ISSN 0025-7680

BRIEF COMMUNICATION MEDICINA (Buenos Aires) 2003; 63: 299-302

VARIABLE KAPPA GENE USAGE IN SWINE ANTIBODIES

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Abstract The kappa light chain locus of swine has been mapped to chromosome 3q12-q14 but at this time, there is not enough information comprising the variable region genes or their transcripts. Here we report the sequences of five transcripts of swine kappa light chain variable region obtained from the spleen of two adult Yorkshire pigs. The lengths of the deduced sequences of these transcripts were variable (between 107 to 112 amino acids). Comparisons of the nucleotide sequences of their variable regions with other species like human and murine VL genes shows a high degree of identity, indicating the use of at least two different families of variable light genes. The contribution of these genes to the generation of variability in swine light chain variable genes as well as their therapeutic use in humans is discussed. An interesting possibility would be the development of an antiretroviral vaccine, useful to protect against a potential risk of infections due to xenotransplantation.

Key words: swine, antibody diversity, light chain, xenotransplantation

Resumen ***Genes de cadena variable kappa en anticuerpos porcinos***. El locus de cadenas livianas kappa en cerdos ya ha sido mapeado al cromosoma 3q12-q14, sin embargo no hay suficiente información relativa a los genes de las regiones variables de ésta ni de sus transcriptos. En este trabajo informamos la secuencia de cinco transcriptos de la región variable de la cadena kappa provenientes del bazo de 2 cerdos Yorkshire adultos. Estos tienen un largo variable de 107 a 112 aminoácidos. Las comparaciones con los genes variables de cadena liviana de otras especies tales como humano y murino VL muestran un alto grado de identidad, lo que sugiere el uso de al menos dos familias diferentes de genes variables de cadena liviana. Se discute el aporte de dichos genes en cuanto a la generación de diversidad en las cadenas livianas porcinas y su posible uso terapéutico en humanos. Una posibilidad interesante sería el desarrollo de una vacuna antirretroviral de utilidad frente a los riesgos potenciales de infecciones del xenotrasplante.

Palabras clave: porcino, diversidad de anticuerpos, cadena liviana, xenotrasplante

Immunoglobulin light chains belong to two isotypes named kappa and lambda. These two isotypes are found in most vertebrates. Nevertheless there are differences in the usage of these isotypes in different species of vertebrates. For example, most of the mice light chains belong to the kappa isotype. Likewise, sheep and cattle use mainly lambda light chains. It seems that swine use both, kappa and lambda light chains with a frequency of 60% and 40%, respectively[1]. The porcine kappa and lambda loci have been mapped to chromosome 3q12-q14 and 14q17-q21, respectively[2, 3].

In contrast to the heavy chain variable region genes of immunoglobulins, a thorough analysis of vertebrate light chain needs to be done yet. The usage of the different light chain genes in species with known VH genes will help in understanding the role of the light chain of antibodies in the generation of diversity in the immune response.

Currently, several mouse x human therapeutic chimeric antibodies are used in human medicine[4]. In some applications, the immunogenicity of the mouse region of these antibodies does not allow for the use of chimeric antibodies. In these cases, it is necessary to humanize the antibodies[5]. This procedure is costly and time-consuming. The high homology between pig and human antibodies could be advantageous in the production of chimeric pig x human antibodies because of the lower immunogenicity in human patients. To produce these antibodies it is necessary to know the genes encoding for the variable domains of swine antibodies.

Herein we report five variable region (VK) and JK transcript sequences from swine kappa light chain. The analysis shows the use of at least two families of variable genes and two families of J genes.

Received: 4-IV-03 Accepted: 18-VI-03

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The sequences were obtained using a 5' RACE-PCR (rapid amplification of cDNA ends-polymerase chain reaction) approach as previously described for VH transcripts of bovine antibodies [6]. Briefly, tissue samples were collected from the spleens of two adult Yorkshire pigs and maintained at –80°C until use. Total RNA was isolated from 100-600 mg of spleen tissue using TRIzol reagent (*Gibco BRL*, Gaithersburg, MD). The mRNA was purified with oligo(dT)-cellulose.

The cDNA was synthesized using 1µg of mRNA as template, in a reaction volume of 50µl containing 1unit (U) of RNasin (*Promega*, Madison, WI), 1mM dNTPs, 4mM Na pyrophosphate and 2mM primer *pka1* (CCACAGAGACAGTTGGGGTC). The reagents were incubated during 10 minutes at 65°C and then were transferred to ice. Then 1.5 units of AMV reverse transcriptase (*Promega*) were added and incubated at 42°C during 1 hour. This primer anneals with codons 19 to 26 of a published porcine constant kappa light chain sequence[7].

Copy-DNA was glass bead-purified and tailed using terminal deoxinucleotidyl transferase and dCTPs. The tailed cDNA was amplified by PCR using a 5' RACE Anchor primer (*Gibco BRL,* Gaithersburg, MD) which anneals with the cytosine tail in the 3' of the cDNA and primer pkp (AACTGCTCCTTCGATGGC) encoding amino acids 13 to 18 of the kappa constant chain. All PCR products were cloned into pCR II (Invitrogen, San Diego, CA) and the inserts were sequenced by the dideoxy-termination method.

The sequences obtained were compared against human germline immunoglobulin sequences using VBASE (http://www.mrc-cpe.cam.ac.uk/vbase), Kabat (http://immuno.bme.nwu.edu), IMGT (http://imgt.cines.fr:8104) and GenBank (http://www.ncbi.nlm.nih.gov) databases. Analyses were performed using Vector NTI *(Informax Inc.)*, *BioEdit*[8], *BLAST*[9] and *V-QUEST*[10] softwares .

Five cDNAs were sequenced and named 1-1L, 2-4L, 4-3L, 4-5L and G502L (*GenBank* accession # AF334738, AF334739, AF334740, AF334741 and AF334742 respectively). They have 109, 107, 107, 110, 112 amino acids spanning from framework 1 (FR1) to FR4 regions.

Comparisons of swine kappa light chain variable region transcripts (VK) with their human counterparts at the nucleotide and protein levels show that pigs express at least two families of VL genes (Figure 1). Transcripts 2-4L and 4-3L are homologous to human VK1 and mouse VK11 whereas transcripts 4-5L, G502L and 1-1L are homologous to human VK2 and mouse VK1, (Table 1). Based on these comparisons, we named those families as SwVK1 and SwVK2.

The five putative JK sequences and the only JK segment reported before[7] were compared at the nucleotide level with human and mouse germline genes and sequences from rabbit and cat (Figure 2). Compared to human sequences, at least three JK minigenes are present in the variable kappa locus of swine. One related to human JK2 (2-4L, 4-3L, 4-5L), another related to JK4 (1-1L, G502L) and the reported JK sequence related to human JK1 group. Compared to mouse at the nucleotide level, transcript 1-1L and the reported sequence are highly homologous to murine JK1 whereas transcripts 2-4L, 4-3L, 4-5L and G502L are related to murine JK5 (Table 1). Additional comparisons with other species such as rabbit and cat showed less identity (Table 1 and Figure 2).

Thus, swine use at least two families of kappa light chain variable region genes and three of JK to generate diversity in their VK repertoire.

Figure 1: Clustal alignment of sequences of SwVK1 and SwVK2 groups

SwVK1	FR1	CDR1	FR2	CDR2	FR3	CDR3
HuVK1*	DIQMTQSPSSLSASVGDRVTITC	RASQ-----GISNYLA	WYQQKPGKVPKLLIY	AASTLQS	GVPSRFSGSGSGTDFTLTISSLQPEDVATYY	CQKYNSAP
2-4L	E.V.....A..A.....T.....	....-----S..S...	....Q...A......	TI.....	......K............G..A.......	.LQHS...
4-3L	E.RD....A..A.....T.....	....-----S..SS.G	....Q...A......	....	......K............G..A.......	.LQH....
RabVK*	..V.......K..A...T...K.	Q...S..S------..S	.......QP......	R......	......K......Q.....NG..CD.A....	.AARY.GN
CatVK*	..V...T.L...VTP.EPAS.S.	....SLLYSDGNT..N	..L....QS.RR...	LV.NRD.	...D.............R..RVEAD..GV..	.GQGLQH.
MuVK11	....I...........I.....	Q...-----.T.IN.N	........A......	G..N..D	.........RY...........D.......	.LQHSYL.
SwVK2						
HuVK2*	DIVMTQSPLSLPVTPGEPASISC	RSSQSLLHSNGYNYLD	WYLQKPGQSPQLLIY	LGSNRAS	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYY	CMQALQTP
G502L	A.........S...........	.........T.K....	...........R...	QA...D.	..........................A....	...FK...
1-1L	A.........S...........	...H.-.EIY.S.L.S	..Q.......R....	FA.....	..........................A....	.Q.HK..-
4-5L	E.........S...........	.....-.EIW.N...S	..Q............	EA.....	..........................A....	.Q.FK.L-
PIGKV*	-----------------------	----------------	----..........V	EA.....	....................NS.....A....	.H.FK.F-
RAB*	.......ASP.SAAV.STV....	QA....YNN.---N.A	..Q.....P.K....	AA..L..	...S..K......Q...T.NG..CD.AAT..	.AARYSGN
CATVK*	..........S...........	.A.....Y...NT...	..........RR...	.V...D.	.......................D......	.G.G..H.
MUVK1	.A............L.DQ.....	......EN...NT...	....	RV...F.	..L...........................	...VTHV.

*Accession numbers: HuVK1 (X93622), HuVK2 (X93632), PIGKV (M59321), MuVK11 (AJ231256), MuVK1 (D00082), CatVK (AF198257), RabVK (AF135643), RAB (AF135605). FR: Framework region, CDR: complement determining region

TABLE 1.– *Variable and joining region sequence identity at nucleotide level*

	Variable region							Joining region					
	1-1L	2-4L	4-3L	4-5L	G502L	M59321		1-1L	2-4L	4-3L	4-5L	G502L	M59321
HuVk1*	66.1	83.9	83.2	68.1	63.8	69.2	HuJk1*	75.7	68.4	71.1	71.1	71.1	86.5
HuVk2*	80.7	64	64	82.2	83.7	81.4	HuJk2*	72.2	77.8	80.6	77.8	75	75
MokK1*	76.1	63	62.1	79	80	82	HuJk4*	75	72.2	75	75	77.8	75
MoVk11*	62.4	76.8	77.6	62.3	61.3	66.9	MoJk1*	81.1	69.4	69.4	71.1	71.1	81.1
CatVk	79.5	60.9	59.7	79.5	83.8	81	MoJk5*	75	77.8	80.6	75	77.8	73
RabVk*	64	74.9	75.6	63.7	62.8	65.7	CatJk	72.2	75	75	75	77.8	78.4
RAB*	65.7	71.2	71.6	63.1	63.2	66.9	RabJk	63.9	63.9	66.7	66.7	66.7	67.6

**Accession numbers: HuVK1 (X93622), HuVK2 (X93632), HuJK1 (V00556), HuJK2 (J00242), HuJK4 (J00243), MoVK1 (D00082), MoVK11 (AJ231256), MoJK1-5 (V00777), CatVk and CatJk (AF198257), RabVk (AF135643), RAB (AF135605), RabJK (X00032), M59321 (Lammers et al[7]).*

Figure 2. Nucleotide alignment of JK sequences.

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HuJK1*      -TGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAA
1-1L        -CTTGT......TAGC.........C..........
PIGIGKVJC*  TC................A......C.....C.....
MuJK1*      -..........TGG...C......C..........

HuJK2*      TACACTTTTGGCCAGGGGACCAAGCTGGAGATCAAA
2-4L        ..TGG...C...GC...A............C.....
4-3L        ..TGG...C...GC................C.....
4-5L        A.TGG...C...GC................C.....

HuJK4*      CTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA
G502L       .CTGG........CG.........C.....C.....
MuJK5*      .....G.....T.CT.........C.....C.G...
CatJK*      ............CC...T......C...........
RabJK*      G.G.........................TCG..G..
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*Accession numbers: HuJK2, HuJK4, HuJK1 (J00242), PIGKVJC (M59321), MuJK1 and MuJK5 (V00777), CatJK (AF198257), RabJK (X00032).

As well as other members of Artiodactyla such as sheep and bovine[6, 11, 12], swine show a limited usage of variable heavy genes, belonging to a family homologous to human VH3, to generate antibody diversity[13]. On the contrary, there is more diversity in the variable light chain of members of Artiodactyla. Sheep use at least six families of V lambda genes[14] and bovine at least two families[15]. Thus, it is possible that the existence of several families of V light genes in Artiodactyla is making an important contribution to the generation of diversity in these species.

In conclusion, we report herein five cDNA clones from swine kappa variable region immunoglobulin transcripts. The sequence analysis shows that swine use of at least two families of VL genes homologous to human VK1 and VK2. We named these families as SwVK1 and SwVK2 respectively. The JK sequences encoded by those sequences are homologous to human JK2 and JK4 gene families.

As a specie of economical importance, swine is a valuable model to investigate the generation of antibody primary repertoire. The availability of gene sequences for variable heavy and light chains of swine will help in making antibodies by recombinant DNA technology to construct pig x human chimeric antibodies. The high identity of VH and VL with human antibodies allows using these chimeric antibodies for therapeutic purposes decreasing the anti-chimeric antibody humoral response of the patient. In addition this approach could be of interest in the clinical field of xenotransplantation[16]. Xenotransplantation may be associa-ted with the risk of transmission of microorganisms, in particular of porcine endogenous retroviruses (PERVs). A possible strategies to prevent virus transmission include the development of an effective vaccine. Neutralizing pig x human chimeric antibodies engineered by the approach here proposed could be an original approach to create an antiretroviral vaccine.

References

1. Butler JE. Immunoglobulin gene organization and the mechanism of repertoire development. *Scand J Immunol* 1997; 45: 455-62.
2. Rettenberger G, Bruch J, Leeb T, Klett C, Brenig B, Hameister H. Mapping of the porcine immunoglobulin lambda gene, IGL, by fluorescence in situ hybridization (FISH) to chromosome 14q17-q21. *Mamm Genome* 1996; 7: 326.
3. Rettenberger G, Bruch J, Leeb T, Brenig B, Klett C, Hameister H. Assignment of pig immunoglobulin kappa gene IGKC, to chromosome 3q12-q14 by fluorescence in situ hybridization (FISH). *Mamm Genome* 1996;7: 324-5.
4. Mey U, Strehl J, Gorschluter M, et al. Advances in the treatment of hairy-cell leukaemia. *Lancet Oncol* 2003; 4: 86-94.
5. Subramanian KN, Weisman LE, Rhodes T, et al. Safety, tolerance and pharmacokinetics of a humanized monoclonal antibody to respiratory syncytial virus in premature infants and infants with bronchopulmonary

dysplasia. MEDI-493 Study Group. *Pediatr Infect Dis J* 1998; 17: 110-5.
6. Berens SJ, Wylie DE, Lopez OJ. Use of a single VH family and long CDR3 in the variable region of cattle Ig heavy chains. *Int Immunol* 1997; 9: 189-99.
7. Lammers BM, Beaman KD, Kim YB. Sequence analysis of porcine immunoglobulin light chain cDNAs. *Mol Immunol* 1991; 28: 877-80.
8. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp Ser* 1999; 41 95-8.
9. ltschul SF, Madden TL, Schäffer AA, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl Acids Res* 1997; 25: 389-402.
10. Lefranc MP, Giudicelli V, Ginestoux C, et al. IMGT, the international ImMunoGeneTics database, *Nucl Acids Res* 1999; 27: 209-12.
11. Dufour V, Malinge S, Nau F. The sheep Ig variable region repertoire consist of a single VH family. *J Immunol* 1996; 156: 2163.
12. Sinclair MC, Gilchrist J, Aitken R. Bovine IgG repertoire is dominated by a single diversified VH gene family. *J Immunol* 1997; 59: 3883.
13. Sun J, Kacskovics I, Brown VR, Butler JE. Expressed swine VH genes belong to a small VH gene family homologuos to human VHIII. *J Immunol* 1994; 153: 5618.
14. Reynaud CA, Dufour V, Weill JC. Generation of diversity in mammalian gut-associated lymphoid tissues. Restricted V usage does not preclude complex gene organization. *J Immunol* 1997; 59: 3093.
15. Sinclair MC, Gilschrist J, Aitken R. Molecular characterization of bovine V lambda regions. *J Immunol* 1995; 155: 3068.
16. Argibay P. Xenotrasplantes. La próxima frontera en el reemplazo de órganos. *Medicina (Buenos Aires)* 2003; 63: 344-6.

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Do not part with your illusions. When they are gone you may still exist, but you have ceased to live.

No abandone sus ilusiones. Cuando ellas se hayan ido, Ud. quizás todavía existirá, pero habrá dejado de vivir.

Mark Twain (1835-1910)