The swine Ig heavy chain locus has a single JH and no identifiable IgD

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Abstract

Sequence analysis of 84 V region cDNAs expressed with IgM, IgG and IgA from both adult and newborn swine suggested that their JH segments had been derived from the same germline JH. Only a single hybridizing JH segment could be identified in genomic DNA, in a JH-C\(^\gamma\) cosmid and in a Sad fragment of the cosmid extending 5 kb 5' of the Enh\(\text{H}\). The single germline JH segment mapped 6 kb 5' to C\(^\gamma\). This JH had a sequence identical to 40 of 42 JH segments expressed in a newborn piglet and 25 of 42 expressed by adult swine. None of the 19 JH segments which varied from the germline sequence were identical to each other and half of the nucleotide changes were silent. No cosmid DNA hybridizing with heterologous probes for C\(\delta\) could be found within 20 kb 3' of C\(\mu\) and C\(\delta\) could not be cloned from genomic or cDNA libraries. A conserved IgD fragment could be amplified from human, mouse and rat genomic DNA but not from rabbit, swine or cattle. We hypothesize that heavy chain organization and constituency in homeothermic vertebrates is correlated with the site of secondary antibody repertoire development and the mechanism(s) used.

Introduction

Emerging data in the last decade reveal considerable diversity among vertebrates in both Ig gene diversity and antibody repertoire development. With regard to the former, rodents and humans have five heavy chain classes, IgM, IgD, IgG, IgE and IgA, and each have four subclasses of IgG and one or two subclasses of IgA, while rabbits have a single IgG heavy chain gene but 13 genes for different IgA subclasses (1,2). Swine have at least six IgG subclasses (3), and a gene encoding IgD has only been described from rodents and primates. Diversity in the mechanism of repertoire development is exemplified by contrasting the pattern seen in rodents and primates with that reported for chickens, rabbits, swine and the domesticated Bovidae. Whereas the former group have a large number of V\(H\) genes belonging to seven to 10 families (4), the V\(H\) genes of each member of the latter group belong to a single, homogeneous V\(H\) gene family (5-7; A. Kaushik, pers. commun.). With the exception of the rabbit, this family is composed of <25 genes. Whereas rodents and primates can utilize four to six JH segments, only a single JH is available for repertoire development in the chicken (8). Since combinatorial joining of >100 V\(H\), 20-30 D\(H\) and four to six JH gene segments is a major mechanism of generating the antibody repertoire, as described in immunology textbooks, species with fewer V\(H\), D\(H\) or JH segments must: (i) generate a smaller repertoire, (ii) use alternative mechanisms or (iii) both. In addition to combinatorial joining and junctional diversity, somatic, non-templated (point) mutations also contribute to V region diversity.

Studies in chickens (9,10) indicate that templated mutations (somatic gene conversion) play a major role in repertoire development; this process also occurs in rabbits (11), swine (12) and cattle (13,14). Convincing evidence for gene conversion in rodents and primates has not been forthcoming. Additional diversity in chickens and cattle may also be generated by extensive size and length variability in CDR3 (14). Thus it appears that repertoire development varies with the species, suggesting the need to study different vertebrate species before allowing concepts to become institutionalized.

The high degree of sequence similarity (15) and MHC gene organization (16) between swine and humans, their physiological, digestive and nutritional similarities (17), and the recent interest in the use of swine in xenotransplantation (18,19) stimulated our interest in examining the Ig genes of swine. These studies have revealed that although swine and humans are not closely phylogenetically related, the C\(\gamma\) (3), C\(\mu\) (20), C\(\delta\) (21) and V\(H\)III (5) genes of swine are most similar to humans of all non-primate species studies to date (the author prefers the use of similarity rather than homology, since the latter assumes knowledge about evolutionary lineage and ignores convergent evolution). Therefore it seemed natural to
speculate that swine and humans would also show similarities in their Ig gene constitution and mechanism of repertoire diversification. Data reported here indicate that, surprisingly, the expected similarities between humans and swine extend neither to the level of JH diversity nor to the occurrence of IgD. Sequence analysis of V regions expressed with swine IgM, IgG and IgA suggested that all JH regions were derived from a single germline JH. Using a \(^{32}\)P\-DNA probe identical to the JH in newborn piglet cDNAs, as well as a probe prepared from overlapping oligonucleotides of this JH, only one hybridizing nucleotide was observed and a single cosmid containing JH and C\(\mu\) was cloned from a genomic cosmid library. The JH gene was located 6 kb upstream from C\(\mu\). A porcine homolog of human/mouse C\(\delta\) could not be cloned from this cosmid or from genomic and cDNA libraries.

Using conserved primers, a C\(\delta\) fragment could be amplified from rat, mouse and human DNA but not from swine, cattle or rabbit DNA. The data support the conclusion that swine have a single gene for JH but none for C\(\delta\).

**Methods**

**Cloning of cosmid and EMBO4 libraries and physical mapping**

A porcine genomic DNA (liver) cosmid library in pWE15 was purchased from Clontech (Palo Alto, CA) and cloned using a \(^{32}\)P-end labeled JH oligo (5'-tctctgccccagcagct-3'). Two genomic EMBO4 phage libraries were kindly prepared from porcine sperm DNAs by Drs M. Suter and K. L. Knight (Loyola University, Chicago, IL). The libraries were amplified to a titer of 3 x 10\(^6\) p.i.f.u./ml and used to infect CES200 cell. Cosmid and phage DNA were purified using Maxiprep and phage purification kits respectively (Promega, Madison, WI). Physical mapping was accomplished using the terminase partial cleavage method (22) as described in detail elsewhere (12,21).

**Genomic DNA subcloning**

The JH-C\(\mu\) cosmid was digested by SacI and a 5 kb fragment containing the JH segment (see Fig. 1) was ligated into a SacI digested pBluescript vector. The subcloned DNA was digested by restriction enzymes following the manufacturer’s recommendation and hybridized with two non-overlapping JH oligonucleotides as described below.

**Southern blot analysis of genomic DNA, cosmid DNA and a JH-containing subclone**

Porcine sperm DNA and cloned cosmid DNA were digested by restriction enzymes and electrophoresed in 1% agarose gel whereas the digested 5 kb SacI fragment was loaded into a 2.5% agarose gel. DNAs were blotted onto nylon membranes and immobilized by heating at 80 for 2 h. A \(^{32}\)P-labeled, 300 bp fragment containing the porcine JH segment and its 5' flanking region, was used in probing of genomic and cosmid DNA, while non-overlapping JH oligonucleotides (5'-tctctgccccagcagct-3' and 5'-tggagcagcagcact-3') were used for probing the 5 kb SacI subclone. The JH oligonucleotides were used sequentially as probes in order to increase the fidelity of hybridization. After overnight hybridization at 65°C with the 300 bp probe, the filter was washed once with a medium stringency solution (1.25 x SSC, 0.1% SDS) at 65°C and twice with a high stringency solution (1 x SSC, 0.1% SDS) at 65°C. The membrane was then dried and the blot exposed to X-ray film. After overnight hybridization at 53°C with the non-overlapping oligonucleotides, the filter was washed twice with low stringency solution (2 x SSC, 0.1% SDS) and twice with a medium stringency solution (1.25 x SSC, 0.1% SDS) at 37°C. The results of these hybridization experiments were recorded on X-ray films by autoradiography.

**cDNA libraries, cloning and sequencing**

The first strand cDNA was synthesized as described by Larrick and Fry (23). Double-stranded cDNA was generated by anchored PCR or regular PCR using leader and anti-sense C\(\mu\), C\(\gamma\) or C\(\alpha\) primers where appropriate (5,12). DNA fragments obtained by PCR were ligated directly into EcoR\(\scriptstyle V\) digested pBluescript vector. Sequencing was done using the fmol sequencing kit from Promega. All duplicate clones were discarded and data from 84 different V\(\gamma\)-regions recorded in Table 1.

**PCR amplification of IgD and IgE fragments**

Taq polymerase and buffer were purchased from Promega. In a 50 \(\mu\)l reaction mixture, 100 ng of genomic DNA was used as template and primer concentrations were 20 pmol. The primer sequences were: sense IgD (5'-GCCTCGAG-TGCTGTGTAGGA-3'), antisense IgD (5'-GCGAATTCTGCTGTGTAGGA-3'), sense IgE (5'-GCCTCGAGTGCCTGATGGAT-3') and antisense IgE (5'-GCGAATTCTGCTGTGTAGGA-3'). The expected lengths of the PCR products were 246 bp for IgD and 536 bp for IgE. The IgD primers amplify a conserved sequence in the C\(\delta\)3 domain whereas the IgE primers amplify a 536 bp C\(\gamma\)-3-C\(\gamma\)4 fragment found in all mammals we have so far studied (24). Rabbit, mouse, bovine and swine DNA were prepared from adult liver. Rat DNA was kindly provided by Dr Kevin Campbell, Department of Physiology and human genomic DNA provided by the Human Genome Mapping Center, both of the University of Iowa.

**Homologous and heterologous C\(\mu\) probes**

Homologous probes for porcine C\(\gamma\), C\(\mu\), V\(\gamma\) and D\(\gamma\) were described in previous publications in which their parent genes were sequenced and characterized (3,5,12,20). The probe for porcine C\(\delta\) is from a truncated cDNA encoding the 3' two-thirds of C\(\delta\)3 and the 5' two-thirds of C\(\delta\)4. It is highly homologous to ovine and equine IgE (24). A 960 bp cDNA containing the complete mouse C\(\delta\)3 gene, and a 1.5 kb cDNA clone containing the 3' end of human C\(\delta\)3, \&m1, \&m2 and a 960 bp 3' UTR, were kindly provided to us by Professor Philip Tucker (University of Texas, SW Medical School, Dallas, TX). In addition, Dr Tucker provided an 800 bp XbaI fragment containing the human heavy chain enhancer (Enh\(\delta\)).

**Results**

Table 1 presents data on the JH sequences found in 84 different expressed V\(\gamma\) genes obtained from newborn and adult swine. Table 1 is organized so that all V region sequences having the same JH sequence, same V\(\gamma\) gene and same D\(\gamma\)
segment are grouped together. Since little somatic mutation (non-templated) has occurred in the newborn piglet, the germline origin of both the V$_H$ and D$_H$ segments could be identified, whereas extensive mutation prevented such identification in V$_H$ genes expressed by adult swine; the latter are designated UID. Therefore the first row of Table 1 lists 25 different adult (P = adult) V-region clones whose V$_H$ and D$_H$ segment could not be identified but whose J$_H$ segment was identical to germline J$_H$. Of the 42 neonatal sequences, 12 were expressed with C$_\gamma$ nine with C$_\alpha$ and 21 with C$_\mu$. The constant region associated with the V$_H$ regions reported can be identified by code in the clone name: g = IgG, a = IgA, m = IgM. Forty of 42 neonatal sequences (Np = newborn) utilized either D$_{H1}$ or D$_{H2}$ and five different germline V$_H$ genes were used. Neonatal V$_H$ genes designated V$_H$O are apparent gene conversion products (12). Among the 42 adult sequences, 25 were expressed with C$_\gamma$, five with C$_\alpha$ and 12 with C$_\mu$. Among the 42 neonatal sequences, 40 of 42 were identical to the germline J$_H$ sequence (see later). The two variants both involved non-conservative changes, E $\rightarrow$ G (Npvg12) and W $\rightarrow$ C (Npvm22). Clone Npvm22 is probably a Taq polymerase error. Thus, newborn piglets preferentially use a single J$_H$ segment regardless of V$_H$, D$_H$ or constant region usage. Among the adult sequences, 25 of 42 expressed J$_H$ segments were identical to the germline J$_H$; 12 of 12 expressed with C$_\mu$ were identical to germline J$_H$, whereas half of the J$_H$ segments expressed with C$_\gamma$ differed in sequence from germline J$_H$. The number of adult C$_\alpha$ sequences (five) was too small for serious analysis although three were identical to the germline J$_H$ sequence.

Among those expressed J$_H$ sequences which differed from the germline sequence (30 nucleotide difference in 19 sequences), no two J$_H$ sequences were identical. Fifteen of the 30 changes were silent and 12 of 15 were non-conservative changes. Thus the FR4 regions of all 84 expressed V$_H$ genes appear to be derived from a single germline J$_H$.

The sequence data presented in Table 1 suggested that either swine use only a single J$_H$ segment or that the swine genome contains only one J$_H$ segment. Using a $^{32}$P-labeled DNA probe identical to the J$_H$ in 40 of 42 newborn piglet cDNAs, we were able to clone a single J$_H$-containing cosmid from a genomic cosmid library (Clontech, Palo Alto, CA) which also contained C$_\mu$. The heavy chain enhancer (Enh$_H$) mapped adjacent to J$_H$ and the membrane exons of C$_\mu$ were found 2.2 kb downstream of the C$_\mu$ (Fig. 1; 21). The hybridizing J$_H$ gene was located 3.6 kb from the 5' end of the cosmid and 6 kb upstream from C$_\mu$. This distance between J$_H$ and C$_\mu$ in swine is the same as in humans (4). The heavy chain enhancer (Enh$_H$) mapped adjacent to J$_H$ and the membrane exons of C$_\mu$ were found 2.2 kb downstream of the C$_\mu$ (Fig. 1; 21). The sequence of this porcine germline J$_H$ is given in Fig. 1, and is most similar to mouse J$_H$4 and human J$_H$6 (70%).

We then wondered if a J$_H$ probe would detect additional J$_H$ regions within the J$_H$-C$_\mu$ cosmid (Fig. 1) or in total genomic DNA. Southern blot analyses of cosmid and total genomic DNA digested with a variety of frequently cutting enzymes yielded a single hybridizing fragment of different size for each enzyme. The smallest was a polynucleotide of $\sim 300$ bp obtained with HindIII digestion (Fig. 2A). Since J$_H$ segments in species with multiple J$_H$ segments are typically separated by 0.5–1 kb, this result suggested that only a single J$_H$ gene existed in swine. Nevertheless, we subcloned a 5 kb SacI fragment located upstream of Enh$_H$ where other J$_H$ segments might be expected, digested the subclone with seven different restriction enzymes and hybridized the resulting blot with...
Table 1. The sequence of the J\_H segment in 84 cDNAs

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The following key will help identify the various V region cDNAs. The complete sequences are available in GenBank according to the clone names given. P = adult swine; Np = newborn piglet; 'a' within the name = expressed with IgA; 'g' = expressed with IgG; 'm' = expressed with IgM; 'a' used as a suffix = clone obtained by anchored PCR.

Only the V\_H genes of newborn piglets could be identified (designated V\_H A, V\_H B, etc.). The same is true for the D segments used by newborns. Adult swine V region sequences are so heavily mutated as to make impossible identification of the specific V\_H gene used (designated UID = unidentifiable).
Fig. 2. Southern blot analysis for swine J\(\mu\). (A) Southern blot restriction analysis of total sperm genomic DNA (five lanes on left) or DNA from the J\(\mu\)-C\(\mu\) cosmid (five lanes on right). A nucleotide ladder is given on the left and the various restriction enzymes used are indicated at the bottom of each lane. We propose that the hybridizing polynucleotides generated with Rsal in the J\(\mu\)-C\(\mu\) cosmid is smaller than that in genomic DNA because of restriction site polymorphism. (B) Southern blot analysis of a 5 kb Sacl subclone (see Fig. 1) using non-overlapping oligonucleotides as probes. The seven restriction enzymes used are indicated on the top of each lane and size markers are shown on the right.

In the last 3 years, we have probed both porcine genomic and cDNA libraries with a variety of heterologous DNA probes for C\(\mu\), C\(\gamma\), C\(\alpha\), C\(\epsilon\), S\(\gamma\) and C\(\delta\) from human, mouse and rabbit. cDNA expression libraries have also been cloned with specific antibodies to porcine Ig heavy chains. As reported elsewhere and stated above, the C\(\gamma\), C\(\alpha\) and C\(\mu\) genes of the swine could be readily cloned by this process (3,5,20). All were most similar in sequence to their human counterparts among all non-primate Ig genes for which sequence data are available. A similar pattern had been observed for porcine C\(\alpha\) and C\(\epsilon\) (25). However, when a porcine genomic library in EMBL 4, a cDNA library prepared from mesenteric lymph nodes (5) or the J\(\mu\)-C\(\mu\) cosmid (Fig. 1) were probed with DNA probes for mouse C\(\delta\) and a 1.6 kb fragment containing the 3' end of human C\(\delta\), the membrane exons and the 3' UTR, we failed to obtain any positive clones. Furthermore, when the porcine membrane exon (\(\mu\)) was used as a probe to screen the J\(\mu\)-C\(\mu\) cosmid, only a single hybridizing polynucleotide fragment was seen, suggesting that a membrane exon of a second Ig sharing some homology to \(\mu\) was absent from the cosmid.
DNA probes or to amplify the segment of C§ conserved in genic identity in their 5' regions (5). The latter feature would that these IPP are the swine analog of the avian bursa of H family that show virtual inter-
belonging to the ancestral V genes (IPP) which involute after 1 year (26), suggesting perhaps that use gene conversion in repertoire development seems plausible. Furthermore, swine possess ileal Peyers patches segment diversity (e.g. one JH gene) is characteristic of species H exchange (gene conversion; 12), the concept that limited VJH one segment. Since we have now shown that newborn piglets express V genes showing templated somatic cross-hybridization does not occur. We believe the evidence expressed and (ii) differ in sequence to the extent that genes but that: (i) they are not chicken in having a single JH gene. The alternative view is H53-Ce4 fragment of C§. While the conserved IgD fragment can be amplified from all species, the conserved IgD fragment can only be amplified from the genomic DNA of those species in which the IgD protein and gene have been characterized.

We further tested whether C§ was present in swine by PCR amplification of genomic DNA from various species using primers which amplify a conserved segment of IgD (246 bp of C§). Primers for the conserved 536 bp C§-C§-4 fragment of IgE were employed as controls. Figure 3 shows that IgD could only be amplified in those species in which the C§ gene and the heavy chain it encodes, have been reported. The conserved C§ segment amplified from these species, when used as a probe, failed to hybridize with swine or cosmid genomic libraries or ZAP cDNA libraries (data not shown).

Discussion

Sequence data presented in Table 1 and the Southern blot data of Fig. 2 are consistent with the conclusion that the swine is the first mammal characterized which resembles the chicken in having a single JH gene. The alternative view is that swine have other JH genes but that: (i) they are not expressed and (ii) differ in sequence to the extent that cross-hybridization does not occur. We believe the evidence available best supports the conclusion that swine have but one JH segment. Since we have now shown that newborn piglets express VH genes showing templated somatic exchange (gene conversion; 12), the concept that limited VH segment diversity (e.g. one JH) is characteristic of species that utilize gene conversion in repertoire development seems plausible. Furthermore, swine possess ileal Peyers patches (IPP) which involute after 1 year (26), suggesting perhaps that these IPP are the swine analog of the avian bursa of Fabricius. Finally, both swine and the chicken have VH genes belonging to the ancestral VH3 family that show virtual intergenic identity in their 5' regions (5). The latter feature would seem necessary for gene conversion to occur (27).

Our failure to clone porcine IgD (C§) using heterologous DNA probes or to amplify the segment of C§ conserved in rodents and humans suggests that: (i) an IgD homolog is absent in swine or (ii) a gene encoding IgD in swine has diverged in sequence to the extent that it is unrecognizable as a rodent/primate homolog. Our failure to find a homolog of an IgD was especially surprising to us because the sequences of swine C§, C§, C§, C§, and the single swine VH3 family are the most similar to their human counterparts among all non-primates so far studied (3,5,20,21). Definitive proof of the absence of an IgD vestige or the identification of some other porcine heavy chain that could function as a virgin B cell receptor (BCR) in the manner of rodent IgD will eventually require sequencing the entire heavy chain locus (or genome, if not linked). Figure 3 indicates that rabbits and cattle also lack a rodent/primate-like IgD. Thus, we emphasize that our data can be interpreted to mean that swine, rabbits and cattle lack an IgD which shares the conserved sequence common to primate and rodent IgD. Other investigators have so far failed to identify a gene encoding IgD in rabbits (28) cattle and sheep (J. Naessens, pers. commun.) and the chicken. At least in swine, the region 20 kb downstream from µm (which contains C§ in rodents and humans) does not contain any Ig gene that can hybridize with any known swine CH gene, gene probes for human or mouse IgD gene or the membrane exon of swine IgM.

The observation that swine and humans, which are not closely related phylogenetically, nevertheless share a high degree of Ig gene sequence similarity, may reflect parallel evolution due to similarities in life styles (both omnivores) resulting in exposure to a related spectrum of pathogens, e.g. rotavirus, herpes, helicobacter and Escherichia coli serotypes. Humans and mice are also distantly related phylogenetically yet have a similar Ig gene organization including an IgD with shared sequence similarity. Perhaps Ig gene organization reflects lymphoid anatomy and the mechanism of antibody repertoire diversification rather than phylogenetic origin. Chickens, swine, sheep and cattle, but not humans and rodents, have hind gut-associated lymphoid tissue which involute early in life (14,26,29-31). This tissue is called the bursa of Fabricius in birds and IPP in mammals (not to be confused with jejunal Peyer’s patches which are secondary lymphoid tissue associated with mucosal immunity). The appendix of the rabbit appears analogous but does not per se involute. Rather, it becomes converted to a secondary lymphoid structure apparently analogous to the Peyer’s patches of the mucosal immune system (32). In chickens, rabbits and cattle (14), these hind gut-associated lymphoid tissues are the site of secondary repertoire development involving gene conversion in B cells which have rearranged their Ig genes in the bone marrow or elsewhere (32). (Primary and secondary repertoire development are differently defined, depending on the author. Some regard secondary development as that which follows antigenic stimulation. While theoretically convenient, this criterion of distinction is experimentally untestable, as the stimulating antigen could be self or some form of B cell superantigen. Rather, we prefer to consider primary repertoire development as that which results from combinatorial joining. Further diversification through somatic hypermutation or gene conversion is defined as secondary repertoire development in this report.) Unlike the primat
rodent group that can undergo V-D-J rearrangement throughout life (33,34), V(D)J rearrangement in chickens and rabbits terminates early in development (9,35) and this is followed shortly after either by involution of these primary gut-associated lymphoid tissues or their conversion to lymphoid tissues with other functions (32). Although the site and length of B cell lymphogenesis remains unknown in swine and ruminants, they also have ITP which involute in early life (see above). Studies in our laboratory on VH gene expression in newborn piglets versus those from adult swine (5,12) indicate that expressed VH genes in newborn piglets show very little somatic mutation and six of 42 of those sequenced could be ascribed to gene conversion whereas the extensively hypermutated adult sequences make gene conversion events difficult to recognize. Recent data in cattle also demonstrate gene conversion in both the λ (12) and heavy chain loci (14) of fetal animals but, like swine, evidence for this event is heavily masked in older animals by extensive untemplated somatic mutation (14). Reynaud et al. (36) also observed extensive untemplated somatic mutation in adult sheep which probably masked any possibility for these investigators to identify gene conversion events among highly homologous VH genes. Our previous data showed that swine have <20 VH genes (5), comparable to the number in chickens although sheep may have even fewer (6). While swine also resemble chickens in having only a single J_{H} gene, ruminants apparently have more (7; J. Naessens, pers. commun.). Despite variations of this type, the occurrence of small numbers of VH gene elements in chickens, swine and ruminants may be characteristic of species which rely on template gene conversion among a small number of VH genes in early development to diversify their repertoire. The exception among species using gene conversion as regards VH gene numbers is the rabbit. However, rabbits use their 3' VH gene 90% of the time (37), a pattern reminiscent of the chicken with only one functional VH gene. This does not appear to be true in swine (12). Since artiodactyls and rabbits are generally regarded as more closely related to primates than birds, phylogeny does not appear to be a reliable indicator of the mechanism of B cell repertoire development.

The apparent absence of an IgD-like BCR in ruminants, swine and the rabbit raises the question as to the function of IgD in the rodent/primate group. Since the obvious correlation of the presence/absence of IgD is with differences in the mechanism of repertoire development, it is tempting to speculate that IgD is needed for this process in rodents and primates but not in ruminants, swine or rabbits (and chickens if IgD cannot be found in this species). The concept that IgD expression is associated with resistance to B cell tolerance induction (38) appears to be going out of favor (39) but, should it be correct, B cell tolerance should not develop in species which lack IgD. A more likely role for IgD emerges from the observation that IgD deficient mice have normal immune responses, a normal pre-B compartment but a reduced B cell compartment (40). This would suggest rather that IgD has something to do with repertoire expansion in primates and rodents. The recent observation that the V regions of IgD expressed on a CD38+ subpopulation of tonsilar B cells are highly mutated (diversified) suggests a role for IgD in repertoire diversification (41) perhaps through facilitation of affinity mutuation as shown in IgD knock out mice (42). A caveat in the concept that IgD may act as an intermediate step in affinity muturation is that the vast majority of peripheral slgM+/slgD+ B cells display nearly germline V genes (43,44). This discrepancy may be a consequence of differences in the site of sample selection, i.e. germinal centers versus circulating B cells. Perhaps B cells in germinal centers with hypermutated VH genes first display these changes by expressing them with IgD. Thus, IgD-bearing B cells with mutated sequences may require lower amounts of antigen to stimulate their further differentiation and their switch to B cells expressing downstream constant region genes (C_{x}, C_{a}, etc.) than due IgM-bearing B cells. This Mass Law effect would be further abated by the 10:1 slgD:slgM ratio reported for splenic B cells (45). Those which do not display such favorable, mutated sequences may remain unselected and re-enter the circulating pool to account for the peripheral B cells with IgD-bearing germline sequences. Since the transcription of C_{x} does not require switch recombination, but merely RNA splicing, this pathway to affinity maturation is energy efficient. The failure to find peripheral B cells with mutated IgD VH genes may indicate that affinity-maturated B cells leaving the lymph nodes have already switched to downstream C_{H} genes.

If the above hypotheses or some variant of it proves correct, why is IgD not needed in ruminants, swine and rabbits? Perhaps because secondary repertoire development in these species: (i) depends heavily on embryonic gene conversion followed by extensive hypermutation events in primary hindgut lymphoid tissues, and (ii) little or no hypermutation continues in peripheral germinal centers since B cell lymphogenesis and repertoire development is not continuous throughout life as in rodents and primates. Furthermore, the hypermutational events in these species appear to be intrinsic, rather than antigen driven, since gnotobiotic and conventional sheep show the same pattern (46). Especially noteworthy in adult swine is the lack of a correlation between the occurrence of hypermutated VH regions and the switch from C_{H} to C_{Y}(12). This would imply that an 'IgD intermediate' is unnecessary in the transition from C_{Y} BCR expressing germline VH sequences to C_{H} BCR expressing heavily mutated VH sequences in germinal centers. Perhaps for rodents and primates which continue the process of V(D)J rearrangement throughout life, an efficient 'second BCR', i.e. IgD, is needed in the antigen-driven transition to high-affinity antibody as a waypoint to forestall the proliferation of autoreactive clones or to determine negative (apoptosis) or positive selection. In other words, B cells leaving hind-gut follicles already have a diversified repertoire and represent in essence, the equivalent of a memory B cell population.

We believe that data strongly support the conclusion that swine, like the chicken, have a single J_{H} segment, that they lack a gene for the rodent/primate homolog of IgD and that immunological studies on non-traditional species can yield insight into the immunological mechanism used by traditional species.
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