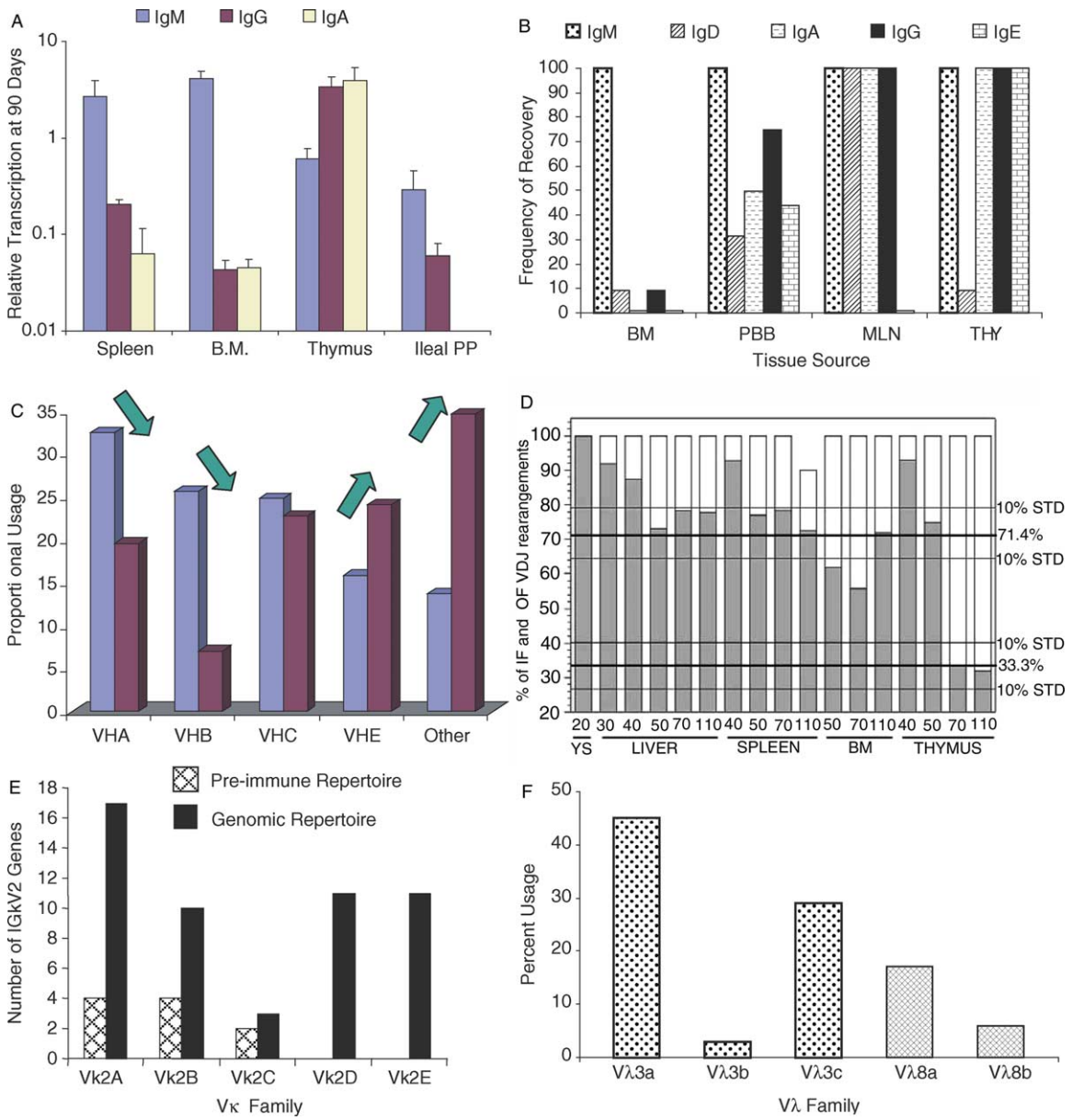

Fig. 2. Development of the porcine pre-immune repertoire. (A) The relative transcription of IgM, IgG and IgA in various fetal lymphoid tissues at 90 days of gestation (gestation is 114 days). Note that the *Y*-axis is a log scale. BM, bone marrow. From Butler et al. [13]. (B) The frequency of recovery of various heavy chain isotypes in 351 clones from primary and secondary lymphoid tissues of newborn piglets. BM, bone marrow; PBB, peripheral blood B cells, MLN, mesenteric lymph node; THY, thymus. From McAleer et al. [17]. (C) V_H usage in 251 clones of the pre-immune IgM repertoire (light bars) and 295 clones from the repertoire of switched isotypes after colonization (dark bars). From McAleer et al. (2004). Arrows indicate trend lines that are significant at the $p = 0.00001$ level. (D) Proportion of B cells with in-frame (IF) rearrangements in various tissues during fetal development. Values on *X*-axis indicate fetal age in days. YS, yolk sac. From Sinkora et al. [68]. The upper horizontal line (71.4%) indicates the proportion of VDJ rearrangement that should be IF, if rearrangement is a random event. STD, standard deviation. The lower horizontal line (33%) is the expected value for IF rearrangements if no selection for B cells with IF rearrangements has taken place. (E) The number of IGKV2 gene used to form the pre-immune repertoire compared to the IGKV2 genomic potential. A-E are subfamilies of IGKV2. (F) Frequency of usage of IGLV3 and IGLV8 genes in the pre-immune repertoire. a, b, c, etc. indicate subfamilies of V_λ genes.

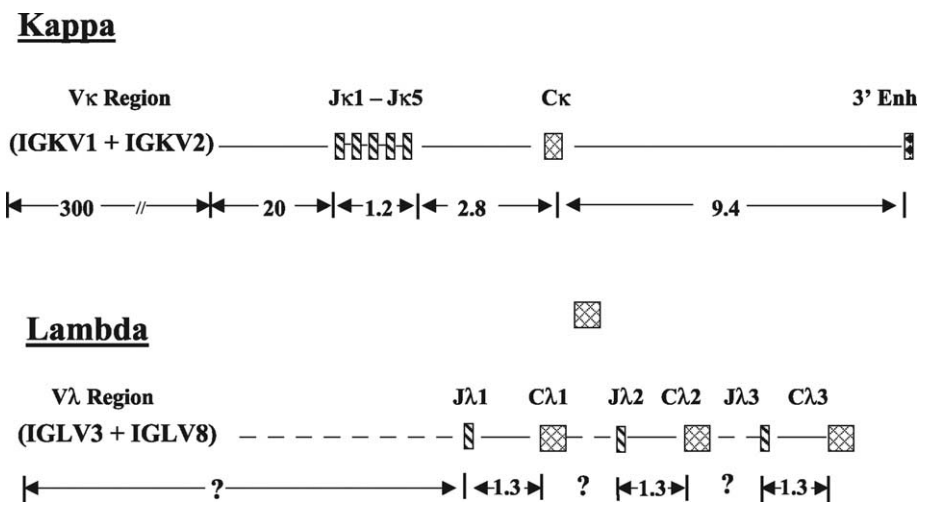

Fig. 3. Partial genomic map of the porcine kappa and lambda loci. More than 250 V_κ and 100 V_λ genes have been cloned and analyzed. The V_κ genes can be mapped to a 300 kb region of the locus. Current evidence indicates that V_κ genes belong to two families (IGKV1 and IGKV2) and that two V_λ gene families (IGLV3 and IGLV8) are used in the pre-immune repertoire (Fig. 2F). Organization of the J–C regions of the kappa and lambda loci are based on BAC clones and contigs amplified by PCR from genomic DNA.

Fig. 2E]. Southern genomic blots and subcloning of BAC clones indicated there are $\simeq$60 V_κ genes in the IGKV2 family [34] and perhaps nearly as many IGKV1 genes [32]. This is larger than the total number of V_κ genes reported for humans by Zachau [35] but closer to the number reported by Kawasaki et al. [36]. Studies on the porcine lambda repertoire are incomplete although sequences from the pre-immune repertoire suggest that two families sharing sequence similarity to human IGLV3 and IGLV8 are used (Butler, Wertz, Sun and Wells, personal communication; Figs. 2F and 3).

Our light chain studies identified six J_κ segments and mapped five of these in the genome (Fig. 3). These were ordered in the locus in the same manner as their apparent $J_\kappa1$–$J_\kappa5$ homologs in human, mouse, horse, sheep and rabbit [34]. This suggests that the J_κ region of the locus is highly conserved among mammals. Interestingly, $J_\kappa2$ was used in $\simeq$95% of all $V_\kappa J_\kappa$ rearrangements of the pre-immune repertoire. Current evidence indicates there is only one C_κ gene in the genome [34; Fig. 3]. Unpublished data on the porcine λ locus indicate that it appears to be organized in a manner similar to that in mice and humans with tandem J_λ–C_λ repeats (Fig. 3). The kappa locus is organized in translocon fashion as in mice and humans (Fig. 3).

2. The concentration and distribution of various antibody isotypes

The diversity of the antibody repertoire is dependent not only on the number of different variable region sequences that can be expressed but also on the number and quantity of different isotypes and subisotypes that express these different variable region sequences. While the former determines the repertoire of antibody specificities, the latter determines the repertoire of effector functions.

The genomic potential of higher vertebrates to express immunoglobulin isotypes and subisotypes is not proportional to the relative concentration of immunoglobulin isotypes in blood. The swine is no exception and Table 3 indicates that serum IgG levels are 10-fold higher than both IgM and IgA levels. Data on IgD and IgE serum levels in human and mice indicate these isotypes are normally present in only trace amounts [26]. Since there are currently no reagents available that are specific for porcine IgD and IgE, there are no data to confirm that the same is true in swine. In any case, it is unlikely that porcine isotype expression can be predicted simply from the swine germline potential.

Heavy chains are synthesized with either a transmembrane tailpiece (mIg) or encoded by a

Table 3
The concentration and relative concentration of porcine Igs in various body fluids

Concentration (mg/ml)[a]					Relative concentration[b]				
Blood									
Isotype	Fetal	Adult	Colostrum	Milk	Parotid saliva	BAL	Bile	Int. wash	Nasal
IgM	0.57	2.5	9.1	0.8	−	±	±	±	−
IgA	0.27	2.0	21.2	1.9	++++	++	++	++++	+++
IgG (total)[c]	3.65	24.0	95.6	0.3	+	+++	++	+	+
IgD[c]	?	?	?	?	?	?	?	?	?
IgE[c]	?	?	?	?	?	?	?	?	?

[a] Value for adults are summarized from Butler [125] without indication of variation among animals or time of collection. For information on variations and changes during gestation and lactation, see [123,124]. Values for adult animals are given in mg/ml, whereas those for 90-day fetal animals are given in μg/ml. The latter from Butler et al. [13].

[b] The 'plusses' correspond approximately to the percentage contribution to the total Ig. Data summarized from Morgan et al. [126], Frenyo and Butler, unpublished.

[c] Reagents are not available for determining individual IgG subclass concentrations or the concentration of IgD and IgE.

different 3′ exon (sIg) that permits their secretion [9, 39; Fig. 1]. The relative transcription of mIg and sIg neither parallels the genomic potential nor the relative occurrence of different isotype-secreting plasma cells. For example, swine IgM transcripts dominate in all fetal lymphoid tissues except thymus yet IgG levels in blood are 6–10-fold higher than those of IgM and most thymic B cells contain IgA (Fig. 2A and B; Table 3) [13]. IgM transcript dominance probably results from the predominant role of IgM as the initial BCR on naive B cells. Therefore, it is not surprising that IgM^+ cells comprise the vast majority of B cells during fetal life in most tissues and in blood (Fig. 2B).

Another apparent source of discrepancy between the genomic potential and the relative levels of different immunoglobulin isotypes in blood, is that secreted isotypes may be differentially distributed throughout the body. This is well known for IgA in most mammals and in humans; IgA levels are 10-fold higher than IgG concentrations in colostrum, whereas IgG exceeds IgA in blood 5-fold. In swine and in many mammals, 80–90% of all Ig in intestinal fluids, tears and parotid saliva is IgA (Table 3). However, total and relative Ig levels in a particular body fluid may vary with physiological condition. A good example is the lacteal secretions of swine in which IgA comprises only 14% of total Ig in colostrum but 80% of total Ig in mature milk (Table 3; Fig. 4B). During late gestation and in the first day postpartum, IgG is selectively transferred from blood across the alveolar epithelial cells of the mammary gland presumably utilizing the FcRn transport receptor. This difference reflects a change in the role of the mammary gland during the first day postpartum. The selective transport of IgG from maternal blood to lacteal secretions during late gestation results in a remarkable drop in serum IgG levels at this time (Fig. 4A). In the first 12–24 h after birth, the columnar enterocytes of the newborn piglet as well as those in the offspring of other Group III mammals [37,40,41; see Preface, this volume] indiscriminately absorb all colostral immunoglobulin intact so their cytoplasm is filled with IgG (Fig. 5A). Following contact with nutrients and proteins, these enterocytes are responsible for so-called 'gut closure' in which they no longer permit intact proteins to be absorbed into lymph and blood (Fig. 5B). This 'closure event' is physiologically timed to the cessation of the transport of IgG from maternal blood into clostrum/milk thus resulting in a dramatic drop in absolute (Table 3) and relative IgG levels in lacteal secretions (Fig. 4B). This is not surprisingly associated with a rebound in serum IgG levels in the mother (Fig. 4A). IgM levels also decline in maternal serum in late gestation (Fig. 4A) suggesting that most IgM in colostrum may also be serum-derived. Consistent with studies showing that IgM is less well transported via the poly-Ig receptor than is dimeric IgA due to diffusion rate differences [42], we found little evidence for transport of IgM from blood to milk in cattle [43]. Nevertheless, enterocytes transporting IgM in the gut can be seen (Fig. 5D). Thus, uncertainty exists regarding the

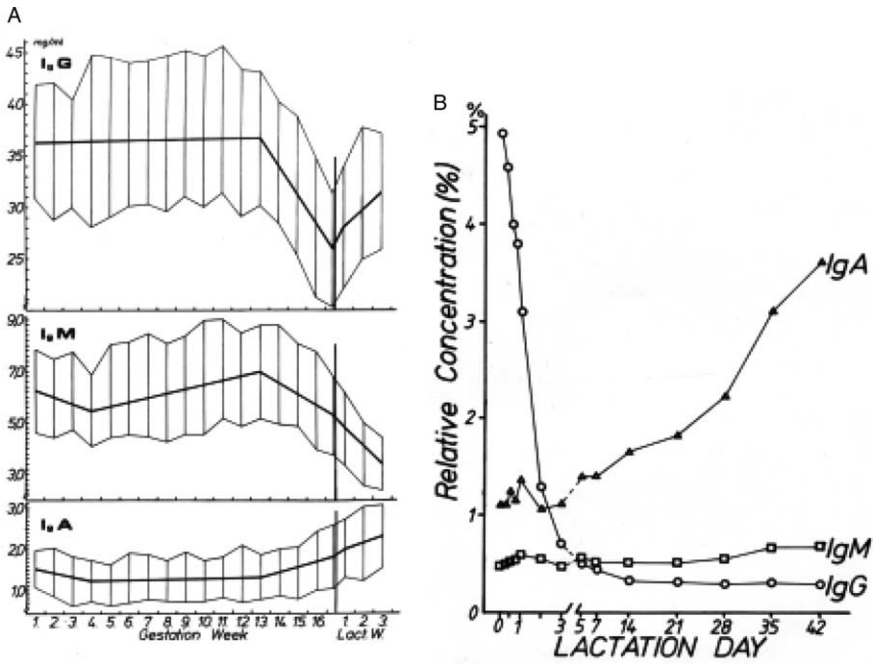

Fig. 4. Changes in immunoglobulin levels during reproduction. (A) Changes in serum IgG, IgM and IgA levels in >1000 sows during the reproductive cycle. Heavy solid lines give mean values with the range indicated. From Klobasa et al. [123]. (B) Changes in the relative levels of IgG, IgA and IgM in lacteal secretions during the first 42 days after parturition in 80 German Landrace sows. Concentrations are normalized to albumin levels to correct for transudation and dilution [124].

molecular mechanism of transport of serum IgM into lacteal and perhaps other secretions.

IgA synthetic activity immediately increases in the mammary gland postpartum and elsewhere in the mother resulting in elevated postpartum serum IgA concentrations (Fig. 4A). This may be a consequence of the gut-mammary gland axis [37,41,44,45]. The 8-fold higher relative level of IgA in mature porcine milk is generally believed to result from local synthesis [46]. Our studies in mice and cattle are consistent with this concept since we found little evidence for the transport of IgA from blood to milk [47,48]. In any case, the lamina propria of conventional newborn piglets soon becomes enriched in IgA-containing cells and the crypt epithelia becomes very active into the transport of IgA into the gut lumen (Fig. 5C). Conceptually, this entire process indicates that IgG transfers the *systemic immunological experience* of the mother to the offspring while IgA transfers the *local immunological experience* (e.g. mucosal) immunity of the mother to her offspring [Fig. 4B; 37,41,49].

Fig. 4B demonstrates a dramatic example of the unequal physiological distribution of immunoglobulin isotypes. Table 3 shows that such unequal distribution is not unique to lacteal secretions but is a common theme at various local sites so that each body fluid is characterized by a particular distribution of Ig isotypes. Missing from the important data on physiological distribution of swine Ig isotypes are data on the distribution of IgG subclasses. Major differences are well documented in other species, especially cattle [40,49,50]. Since subclass-specific reagents are not available for swine, differential subclass distribution remains unknown. When such information becomes available it is likely that hypotheses regarding subclass function can be more properly tested. It will be interesting to know if a single IgG like IgG1 in ruminants, dominates the transfer of passive IgG antibodies to the suckling piglet. While it is generally believed that IgE and IgD represent a minor proportion of secreted immunoglobulin in blood or any body fluid of mammals, the unavailability of reagents prevents this to be empirically tested in swine.

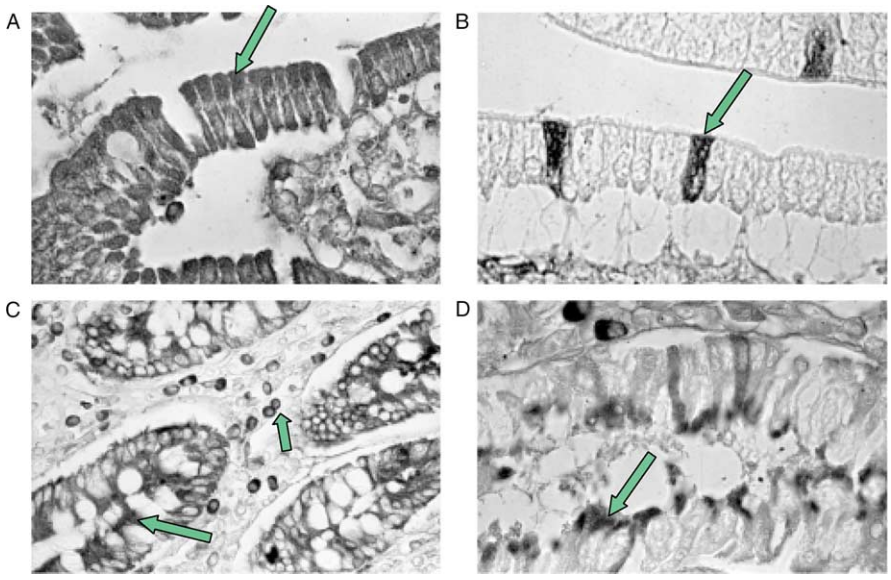

Fig. 5. Immunoglobulin absorption, synthesis and transport in the gut of newborn and young pigs. (A) Uptake and transport of maternal IgG by enterocytes of the gut of the newborn piglet (arrow). The dark stain within the cytoplasm of enterocytes identifies IgG detected using HRP-conjugated anti-IgG followed by the use of bis-diazobenzidine to produce an insoluble cleavage product. (B) Near absence of enterocytes containing IgG 24 h postpartum determined using the same technology as in A (arrow). (C) Synthesis (arrow) and epithelial transport (arrow) of IgA by crypt epithelial cells in the gut of 5 week old conventional piglets. (D) Detection of a few IgM producing cells in the lamina propria of conventional 4-week-old piglets and evidence for IgM transport into the gut lumen by crypt epithelial cells (arrow).

Fig. 4B shows that the relative concentration of IgG and IgM in lacteal secretion after five days (generally recognized as the transition point from colostrum to milk) is still 0.5 [52]. This might indicate either some continued low-level transport from blood or that these Igs are also synthesized at low levels within the gland. Either of these possibilities could also account for the IgG and IgM in other body fluids (Table 3).

An interesting feature of the porcine mammary gland that should be of interest to mucosal immunologists, is the observation that while the 'free secretory component (SC)' of the poly-Ig receptor is abundant in both human and bovine colostrum and whey [38,40,50], it appears virtually absent from the lacteal secretions of swine. The excess SC in cattle suggests that the much lower transport of dimeric IgA into bovine colostrum is not due to an apparent SC deficiency. Rather, since the promoter for the poly-Ig receptor is especially sensitive to INFγ, the well-known periodic inflammation of the bovine gland (mastitis) may explain the abundance of free SC.

Swine serum IgA levels are much lower than in humans [37,40,41] but similar to those in mice and most other mammals. This discrepancy with humans is a consequence of the fact that $>80\%$ of serum IgA in humans is monomeric and is derived from the bone marrow [53]. In non-primates, serum IgA is mostly polymeric and in swine, one-third is derived from the intestinal mucosa [54]. Thus, the relatively low concentration of serum IgA in swine compared to humans, is the rule rather than the exception for non-primate mammals [41].

The relative concentration of the various Ig isotypes in exocrine body fluids is typical for many mammals such as humans. IgG typically predominants in lower respiratory fluids such as the BAW from the lung while IgA predominates in bronchial fluids, tears and parotid saliva as in humans [Table 3; 56]. Whole saliva is typically a mixture of IgA and IgG that enters via transudation from blood or is synthesized by plasma cells in the salivary glands. Whole saliva contains equal amounts of IgG and IgA with low levels of IgM whereas parotid saliva is $>85\%$ IgA and IgM is typically absent [55,56].

In species with multiple IgG subclasses, distribution is not uniform. As indicated above, bovine IgG1

predominates in most exocrine body fluids [49,57,50,40]. Since subclass-specific antibodies of defined specificity are currently unavailable for swine IgG, it is not known whether the various subclasses are equally distributed in blood and in the various exocrine body fluids. Knowing the distribution of IgG subclasses might provide insight into subclass-specific IgG transport and how subclass-specific antibodies may be involved in Th1 and Th2 immune responses since these T cell mediated events are often correlated with the expression of certain IgG subclasses in mice [58,59], cattle [60] and swine [61,62].

3. B cell lymphogenesis and the pre-immune repertoire

3.1. Organization of the major B cell lymphoid tissue in swine

The organs and tissues involved in B cell development in swine differ only slightly from those described for mice and humans, so with the exception of lymphocyte recirculation pathways and two particular lymphoid organs, the general pattern approximates that of mice and humans. First, it is important to realize that swine have so-called 'inverted lymph nodes', resulting in relatively little recirculation of lymphocytes in lymph, so lymphocyte levels are 5-fold higher in blood than in mice and humans [63]. The relevance of this to antibody repertoire development has not been examined and will not be further discussed. The two exceptional organs involved in B cell lymphogenesis in swine are the ileal Peyers patches (IPP) and the thymus (Fig. 6).

3.2. B cell development in swine: variations on the theme from mouse/human immunology

The initial sites of early B cell lymphogenesis in the fetal piglet are the same as those in well-studied mammals and the chicken [64,26]. For example, the YS and fetal liver are early sites of B cell lymphogenesis in both mice and swine before the

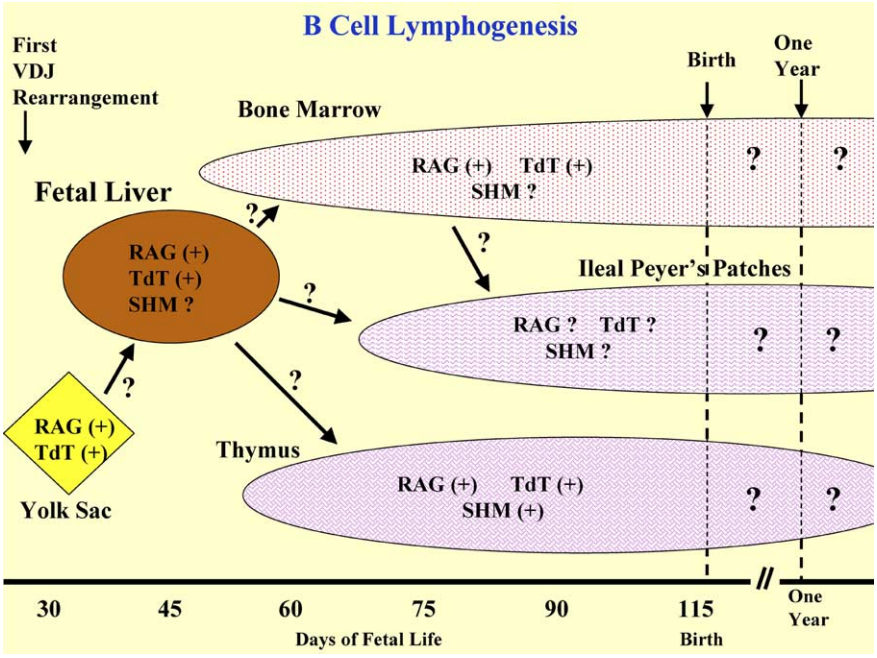

Fig. 6. Current model of B cell lymphogenesis in piglets. RAG, recombinase activation genes; Tdt, terminal deoxynucleotide transferase; SHM, somatic hypermutation. Arrows with question marks indicate possible trafficking pathways for developing B cells. The diversification mechanisms in certain tissues as well as the extent and duration of B cell lymphogenesis after birth, have not been quantified which explains the question marks.

BM becomes the apparent major site of B cell lymphogenesis (Fig. 6). In mice, pro-B cells developing in the YS/fetal liver may be under slightly different control and/or selection then those developing later as indicated by their lower dependence on IL-7. Perhaps, this is somehow related to our observation on the higher proportion of B cells in YS/fetal liver with in-frame rearrangements than those from BM (Fig. 2D; see below). Our data suggest that λ5 might also be expressed on early large pre-B cells while on mature porcine B cells, there is equal expression of κ- and λ-in a pattern similar to humans [31]. Relative usage of κ- and λ-in mice and ruminant artiodactyls seems to be correlated with the size of the V_κ and V_λ repertoire. This is opposite the situation in horse in which kappa is under-expressed relative to the size of the V_κ genome [66,67]. Data from swine suggest there are $\simeq$60 V_κ IGKV2 genes and probably 20–30 IGKV1 genes [32,34], whereas the number of V_λ genes is still unknown but seems smaller (Fig. 2F; Butler, Wertz, Wells, unpublished). This suggests that the type of light chain expressed, especially in the pre-immune repertoire of swine and perhaps other species, is a regulatory phenomenon not related to genomic availability. This is consistent with the preferential usage of $<$20% of the available IGKV2 genes in the pre-immune repertoire of swine [Fig. 2E; 32,34].

The earliest rearrangements of VDJ in the swine heavy chain loci can be found on day of gestation (DG) 20 (i.e. the first hexamester of the 114 day gestation of the piglet) in the yolk sac (YS; Figs. 2D and 6) [68]. At DG30, B cell lymphogenesis also occurs in the fetal liver although like YS, heavy chain transcripts are difficult to find. Not surprisingly, the early repertoire in YS and fetal liver is highly oligoclonal [68] and of special interest is the ratio of in-frame (IF) to out-of-frame (OF) rearrangements (Fig. 2D; see below). In both of these early sites of B cell lymphogenesis, 90–100% of all rearrangements are IF. However, as the bone marrow becomes active at DG45 (Fig. 2D; 6), the ratio of IF:OF rearrangement changes so that approximately 71% of all rearrangements are IF (Fig. 2D). Seventy-one percent is the proportion expected if the process of VDJ rearrangement is entirely random. When individual mature B cells arising from BM are sampled, many show two rearrangements, only one of which is in-frame (Sinkora and Butler, unpublished). This raises the question as to why the pattern seen in YS and fetal liver is different from that in BM. Is the process not random or is there something about the sampling procedure and analyses that results in this unexpected ratio? One hypothesis is that the rate of VDJ rearrangement in the fetal liver is slower than in BM so that cells initially producing OF rearrangements on the first allele receive an apoptotic signal before a productive rearrangement is generated from the second allele. The hypothesized faster rate of B cell lymphogenesis in BM might allow BM cells that were unsuccessful in their first attempt to be rescued from apoptosis by virtue of a second successful productive rearrangement on the other chromosome. Alternatively, germline transcriptional activation may act on a single chromosome in YS and FL as compared to BM. It is unlikely that the predominance of B cells with a single productive rearrangement could result from some type of secondary receptor revision. Receptor editing is primarily a feature of light chains [69] although genomic organization permits it to occur in TCRα and TCRβ in thymus and even in some peripheral T-cells [70]. Although the organization of V_H and D_H segments is not resolved, conventional receptor editing in a species with a single J_H, would have to be by a separate mechanism.

B cell development in mice, humans and apparently also rabbit [71], involves the expression of a pre-BCR that contains a surrogate light chain composed of VpreB and lambda 5 (λ5). Assemblage of this pre-BCR seems to be a pre-requisite for further B cell development [72,73]. λ5 shares sequence similarities with authentic λ-chains and some anti-λ chain mAb also recognize λ5 (Rolink, pers. comm.). The pre-B cell stage in mice and humans is followed by the appearance of B cells with a mature BCR containing a kappa chain so that newly formed B cells display a μ–κ BCR. We also observed preferential expression of λ-transcripts in fetal liver and bone marrow [32]. However, the competitive PCR assay we used did not distinguish λ5 from authentic λ. The same technology comparatively showed that secondary lymphoid tissues (e.g. thymus and IPP) express equal amounts of λ and κ transcripts [32]. When fetal bone marrow cells were studied by flow cytometry (FCM) and identified using anti-Igα (CD79α), the large pre-B population stained with anti-λ but not anti-κ

(J. Sinkora, unpublished). This large pre-B population also stained weakly with anti-porcine VpreB prepared in our laboratory.

Studies in mice recognize several different B cell subsets. B-1 and B-2 subsets are generally defined based on the expression (B-1) or lack of expression (B-2) of CD5, a marker common to all T-cells. Additional markers now distinguish these subpopulations (see below). B-1 cells were believed to arise early in ontogeny and especially populate the peritoneum where they are believed to account for the broad, cross-reactive natural antibody repertoire that often typifies the response to TI-2 antigens like bacterial polysaccharides [74,75]. These correspond to the Category I antibodies in the conceptual scheme of Cohen [76]. The controversial B1/B2 concept [75,77] is also related to the concept of transitional B cell subsets which could also explain phenotypic differences [78–79]. B-1 cells are characterized by IgD^{low} CD5(+), whereas B-2 cell expressed IgD^{high}, CD21 and CD23, although some consider true B1 cells those that express kinase Lck [80]. The transitional concept (T-1 and T-2) recognizes the gain in expression of IgD, CD21 and CD23 as part of an orderly transition to immunocompetent B cells [81]. In swine, all B cells are weakly CD5(+) and although no mAb exists to recognize porcine IgD, the transcript for this Ig does not appear until B cells reach secondary lymphoid tissues (Fig. 2B) [17]. There is also a pronounced increase in the proportion of porcine B cells expressing CD21 after birth (M. Sinkora, pers. comm.). The latter phenomenon has also been reported for sheep [18]. Therefore, although porcine B cells cannot be separated into subsets based on CD5 or IgD expression, they do appear to follow a transition in development that involves changes in phenotype and transcript expression that is somewhat similar to that described for mice. It is of some interest that chicken B cells diversifying in the bursa of Fabricius and those of rabbit from appendix can also show 100% IF rearrangements. Chickens, swine and rabbits exclusively use the V_H3 family for their repertoire. Perhaps, a careful analysis of the VDJ rearrangement status of mouse B1 cells, not merely their surface phenotype, should be undertaken.

Two lymphoid organs in swine represent a departure from the pattern seen in mice and humans on which current paradigms of B cell development are based. A lymphoid organ that is not found in mice and humans is the one composed of an extensive linear array of follicles found at the terminal end of the ileum, immediately adjacent to the ileal–caecal junction (Fig. 7A and B) [82,83]. This organ is called the ileal Peyers patches (IPP) and a similar collection of follicles is found in sheep [33,84]. Studies in sheep suggest that the IPP are a type of primary B cell organ [83–86] characterized by rapid lymphocyte turnover and increased apoptosis [85,88]. There are also distinct follicles in the jejunum and upper ileum (JPP; Fig. 7A) that are similar to those simply referred to as Peyers patches and are part of the mucosal immune system of all mammals. These appear functionally distinct since the IPP reach maximum size early in the postnatal period and thereafter proceed toward involution [84,89], whereas JPP expand in number and are active throughout life. Cells from individual follicles can be recovered by micromanipulation (Fig. 7B) and their VDJs recovered by PCR and cloned. Data obtained in this manner suggest that most follicles exhibit a spectratype indicating that the cells present are derived from one or few B cell clones (Fig. 7C). This is not dissimilar to follicles of the chicken bursa of Fabricius in which each is believed to be formed from 2–3 founder B cells [27]. Sequence analyses of cloned VDJs from individual follicles allow lineage studies to be done (Fig. 7D). These lineage studies further confirm that the B cells present are only slightly diversified descendents of one or two B cells that formed the follicle. We suppose that the IPP may be the site of the transition from T-1 to T-2 B cells [78]. The argument that favors this hypothesis is the absence of expression of IgD in BM cells or the near absence of IgD in the blood of newborn piglets versus its uniform appearance in secondary lymphoid tissue (Fig. 2B) [17]. It has been shown that mutations accumulate in IgD transcripts that presumably represent transitional cells [87,90]. We propose to test this hypothesis by sequence analyses of B cells in IPP follicles and by resection of the terminal ileum in newborn piglets.

A second feature of porcine B cell development that deviates from the mouse-primate model concerns the thymus. The porcine thymus in fetal and early neonatal life contains B cell in three stages of development. The medulla contains numerous plasma cells that can be detected by immunohistochemistry or ELISPOT [13,

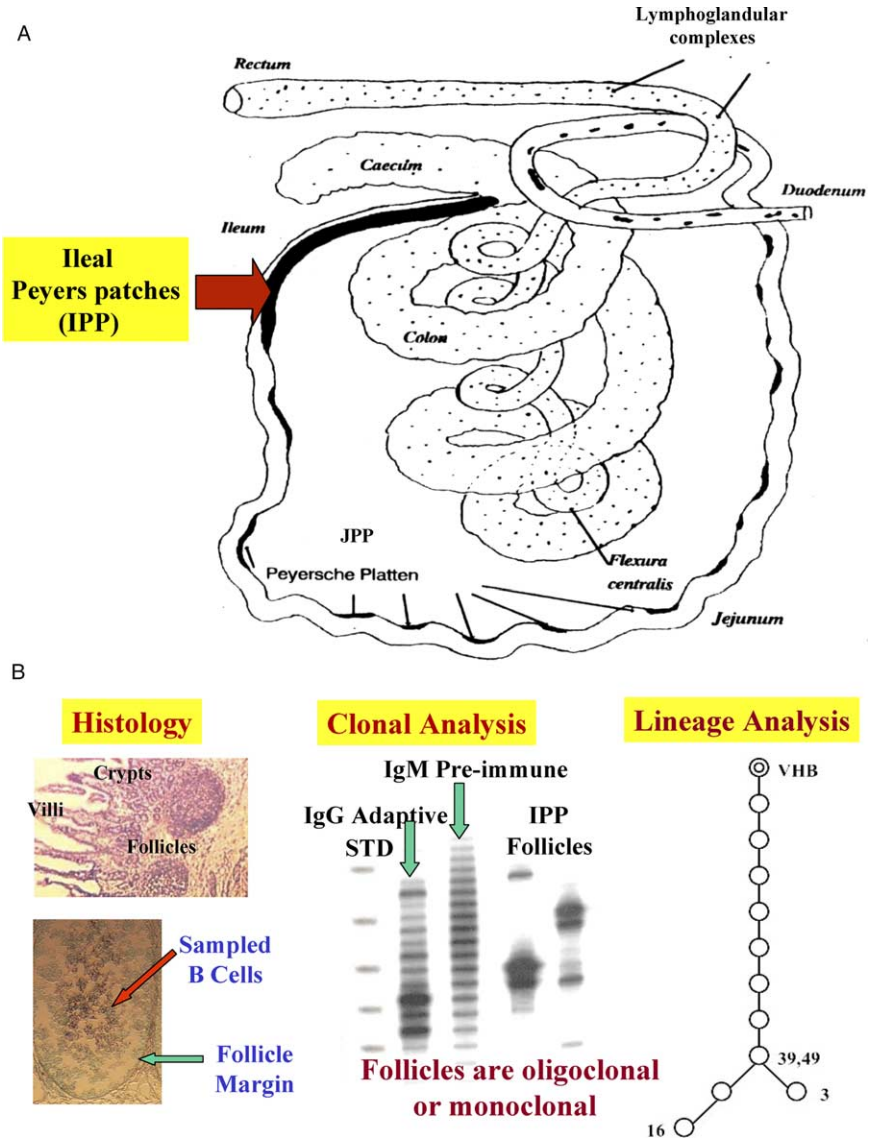

Fig. 7. (A) The lymphoid anatomy of the porcine gastrointestinal tract. Some terms are expressed in German. From Uhr [109]. Attention is focused on the ileal Peyers patches which are described in greater detail in Part B. (B) Histology of the IPP (top) showing B cells within a follicle (bottom) that can be collected singly or as a group by micromanipulation. (C) Spectratypic analyses of the rearranged VDJ from two IPP follicles (two right-hand lanes) compared to the unselected spectratype of the IgM pre-immune repertoire from blood (middle lane) or that of the selected IgG repertoire from the MLN following an adaptive response (Left lane). STD, length standards for CDR3. (D) Lineage analyses of B cells recovered from a single follicle in which all clones use V_HB (see Table 2 and Fig. 1). Numbers denote V_HB clones.

91] as well as surface Ig(+) B cells (M. Sinkora, unpublished data). Individual plasma cells from the medulla can be sampled by micromanipulation and about half contain both productive and non-productive VDJ rearrangements. Among these cells, switched isotypes predominate (Fig. 2A and B) [13,17,91]. Noteworthy is that IgD transcripts are rare in thymus, occur in 30% of PBBs, whereas they are present in all samples from the mesenteric lymph node (MLN; Fig. 2B) [17]. However, if total DNA is collected from

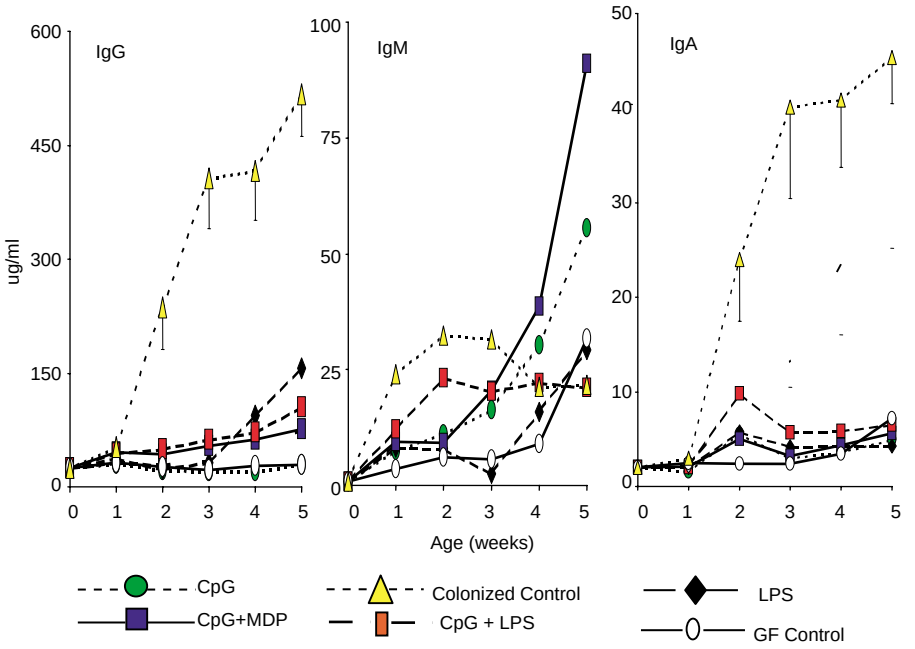

Fig. 8. The effect of colonization and selected PAMPS on serum Ig levels in isolator piglets. Noteworthy is that PAMPS given to GF animals have comparatively little effect on IgG levels whereas bacterial DNA (CpG) alone or co-administered with LPS or MDP, significantly raises IgM levels on weeks 4 and 5. All treatments cause a 'primary increase' in IgM and IgA on week No. 2. From Butler et al. [107].

thymus and the VDJ spectratype examined, it is dominated by rearrangements characteristic of a non-selected spectratypic pattern [15]. Specifically, only one-third of all rearrangements are IF (Fig. 2D). These constitute the third developmental stage of thymic B cells. This 'pro-B like cell population' (they express no sIg and make no VDJ transcripts) is responsible for this dominant spectratypic pattern when whole thymus is examined. These cells reside under the thymic capsule between thymic lobules (M. Sinkora & J.E. Butler, unpublished). It remains unknown whether the medullary B cells originate from the pro-B-like cells beneath the thymic capsule or are immigrants.

Recent studies on postpartal repertoire diversification indicate the thymic repertoire is diversified already at birth and that colonization of the GI tract does not affect clonal selection, repertoire diversification or cell numbers (Fig. 8B) [17,92]. Thus, thymic B cells represent an autonomous population that is perhaps formed in the thymus. The apparent lack of selection for pro-B cells in thymus that have productively rearranged their VDJ (Fig. 2D), suggests that the machinery for selection and for the promotion of cells with productive rearrangements is lacking in this organ. Thus the porcine thymus may be a 'dead-end' organ for B cell development and may be an organ isolated from further environmental influences. It is possible that B cell development in the thymus is a developmental pathway gone wrong, perhaps because of a delay in Notch I expression [93]. Alternatively, B cells in thymus may be part of a process for negative selection of medullary T cells [94].

3.3. Development of the porcine pre-immune repertoire

We define the pre-immune repertoire[1] as the repertoire that forms in fetal life in the absence of environmental factors, i.e. before the appearance of

[1] There may be some disagreement about the meaning of pre-immune repertoire. In swine, we consider it to be the result of VDJ rearrangement that appear in fetal life in the absence of environmental antigens or PAMPS. However, in rabbits, colonization of the appendix is thought to generate the pre-immune repertoire [102].

foreign antigens or commensal or pathogenic microbes that express PAMPs (pathogen-associated molecular patterns) [16,95,96]. The pre-immune repertoire as we have defined it, may develop in a regulated manner as a consequence of evolutionary selection for a repertoire that recognizes environmental antigens that threaten the host. Alternatively, it may be dictated by gene segment proximity; early rearrangements favor 3′ V segments and 5′ J segments. Arguments favoring the use of certain gene segments in early rearrangement based on their position in the locus have been made from studies in mice and humans [26,97–98] and in rabbits that initially and mostly use their most 3′ V_H gene [2,99]. In swine we know that V_HA and V_HB often comprise $\simeq$50% of early V_H usage (Fig. 2C) [14–17] although $>$20 porcine V_H genes have been described (Table 2) [19]. Both V_HA and V_HB are in the 3′ region of the V_H locus (Fig. 1). In mice, J_H1 often initially rearranges to the shortest and/or most 3′ D_H segment [100,101]. In swine D_HB is shorter than D_HA (Fig. 1) and is most frequently used in VDJ rearrangements before DG60 [15]. However, we do not know that D_HB is the most 3′ D_H segment since the region from D_HB to J_H has not been mapped. In any case, the molecular make-up of the V_H repertoire in swine is clearly distinct from development in mice and humans since the latter species use V_H gene segments from many families while swine use $\simeq$5 V_H gene from one family to form their pre-immune repertoire (Fig. 2C). While mice and humans rely heavily on combinatorial diversity, swine use $<$15 combinations which makes junctional diversity relatively more important for repertoire diversification in swine than in mice and humans [15].

The pre-immune V_κ repertoire of swine also uses certain selected IGKV2 genes (Fig. 2E) and one of five J_κ segments in $>$95% of the rearrangement [32, 34]. The lambda locus has not yet been mapped or the genomic V_λ repertoire determined (Fig. 3) but only two V_λ families (IGLV3 and IGLV8) are used to form the pre-immune repertoire (Fig. 2F). These findings indicate that V segment usage in the heavy and light chain loci is selective and conserved in the pre-immune repertoire. The pattern of V-gene usage may differ in older animals exposed to environmental influences since there are $\simeq$80 V_κ genes [32] and $\simeq$20 V_H genes (Table 2) available.

4. The role of environmental and maternal factors

4.1. The pre-immune repertoire diversifies following GI tract colonization

The porcine pre-immune B cell repertoire changes upon exposure of germfree (GF) piglets to colonizing bacterial gut flora. This causes $>$20-fold increase in serum IgG and IgA levels and $>$6-fold increase in IgM (Fig. 8). Relative to adult swine, serum IgA levels are most affected [95]. Colonization is also associated with diagnostic changes in V_H usage in which usage of V_HA and V_HB decreases and V_HE and "other" usage increase (Fig. 2C) [17]. The highly significant predictability of these changes ($p=$ 0.00001) allowed the construction of a Repertoire Diversification Index (RDI). Using this index, colonization-dependent increases in the RDI for IgA and IgG transcripts in MLN and blood are significant but the RDI for IgM is not. Interestingly, the RDI for IgG and IgA in thymus is higher at birth than in the MLN after colonized piglets but in thymus decreases after colonization [17]. Perhaps in late gestation, the thymus ceases to function as a 'secondary B cell organ' and the diversified residents represent long-lived plasma cells that reflect much earlier events.

4.2. Colonization is required for immunoresponsiveness to TD and TI-2 antigens

Newborn piglets maintained GF in isolators are unable to mount antibody responses to TD or TI-2 antigen but primary IgG and IgM responses to TD and TI-2 antigens are observed in colonized, isolator piglets [96]. Primary IgM responses favor the TI-2 epitope (TNP) and its analog DNP whereas IgG antibodies equally recognize the TI-2 and TD epitopes (Fig. 9A). Secondary responses are only seen for the TD antigen and both IgM and IgG favor the TD epitope (Fig. 9B). This observation fits the paradigm of responsiveness to TD and TI-2 antigens described from studies in rodents [103].

These findings raised the question as to what feature(s) of colonizing bacteria were responsible for promoting immunoresponsiveness. Candidates include bacterial antigens, cytokine released from colonized epithelial cells, stress protein induced by bacteria [104] and activation through PRRs. We

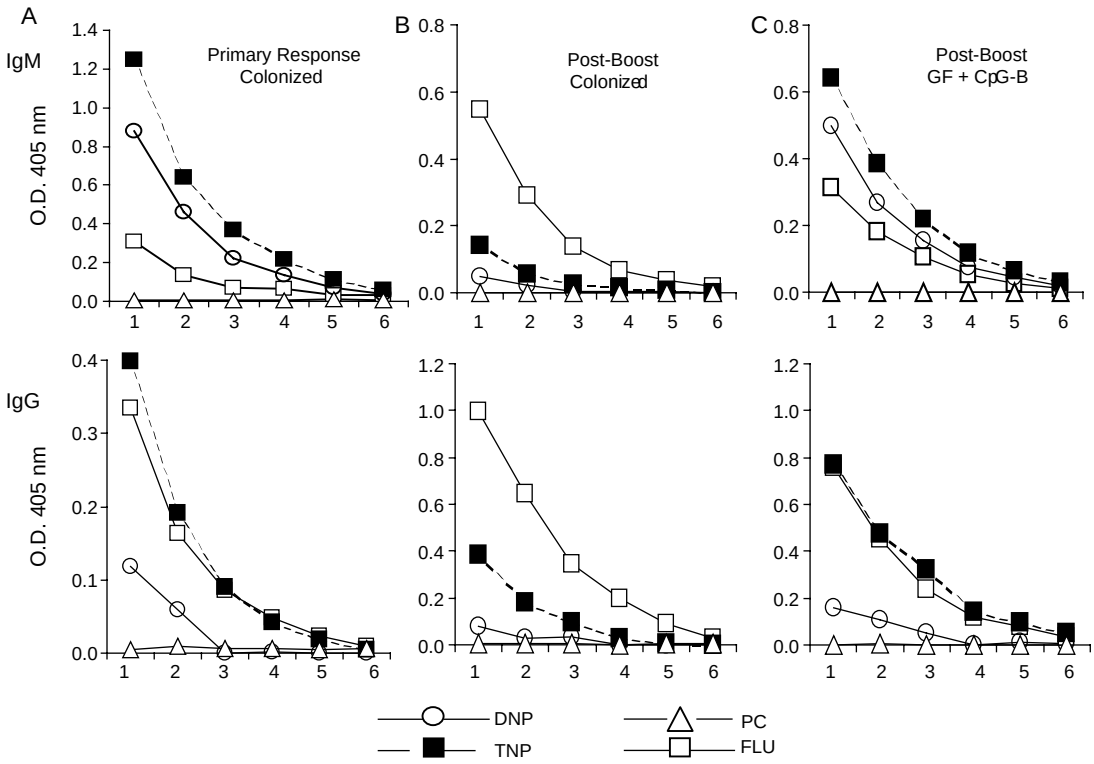

Fig. 9. The immune response of germfree and colonized isolator piglets and germfree piglets given TLR ligands. (A) The IgG response to the epitope (TNP) of the TI-2 immunogen (TNP-Ficoll) and the epitope (FLU) of the TD immunogen (FLU-KLH). Down arrows indicate times of immunization and challenge, respectively. Legend on the figure. Error bar denote SD. (B) Epitope specificity of the IgM and IgG responses after challenge in colonized piglets versus germfree animals given bacterial DNA in the form of CpG-B. Results shown for CpG-B are the same as with CpG-B + MDP and CpG-B + LPS. All animals were simultaneously immunized with TNP-Ficoll and FLU-KLH. Antigen legend is on the figure. PC, phosphorylcholine; DNP, dinitrophenyl.

directly tested the last possibility using defined TLR ligands given i.p. to germfree (GF) piglets. We choose CpG-B ODN (as bacterial DNA) and muramyl dipeptide (MDP; derived from peptidoglycan) because they are chemically synthesized and therefore not contaminated by LPS [105] and bacterial peptidoglycan [106]. No synthetic substitute was available for *E. coli* LPS. Interestingly, CpG-B ODN was the only TLR-ligand that could alone stimulate a response (Fig. 9A) and the IgM response strongly favored the TI-2 epitope and its DNP analog even after challenge (Fig. 9C) [107]. IgG responses (Fig. 9C, lower) resembled the primary response of colonized piglets (Fig. 9A). MDP and LPS when co-administered with CpG-B, acted synergistically to significantly increase serum IgM levels while having a minimal effect on IgG and IgA levels (Fig. 8). We therefore went on to show that CpG-B alone or together with MDP or LPS, promoted a very strong IgM response especially to the TI-2 epitope, but did not support a secondary response [107]. These findings suggest that CpG ODN is able to stimulate antigen-specific, naive B cells (mostly IgM + in swine, see Fig. 2A and B) that differentiate into immunoglobulin secreting cells. It is possible that stimulation through TLR9 (receptor for bacterial DNA) causes up-regulation of TLR2 (MDP) or TLR4 (LPS) that leads to expansion of antigen-specific B cells in the presence of MDP and LPS thus generating a pattern of immunoresponsiveness not unlike that seen after colonization.

4.3. The effect of colonization on changes in gut-associated lymphoid tissue (GALT)

We have shown that when piglets are colonized with a commensal exclusion flora, serum Ig levels increase 5–20-fold (Fig. 8). Colonization especially effects repertoire diversification in the ileum, MLN and preferential effect serum IgA levels [95]. As reviewed above, GALT in swine is found in the colon, IPP and JPP (Fig. 7A). Little is currently known about the colonic patches or lymphoglandular complexes in this region [108,109] but the JPP appear to be equivalent to those described in immunology textbooks as generic Peyers patches that serve as part of the mucosal immune system. It is known that the IPP and JPP differ in post natal development [111]. As we proposed in Section 3.2, the IPP may also have a separate role in antigen-independent repertoire diversification in the manner described for the corresponding lymphoid tissues in sheep [33,110]. Fig. 7B shows the location of the IPP follicles and how both individual B cells or all those in a single follicle may be sampled (Fig. 7B, left). As mentioned in Section 3.2, B cells recovered from individual follicles can be cloned and their VDJ sequenced to allow lineage diversification to be studied (Fig. 7B, right). Although the IPP may function in some type of antigen-independent B cell diversification, individual follicles in colonized piglet express all three major isotypes whereas GF animal express only IgM [96]. Weinstein et al. [112] have shown that the appendix of the rabbit changes from a quasi-primary lymphoid tissue to part of the mucosal immune system [2]. This could explain how the IPP initially functions as a site of antigen-independent B cell repertoire diversification as originally described for chicken and sheep [110,113–115] but later during neonatal development, becomes a secondary lymphoid tissue of the mucosal immune system like JPP.

Mucosal immunologists have shown that B cells stimulated in the JPP first migrate to the MLN so they should reflect environmental exposure that occurred in the gut mucosa [116]. This is consistent with studies by McAleer et al. [17] showing that colonization significantly promotes diversification of the IgA and IgG repertoires in the MLN (Fig. 2C).

5. Special topics

5.1. The piglet model of development

Perhaps, most noteworthy is the natural biological model offered by swine for studies on developmental immunity. Swine belong to the Group III category of mammals on the basis of their mode of maternal–fetal transfer of immunity [37,40,41]. Among this group, which also includes horses, sheep and cattle, there is believed to be no significant in utero transmission of maternal proteins such as immunoglobulins (see Preface). Thus the fetus develops in the absence of maternal regulatory factors so the development of its immune system is totally intrinsic. This is not the case in either Group I (humans, rabbits) or Group II (rodents, carnivores) mammals [40,41]. Thus, pregnancy can be terminated at any time in swine and the degree of development of the intrinsically regulated immune system studied in the 8–16 fetuses that comprise the normal litter. Since the offspring of Group III mammals are precosial, fetuses can also be aseptically recovered by Caesarian and placed into germfree isolators [117,118] or SPF autosows [119]. This allows the *direct effect* of environmental factors such as colonizing bacteria, pathogens and nutritional factors on the newborn's naive immune system to be studied. In addition, maternal factors in the form of milk and colostrum, can be added back to these isolator animals to determine their role in regulating development. The various piglet models for developmental immunology are illustrated in Fig. 10. Data obtained using this system to study the role of colonizing bacteria were presented in Figs. 8 and 9. The system has also been used to show how a virus interferes with the normal development of neonatal tolerance resulting in autoimmunity [120] and how maternal IgG can regulate development [121]. The resurgence of interest in how nutritional factors effect the immune system, especially that of the gut, has lead to the establishment of a dedicated division of the ARS in Beltsville and a renewal of interest in nutritional/neonatal immunology at the NIH. Because of the similar nutritional requirements of humans and pigs, the piglet models (Fig. 10) may be valuable in future research.

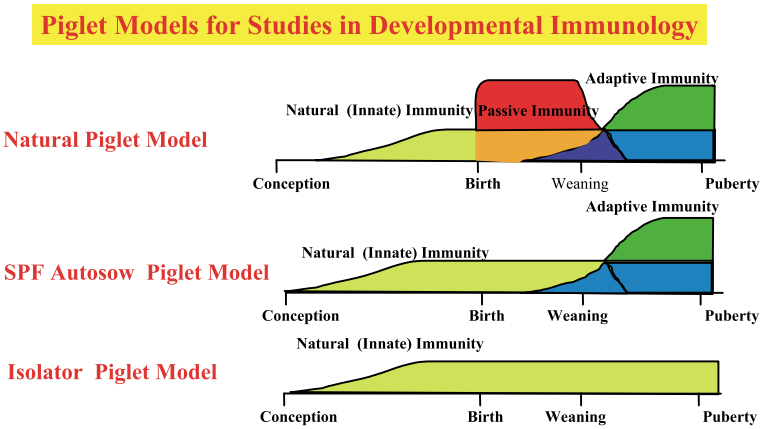

Fig. 10. Piglet models for studying development of adaptive immunity. The virtual absence of Ig transport to piglets in utero allows fetal development to be studied using conventional piglets without ambiguity imposed by passive immunity (Natural Piglet Model). The autosow system allows development of adaptive immunity to be studied after birth in naturally colonized piglets but in the absence of passive immunity (SPF Autosow Piglet Model). The Isolator Model (bottom) allows both the effect of passive immunity, colonizing microbes and PAMPS to be studied in a controlled environment.

5.2. The role of the thymus and the IPP in B cell and antibody repertoire development

The thymus is a special topic in studies of B cells and antibody repertoire development in this species because it has generally been regarded as a T cell, not a B cell organ. However, the features of the porcine thymus that were described in Section 3.2 suggest otherwise. Is it a case of the developmental pathway controlling T- and B-cell development, e.g. Notch I, E2-B, PAX, etc. gone wrong, or do the B cells in the porcine thymus have important roles that heretofore have not been described? Thus, swine may offer a model for understanding the role of thymic B cells.

The IPP of swine and other artiodactyls appears to play a role in B cell development although the details of this role have not been elucidated for swine. It is possible that the porcine IPP serves at least a dual role. Perhaps during late fetal and early neonatal life it serves as a site for antigen-independent repertoire development whereas later it becomes part of the mucosal immune system. The accessibility of the porcine IPP through surgery in living animals and for molecular examination of individual follicle in a species that offers the many possibilities described in Fig. 10, offers an excellent opportunity for developmental studies of this organ.

As our knowledge of comparative immunology broadens to include more and more species, it is likely that involvement of hindgut organs and the thymus in discussion of B cell biology will be revisited. The use of the swine model can therefore help to explain the role of the thymus and IPP in a large number of species in which similar phenomenon occur.

5.3. Phylogeny/taxonomy can be fickle

Information reviewed here indicates that phylogeny is a poor indicator of antibody gene organization and repertoire development. Although swine are artiodactyls, their light chain usage resembles humans not other artiodactyls or ungulates. Furthermore, the C regions of porcine immunoglobulins share a surprisingly high degree of sequence homology with those of humans. Yet, formation of the pre-immune heavy chain repertoire is very different and is characterized by a reduced role for combinatorial diversity and an increased role for junctional diversity. The expressed V_H and V_κ pre-immune repertoires are restricted to a small portion of the available V_H and V_κ genes in the genome and to far fewer genes than in humans. Surprisingly, this restriction is not shared by the porcine V_β repertoire which is similar to humans in terms of V_β families, sequence similarity and conservation of $3'$ V_β locus

(e.g. J_β, C_β) [122]. This suggests that even related genetic systems in the same species do not evolve in parallel. Furthermore, it continues to emphasize that no one organism, even if belonging to the same mammalian order, can serve as a model for others in the same order, not to mention those outside the order.

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