Developmental and Comparative Immunology 33 (2009) 321–333

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

Immunoglobulins, antibody repertoire and B cell development

J.E. Butler [a,*], Y. Zhao [b], M. Sinkora [c], N. Wertz [a], I. Kacskovics [d]

[a] Department of Microbiology, University of Iowa, Iowa City, IA, USA
[b] State Key Laboratories for AgroBiotechnology, College of Biological Sciences, China Agricultural University, Beijing, PR China
[c] Institute of Microbiology, Academy of Science of the Czech Republic, Novy Hradek, Czech Republic
[d] Immunology Research Group of the Hungarian Academy of Sciences at Eotvos Lorand University, Budapest, Hungary

ARTICLE INFO

Article history:
Available online 18 September 2008

Keywords:
Swine
Immunoglobulin
B cell
Repertoire
Review

ABSTRACT

Swine share with most placental mammals the same five antibody isotypes and same two light chain types. Loci encoding λ, κ and Ig heavy chains appear to be organized as they are in other mammals. Swine differ from rodents and primates, but are similar to rabbits in using a single VH family (VH3) to encode their variable heavy chain domain, but not the family used by cattle, another artiodactyl. Distinct from other hoofed mammals and rodents, Cκ:Cλ usage resembles the 1:1 ratio seen in primates. Since IgG subclasses diversified after speciation, same name subclass homologs do not exist among swine and other mammals unless very closely related. Swine possess six putative IgG subclasses that appear to have diversified by gene duplication and exon shuffle while retaining motifs that can bind to FcγRs, FcRn, C1q, protein A and protein G. The epithelial chorial placenta of swine and the precosial nature of their offspring have made piglets excellent models for studies on fetal antibody repertoire development and on the postnatal role of gut colonization, maternal colostrum and neonatal infection on the development of adaptive immunity during the "critical window" of immunological development. This chapter traces the study of the humoral immune system of this species through its various eras of discovery and compiles the results in tables and figures that should be a useful reference for educators and investigators.

1. Historical perspective

Characterization of the porcine Igs, like those most other species, falls into three eras: (a) the era of protein chemistry, chromatography and polyclonal antibodies (pAbs), (b) the era of molecular genetics and monoclonal antibodies (mAbs) and (c) the future era. Discoveries in the first two will dictate the direction of the third.

The first era began in the late 1960s and early 1970s with characterization of the porcine Igs by size-exclusion and ion-exchange chromatography, SDS-PAGE and gel precipitin assays using pAbs. In this period IgM and IgA would be initially characterized and the heterogeneity of IgG recognized. PAb were used to determine the concentration of different isotypes in blood, colostrum/milk and other external secretions and to localize antibody-containing cells (ACCs).

The lack of naturally occurring myeloma proteins in swine and the unsuccessful attempts to induce B cell tumors in this species delayed the complete characterization of the Igs of swine and many other species until molecular genetics emerged in the mid-1980s. The application of molecular genetics roughly parallels the use of mAbs in immunohistology and ELISA-based assays and dominates the second era during which time the genes encoding IgA, IgG subclasses, the VH genes, DH and JH gene segments, IgM, switch μ region (Sμ), IgE and finally IgD, would be characterized. Also during this period, porcine Cλ and Cκ would be cloned, the variable region genes of kappa (Vκ) characterized and partial maps obtained for both light chain loci.

The immediate future era will hopefully be a period when the functional role of the many IgG subclasses will be defined, the heavy chain locus mapped and the presence of regulatory motifs, identified. The true role of the hindgut lymphoid tissue, e.g. ileal Peyers patches in antibody development will hopefully be clarified including the mechanisms involved. With the availability of new B cell markers, B cell subsets will finally be defined and lymphogenesis studied in the manner that is routine in mice and humans. The future era should also provide more insight into how gut colonization and maternal factors in milk/colostrum shape the antibody repertoire of the developing neonate and influence development of neonatal immune homeostasis. Either through

* Corresponding author. Tel.: +1 319 335 7776; fax: +1 319 335 9006.
E-mail address: john-butler@uiowa.edu (J.E. Butler).

0145-305X/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dci.2008.06.015

competition, user demand, government regulation or a combination of these, immunological reagents will become available to meet the needs of investigators. The future era is likely to be one when swine will be increasingly used as models for human disease and as a source of humanized antibodies for immunotherapy.

This review puts into perspective the antibody system of swine by comparing it with other contemporary mammals as regards: (a) isotypes, subclass and light chain diversity and (b) variable region diversity. Information from these comparative studies allows phylogenetic schemes to be constructed that can provide insight into the evolution of the antibody system of swine.

2. Igs, Ig genes and Ig gene expression

2.1. CH isotypes, subisotypes and allelic variants

2.1.1. IgM

The major Igs of swine were initially recovered by size-exclusion and ion-exchange chromatography followed by SDS-PAGE to identify their polypeptide composition and by using pAbs in immunodiffusion and immunoelectrophoretic assays [1–4] (Fig. 1A). The high MW fractions from size-exclusion columns contain predominately IgM (≈10^3 kDa; Table 1) with the H- and L-chains of IgM estimated by SDS-PAGE at 69–74 and 25 kDa, respectively (Fig. 1B). Deduced amino acid composition would place these values at 64 and 24 kDa, respectively. Since the IgM heavy chain is heavily glycosylated, circa 10–12%, the molecular mass based on the nascent polypeptide is significantly lower than the value obtained by SDS-PAGE. In addition, minor polypeptides of 16, 42 and 45 kDa are sometimes seen during SDS-PAGE analysis [5,6]. The 16 kDa band is consistent with the size expected for J-chain. The putative J-chain isolated by Zikan [2] migrated in SDS-PAGE with the molecular size of L-chains, which was believed to be due to glycosylation or conformation resulting from unreduced intrachain disulfide bonds. However in studies on human SIgA, J-chain migrated faster in SDS-PAGE than L-chains but after sulfotolysis could be separated from other polypeptide chains by ion-exchange chromatography [7]. The 42 and 45 kDa bands are also seen in preparations of bovine IgM and SIgA (Fig. 1B) but their identity has not been established. The major contaminant in such preparations is α2-macroglobulin (α2-M; MG in Fig. 1B) which in some species can be removed by Pevikon electrophoresis [8]. IgM purity can be ascertained because on SDS-PAGE, α2-M subunits are much larger [6] (Fig. 1B). A method based on chelation by zinc has also been described that can reduce contamination by α2-M [4]. Nevertheless, α2-M is highly immunogenic and pAb to anti-IgM may need to be absorbed on fetal serum affinity matrices which contain high levels of α2-M but only trace amounts of IgM [9]. IgM is phylogenetically conserved with homologs in fish [10–12] (Suppl. Fig. 1).

Mapping a cosmid clone allowed porcine Cμ, two Cμm exons and Sμ to be characterized [13]. The secreted tailpiece and membrane exons were highly conserved (>90% with other species) and homology to other species decreased from 3′ to 5′. Porcine Sμ is 3.2 kb in length and contains the three major pentameric repeats known to occur in the switch regions of other species.

2.1.2. IgA

IgA like IgM was initially purified by size-exclusion and ion-exchange chromatography and its polypeptide composition determined by SDS-PAGE [1,3–18]. The predominant IgA in exocrine secretions is polymeric SIgA (11S; Table 1) and by SDS-PAGE can be shown to be comprised of Cα chains (58 kDa), light chains (25 kDa) and secretory component (71 kDa) [5] (Fig. 1B; Table 1). The heavy chain mass based on the nascent polypeptide chain is less than that observed by SDS-PAGE since the Cα chain contains 6–8% carbohydrate.

Unlike primates, a substantial portion of serum IgA is polymeric and it can be purified from serum [19]. However, half of the IgA in serum can be monomeric [14,20] which is a much higher proportion than in cattle [12]. While free secretory component (SC) is abundant in the colostrum of cattle and humans, this appears not to be true in swine (Butler, unpublished). Excess SC in cattle and humans may reflect differences in the regulation of transcription [21]. Purified porcine SC was reported in the PhD thesis of Bourne [22] and the poly-IgR has been cloned from colostral epithelial cells [23].

IgA-specific pAb have been used to determine the level of IgA in serum and secretions [1,5,19,20,24–31] and to study the distribution of IgA-containing cells [32–37].

The gene encoding porcine Cα was recovered from a cDNA library using a probe for rabbit Cα; lowest homology to other species was in Cα1 domain and highest was found in the Cα3 domain [38]. A mutated splice acceptor site in Cα2 is found only in the IgA^b allotype, resulting in a "hingeless" IgA [39]. While the IgA^a and IgA^b allotypes are equally expressed in heterozygotes, there is

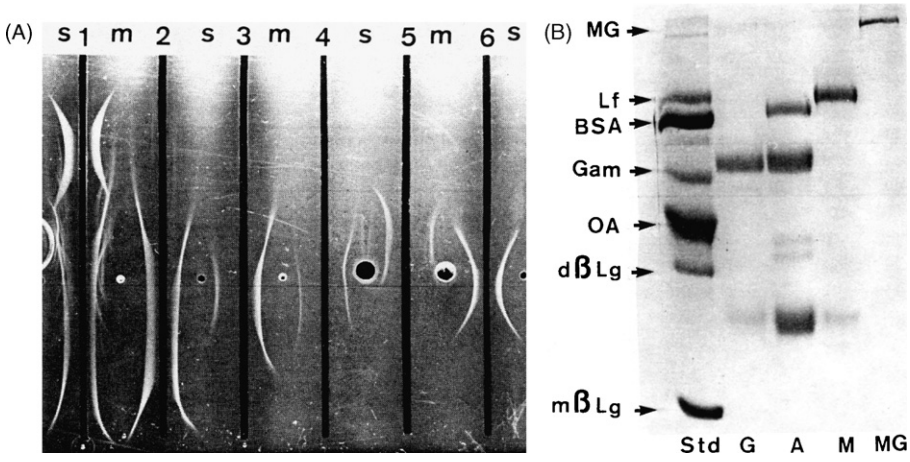

Fig. 1. Characteristics of the major classes of porcine Igs and their polypeptide chains. (A) Immunoelectrophoretic analysis of porcine IgM, IgA and IgG using isotype-specific pAbs. Wells contain serum (s) or colostrum (m). Troughs contain: 1 = anti-whole serum; 2 = anti-IgG; 3 = anti-IgA; 4 = anti-IgM; 5 = anti-α2; 6 = anti-transferrin. (B) Characterization of the polypeptide constituency of IgM (M), SIgA (A), IgG(G) and α2-M (MG) by SDS-PAGE. The left-hand column is a mixture of common proteins of known molecular mass including: mβLg = monomeric β-lactoglobulin (18 kDa), dβLg = dimeric betalactoglobulin (36 kDa) OA (43 kDa) GAM (55 kDa) BSA (67 kDa) Lf (74 kDa) and monomeric α2-M (110 kDa).