Porcine CD58: cDNA cloning and molecular dissection of the porcine CD58–human CD2 interface

Angélique Brossay, Florent Hube, Thierry Moreau, Pierre Bardos, and Hervé Watier

a EA 3249 “Cellules Hématoopoïétiques, Hémostase et Greffe,” Université François Rabelais, Tours, France
b IFR 120 “Imagerie et Exploration fonctionnelles,” Université François Rabelais, Tours, France
c INSERM EMI-U 0010, Protéases et Vectorisation, Université François Rabelais, Tours, France

Abstract

The porcine ligands of human CD2 remain unknown in xenotransplantation despite being an important pathway of T cell costimulation. Of the two main candidates, i.e., CD48 and CD58, the cDNA of the most likely ligand poCD58 was cloned from CD48-negative endothelial cells costimulating human CD4+ T cells through the CD2 pathway. The deduced protein sequence is 244 residues long and is 43% homologous to the human sequence. Based on similarity between porcine and human CD58 external V-set Ig-type domains, a structural model of poCD58–huCD2 interaction was built. Most of the charged residues located at the interface with huCD2 are highly conserved. Six putative hydrogen bonds between poCD58 and huCD2 were identified; five involve the same residues as in the syngeneic combination while the sixth is formed between an additional tyrosine in poCD58 and Arg48 in huCD2, increasing the complementarity between the two molecules. These structural data will help us to develop poCD58 blocking agents for xenotransplantation.

Keywords: Porcine CD58; Molecular modelling; Xenogeneic interactions

When pig to human xenotransplantation was considered in the early 1990s, the problem of hyperacute rejection was first investigated and recently solved by using organs from pig transgenic for complement regulatory proteins [3]. Cellular rejection phenomena were initially underestimated, mainly because previous murine/human xenogeneic experiments suggested that phylogenetic divergence prevented the recognition of ligand/receptor and receptor/counter-receptor pairs in xenogeneic systems. In particular, human T cell responses to xenogeneic (murine) antigen presenting cells (APCs) were considered low due to inability of costimulatory receptors (CD28, CD2, and LFA-1) to be engaged by the costimulatory molecules on murine APC. However, phylogenetic divergence between pig and human is less than between murine and human. Indeed, strong xenogeneic T cell responses to pig APC were observed and costimulation blocking experiments clearly demonstrated that human CD28 [5], CD2, and LFA-1 [9] could be engaged by the putative porcine counter-receptors.

Characterization of porcine costimulatory molecules was therefore initiated with the description of poCD86 (also known as B7-2) [7] and then poCD80 [19]. Subsequently, molecular, functional, and structural characterization of porcine CTLA4, one of the poCD86 ligands, led to the unexpected finding that this molecule bound weakly to human CD86 and CD80 [18] and failed to inhibit human T cell responses, when used as a CTLA4-Ig fusion protein. Porcine ICAM-1 was recently characterized [14] and demonstrated a low degree of conservation with human ICAM-1 (41% identity at the protein level). However, despite being divergent, porcine ICAM-1 has kept the ability to bind human LFA-1 and to transmit costimulatory signals to human T cells [9]. Up to now, in xenogeneic pig-to-primate models, porcine cells have demonstrated their capacity to...
Costimulate human CD4⁺ T lymphocytes through the CD2 pathway, but the porcine ligand was not yet identified. Contrary to the situation observed for rodents (Fig. 1), two distinct ligands, CD58 and CD48, could be engaged by the CD2 T lymphocyte receptor in humans. As CD58 does exist in the ovine species and as it was a ligand of huCD2 [11,12], we hypothesized that huCD2 could be engaged by a porcine ortholog of huCD58. To specifically characterize poCD58, we first searched for a porcine cell line able to costimulate human CD4⁺ T lymphocyte through the CD2 pathway without expressing poCD48. The porcine L23 lymphoblastoid B cell line, which costimulates human T cells by the CD2 pathway [1], was demonstrated to express CD48 (Brossay, A., et al., unpublished) and was discarded. By contrast, porcine aortic endothelial cells (PAECs), which express a ligand for huCD2 ([9,15], Brossay A. et al., submitted), do not express CD48 mRNA (Brossay A. et al., submitted) and were therefore selected for CD58 molecular characterization. Porcine CD58 cDNA was entirely cloned from PAEC by a multistep strategy. The amino acid sequence was predicted and aligned with the huCD58 protein sequence. Based on the similarity between porcine and human CD58 adhesion domains, a structural model of poCD58–huCD2 interaction is also presented and provides insight into the key amino acids in poCD58 that are important for CD2 binding.

Materials and methods

Cell culture. PAEC were isolated from miniature swine homozygous for the Swine Leucocyte Antigen (d) haplotype as previously described [21]. Briefly, PAEC were harvested after the treatment of aortas with collagenase A from Clostridium histolyticum (Boehringer–Mannheim, Meylan, France), They were seeded in 25 cm² gelatin-coated culture flasks in RPMI-1640 medium (Life Technologies, Cergy-Pontoise, France) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Life Technologies), 25 mM sodium bicarbonate (Flow, Les Ulis, France), 2 mM L-glutamine (Flow), 1 mM sodium pyruvate (Flow), 60 µg/mL tylosin (Life Technologies), 501U/mL penicillin (Flow), and 50 µg/mL streptomycin (Flow). PAEC were subcultured after trypsin-EDTA (Life Technologies) treatment and used from the second to the eighth subcultures.

Human peripheral blood mononuclear cells (PBMCs) from healthy volunteers were isolated by centrifugation (20 min, 800g) of heparinized blood over a d = 1.077 lymphocyte separation medium (Lymphoprep, Nycomed, Oslo, Norway) and cells were collected from the plasma/Lymphoprep interface. To prepare highly purified CD4⁺ lymphocytes, huPBMC were mixed with anti-CD48-coated magnetic beads (Dynabeads; Dynal, Olso, Norway) following the manufacturer’s instructions.

Xenogenic mixed lymphocyte endothelium coculture (XMLEC) was performed as previously described [17]. Briefly, PAEC were seeded in triplicate in 96-well flat-bottomed culture plates (Falcon 3072, Becton–Dickinson) to obtain confluent monolayers (3 × 10⁶ cells/well) and irradiated (30 Gy). Human CD4⁺ were cultured for 3 days in the presence of PHA (0.05 pg/mL, Sigma), either alone or on porcine adherent cell monolayers, at 37°C, in a humidified 5% CO₂ incubator. One µCi (3.4 × 10⁸ Bq) of [³H]thymidine (Amersham, Little Chalfont, U.K.) was added to each well 18 hours before the end of incubation. Tritiated thymidine incorporation and cell proliferation were quantified using an automated harvester (Filtermate 196, Packard, Rungis, France). Radioactivity on filter plates was measured with a liquid scintillation beta-counter (Tri-Carb 2550 TR/II, Packard). Results were expressed in cpm as means ± SD of thymidine incorporation in triplicate wells. Blocking experiments were performed with a nonblocking agent (anti-CD2b, clone 6F10.3, Beckman Coulter) and a blocking mAb (anti-CD2, clone T11, Beckman Coulter).

mRNA isolation and reverse transcription. Total mRNA was isolated from 10⁶ PAEC by using the Dynabeads mRNA Direct Kit (Dynal) following the manufacturer’s instructions. Total mRNA was then reverse-transcribed in a 50 µL reaction mixture containing 500 µM of each deoxynucleotide triphosphate, 4 µM oligo(dt) 20, 25 U RNase inhibitor, and 25 U of AMV reverse transcriptase (Roche Diagnostics, Meylan, France). After 1 hour incubation at 42°C, the enzyme was inactivated for 5 min at 95°C.

Porcine CD58 cDNA cloning. Human and sheep CD58 cDNA sequences (GenBank Accession Nos: NM001779 and D28584, respectively) were aligned and degenerate forward and reverse primers (A58for, 5’-tggcagatcactc 3’ and A58rev, 5’-aatggtaaatcagatgsctt 3’) were designed. PCR amplification was performed in a total reaction volume of 52 µL containing 10 mM Tris–HCl, pH 9.0, 50 mM KCl, 0.01% (w/v) gelatin, 1.5 mM MgCl₂, 0.1% Triton X-100, 50 µM of each deoxynucleotide triphosphate, 0.5 U Taq polymerase, and 2 µM of each primer synthesized by Genset (Paris, France). PCR was set up in a GeneAmp PCR system 2400 (Perkin–Elmer France SA, Saint Quentin en Yvelines, France) programmed for an initial denaturation step of 3 min at 94°C, followed by 35 cycles for 30 s at 94°C, hybridization for 60 s at 47°C, and elongation for 60 s at 72°C. The final extension was performed for 7 min at 72°C. PCR products were then analyzed by electrophoresis through 1.6% agarose gels in TBE buffer (90 mM Tris–HCl, 90 mM boric acid, and 2.5 mM EDTA) containing 0.05% (w/v) ethidium bromide (Eurobio). Gels were UV transilluminated (Gel Doc Tris–HCl, 90 mM boric acid, and 2.5 mM EDTA) containing 0.05% (w/v) ethidium bromide (Eurobio). Gels were UV transilluminated (Gel Doc 1000 system, Bio-Rad, Ivry sur Seine, France) and the picture was captured on a Kodak Digital Science Imager (Rochester, NY).

A PCR product of 526 bp (poCD58.1) was then purified by the GFX PCR DNA and Gel Purification Kit (Amersham–Pharmacia Biotech Europe, Paris, France) and was cloned into the pCR2.1 vector (Invitrogen, Cergy Pontoise, France). Several clones were selected and both strands of each were sequenced by the dideoxyribonucleotide chain-termination method using a Perkin–Elmer ABI Prism 377 DNA sequencer.

The determined consensus sequence was then used as a probe to screen the porcine EST database from TIGR (The Institute for Ge-
Amino acid sequence analysis. Amino acid sequence was predicted from the entire poCD58 cDNA with Infobiogen Translation Software (http://www.infobiogen.fr/services/analyseq/cgi-bin/traduc_in.pl). Molecular weight (MW), theoretical isoelectric point (pI), and amino acid composition were analyzed and calculated by using the ProtParam Program (http://www.expasy.ch/tools/protparam.html). Multiple sequence alignment of the CD58 proteins was performed using CLUSTALW (http://www.infobiogen.fr/services/analyseq/cgi-bin/clustalw_in.pl). Potential N-glycosylation sites were identified using ScanProsite (http://www.expasy.ch/tools/scnpsite.html).

Structural modeling of porcine CD58. A 3D structural model of the adhesion domain (domain V) of poCD58 was built using the automatic comparative modeling server GENO3D (http://geno3d.phil.ibcp.fr) and the structure of the CD2-binding domain of huCD58 (PDB Accession No. 1.4q9) as a template (48% identity with poCD58 adhesion domain). The resulting model was further energy minimized using Discover as implemented in INSIGHT II, version 2000 software (Accelrys).

All calculations were done with a 10 Å nonbond cutoff and a 2 Å distance-dependent dielectric constant on a Silicon Graphics Octane workstation. The minimization procedure started with a round of steepest descent algorithm until the maximum derivative was <10 kcal/molÅ during this step. The second and third steps of minimization were carried out using the steepest descent algorithm with a tethering force constant on the backbone atoms of 100 and 50 kcal/molÅ, respectively, until the derivative was <5 kcal/molÅ (second step) and <3. Finally, the poCD58 model was minimized unconstrained using the conjugate gradient algorithm until the derivative was <1 kcal/molÅ.

The putative heterophilic complex between poCD58 and huCD2 was obtained by superimposing the Cα atoms of the poCD58 model and huCD2 (coordinates extracted from the PDB file 1q99) on the corresponding components of the huCD58-huCD2 complex (PDB Accession No. 1q99). The few atom overlaps occurring at the complex interface were relieved by energy minimization using 2000 iterations of the steepest descent algorithm, the backbone atoms being fixed during this step. The electrostatic potentials of huCD2 were calculated using DELPHI (Accelrys) using formal charges at pH 7.4 (arginine, lysine, and N terminus, +1; glutamate, aspartate, and the C terminus, –1; and histidine, neutral), an ionic strength in the aqueous environment of 0.15 M, and dielectric constants of 2 and 80 for the interior and the exterior of the protein, respectively. The molecular surface was colored according to the electrostatic potential using Insight II.

Results and discussion

Porcine endothelial cells are known to costimulate human CD4+ T lymphocytes through the CD2 pathway [9] and were previously shown not to express CD48 (Brossay, xenotransplantation, 2002, submitted). To check our PAEC, blocking experiments were performed in a mixed lymphocyte/endothelial cell coculture in the presence of phytohemagglutinin (PHA). While controls and nonblocking anti-human CD2 (T11.1) mAbs were ineffective, the anti-human CD2 blocking mAb T11.2 strongly inhibited the CD4+ T cell proliferation by 85% (Fig. 2), confirming that a porcine ligand of huCD2, differing from poCD48, was expressed on these porcine endothelial cells.

A first PCR amplification was then performed using PAEC-derived cDNA, using degenerate primers A58for and A58rev designed based on human and sheep CD58 cDNA alignment. A 526 bp-fragment was obtained (Figs. 3A and D) purified, sequenced, and named poCD58.1 after confirming homology with human and ovine CD58 (not shown). The TIGR porcine EST database was screened with poCD58.1, leading to the identification of two fragments, EST 141404 and EST 259109, of 540 and 482 bp, respectively (Fig. 3B). The 5' end of EST 141404 aligned with the 5' flanking region of poCD58.1 whereas the 5' end of EST 259109 aligned with the 3' flanking region of poCD58.1 (Fig. 3B). Because the entire poCD58 coding sequence could be deduced from the assembly of these three fragments (Fig. 3C), B58for and B58rev were designed upstream from the initiation codon and downstream from the STOP codon, respectively, and allowed the amplification and cloning of poCD58.2 cDNA (912 bp) (Figs. 3C and E). Eleven clones were sequenced and found to be identical. The sequence has been submitted to GenBank (Accession No. AF469666).

The poCD58 cDNA sequence predicts a 244 residue protein which can be aligned with huCD58 (Fig. 4). As huCD58 has a 28 residue peptide signal sequence, the putative poCD58 signal sequence was supposed similarly to contain 28 amino acids. As its human counterpart, poCD58 is a type I transmembrane protein with an

Fig. 2. CD2 pathway blocking experiment. The proliferation of human CD4+ T cells was tested after 6 days of coculture on irradiated PAEC monolayers, in the presence of blocking agent (anti-CD2b) and non-blocking agent (anti-CD2) at saturating concentrations or their controls. At 18 h before the end of the coculture, wells were pulsed with tritiated thymidine (3.6 × 10^6 Bq/well) and proliferation was measured by thymidine uptake. Results are expressed in cpm as means ± SD of triplicate determinations. This figure is derived from one experiment representative of five.
extracellular portion based on a V set Ig-like domain (92 aa) and a C2 set Ig-like domain (102 aa). Human (226 aa) and porcine CD58 (217 aa) mature proteins share 43% overall identity. This has to be compared with 48% amino acid identity between human and sheep CD58 and suggests higher divergence. The six cysteines in the C2 domain show conserved positions (104, 115, 134, 140, 163, and 181). Four N-linked glycosylation sites in human are denoted by a circle and putative N-linked glycosylation sites in pig are denoted by a diamond. The signal peptide sequence is underlined.
quality control [6]. Indeed, the only conserved N-glycosylation site between the murine, porcine, and human MHC class I sequences (Asn 86) has been clearly demonstrated to be involved in the expression and function of a MHC class I antigen [13]. Nonconservation of the three other N-linked glycosylation sites, together with divergence at the peptide levels probably explains why no anti-huCD58 mAbs have been described to cross-react with the porcine ortholog.

Because the V domain of huCD58 is sufficient for binding to CD2 [8], it is likely that the orthologous V domain is involved in the binding to huCD2 if poCD58 proves to be a ligand. Only one amino acid residue insertion was observed in the porcine sequence, a tyrosine at position 42. This tyrosine insertion is located in the putative C'–C' loop of poCD58, towards the CD2 molecule, and probably does not alter the backbone conformation which is likely to be conserved between huCD58 and poCD58. However, this extra amino acid at position 42, together with an extra N-linked glycosylation site at position 43, could prevent or modify the putative interaction with CD2.

Therefore, to gain additional insight into the interaction of poCD58 with huCD2, a structural model of poCD58 V domain was prepared by homology modeling, relying on the high resolution structure of huCD58. The homology-based poCD58 model displayed the same V set Ig-like fold as the huCD58 template (Fig. 4), the rmsd (root-mean-square-deviation) between the two structures being 1.29 Å after superimposing 88 Ca atoms, a value in agreement [4] with the 43% identity between poCD58 and huCD58. The only significant conformational difference between both structures occurred in the loop between β strands C' and C' due to the insertion of a tyrosyl residue at position 42.

The complex between poCD58 and huCD2 was obtained by superimposing both molecules onto the corresponding components of the X-ray structure of the huCD58–huCD2 complex [20]. Surprisingly, compared to huCD58, most of the charged residues located at the interface between poCD58 and huCD2 are highly conserved (Table 1) and could be engaged in salt bridges with complementary charged residues of CD2 (Fig. 5A).

This suggests that, as in the human heterophilic complex, the major source of binding energy would originate from these charged residues. Charge complementarity occurring at the poCD58–huCD2 interface is clearly evidenced in Fig. 5B, which depicts the electrostatic potential surface of huCD2 in the poCD58–huCD2 complex. In addition, six hydrogen bonds were identified at the interface between poCD58 and huCD2 (Table 1). Five of the hydrogen bonds are formed between the same residues as in the human interaction (Table 1) while the sixth is formed between the additional tyrosyl residue 42 of poCD58 and Arg48 of huCD2. Instead of preventing the interaction between poCD58 and huCD2, it is thus likely that this extra amino acid contributes significantly to the interaction between the two molecules.

Amino acid sequence homology with huCD58 was used to model successfully poCD58, and the possible interaction with huCD2 leading to confirm that the cDNA isolated here is the porcine CD58 ortholog. Moreover, conservation of the charged residues involved in the electrostatic interaction and of the hydrogen bonds suggests that poCD58 is the huCD2 li-
gand expressed by porcine endothelial cells and involved in human CD4+ T lymphocyte costimulation. Developing anti-poCD58 specific antibodies would therefore be a promising approach in pig-to-human xenotransplantation to inhibit human lymphocyte activation triggered by porcine antigen-presenting cells, leaving intact the interactions with human APCs, which are required for anti-infectious defenses. Whether poCD48 constitutes another human CD2 ligand remains an open question, notably in porcine cells presenting costimulatory functions and co-expressing CD48 and CD58, such as the L23 lymphoblastoid cell line (see Fig. 6).

More generally, taking into consideration the obvious species-specific differences in the CD2 system between human and mice (summarized in Fig. 1) and the still debated phylogenetic history of this system [20–22] increasing the knowledge in a third, intermediate species, is of primary importance, and could lead to unexpected finding concerning xenogeneic interactions. While CD244 (2B4) is still totally unknown in the pig species, a cluster of mAb recognizing CD2 has already been defined for several years [10], and a partial putative porcine CD2 cDNA sequence has been recently identified (A. Brossay et al. unpublished). Moreover, this family also includes a CD2 homolog which has been found in the genome of the African swine fever virus (ASFV), an infectious agent which specifically infects porcine macrophages and endothelial cells [2,16]. In the context of several putative poCD58 ligands, it is remarkable that porcine and ovine CD58 comprise an extra amino acid at position 42 (Tyr and Ser, respectively), and that this residue in poCD58 could be involved in electrostatic interactions with its ligands as suggested by our model. Extending the characterization of the porcine ligands of poCD58 and their analysis by molecular modeling would be very useful.

Acknowledgments

A. Brossay and F. Hubé are supported by Hrant Nos. 9952 and 12906 from the Ministère de l’Education Nationale, de la Recherche et de la Technologie. This work was funded by INSERM (“Action Concertée Coordonnée xénogreffe”). We thank Mrs. C. Cherpi-Anthar and Prof. C. Andres (INSERM U316, Tours, France) for nucleotide sequencing. We are grateful to Dr. P. Chardon and Dr. C. Renard for useful discussions and Dr. J. Lund and Prof. R. Jefferis (University of Birmingham, UK) for critically reading the manuscript.

References

[1] C. Bonenfant, I. Vallée, J. Sun, A. Brossay, G. Thibault, J.M. Guillaume, Y. Lebranchu, P. Bardos, J.E. Butler, H. Watier, Analysis of human CD4 T lymphocyte proliferation induced by porcine lymphoblastoid B cell lines, Xenotransplantation 9 (2002) 1–13.
[2] M.V. Borca, G.F. Kutish, C.L. Afonso, P. Irusta, C. Carrillo, A. Brun, M. Sussman, D.L. Rock, An African swine fever virus gene with similarity to the T-lymphocyte surface antigen CD2 mediates hemadsorption, Virology 199 (1994) 463–468.
[3] L. Buher, T. Friedman, J. Iacomini, D.K. Cooper, Xenotransplantation—state of the art—update 1999, Front. Biosci. 4 (1999) D416–D432.
[4] C. Chothia, A.M. Lesk, The relation between the divergence of sequence and structure in proteins, EMBO J. 5 (1986) 823–826.
[5] T.A. Davis, N. Craighead, A.J. Williams, A. Scadron, C.H. June, K.P. Lee, Primary porcine endothelial cells express membrane-bound B7-2 (CD86) and a soluble factor that co-stimulate cyclosporin A-resistant and CD28-dependent human T cell proliferation, Int. Immunol. 8 (1996) 1099–1111.
[6] L. Ellgaard, A. Helenius, ER quality control: towards an understanding at the molecular level, Curr. Opin. Cell Biol. 13 (2001) 431–437.

[7] S.E. Maher, K. Karmann, W. Min, C.C. Hughes, J.S. Pober, A.L. Bothwell, Porcine endothelial CD86 is a major costimulator of xenogeneic human T cells: cloning, sequencing, and functional expression in human endothelial cells, J. Immunol. 157 (1996) 3838–3844.

[8] G.T. Miller, P.S. Hochman, W. Meier, R. Tizard, S.A. Bixler, M.D. Rosa, B.P. Wallner, Specific interaction of lymphocyte function-associated antigen 3 with CD2 can inhibit T cell responses, J. Exp. Med. 178 (1993) 211–222.

[9] A.G. Murray, M.M. Khodadoust, J.S. Pober, A.L. Bothwell, Porcine aortic endothelial cells activate human T cells: direct presentation of MHC antigens and costimulation by ligands for human CD2 and CD28, Immunity 1 (1994) 57–63.

[10] A. Saalmuller, G. Kuebart, E. Hollemweguer, Z. Chen, J. Nielsen, F. Zuckermann, K. Haverson, Summary of workshop findings for porcine T-lymphocyte-specific monoclonal antibodies, Vet. Immunol. Immunopathol. 80 (2001) 35–52.

[11] P. Selvaraj, M.L. Dustin, R. Mitsnacht, T. Hunig, T.A. Springer, M.L. Plunkett, Rosetting of human T lymphocytes with sheep and human erythrocytes. Comparison of human and sheep ligand binding using purified E receptor, J. Immunol. 139 (1987) 2690–2695.

[12] P. Selvaraj, M.L. Plunkett, M. Dustin, M.E. Sanders, S. Shaw, T.A. Springer, The T lymphocyte glycoprotein CD2 binds the cell surface ligand LFA-3, Nature 326 (1987) 400–403.

[13] T. Shiroishi, G.A. Evans, E. Appella, K. Ozato, In vitro mutagenesis of a mouse MHC class I gene for the examination of structure-function relationships, J. Immunol. 134 (1985) 623–629.

[14] C.J. Stocker, K.L. Sugars, H. Yarwood, A. Delikouras, R.J. Lechler, A. Dorling, R.C. Landis, B.J. Morley, D.O. Haskard, Cloning of porcine intercellular adhesion molecule-1 and characterization of its induction on endothelial cells by cytokines, Transplantation 70 (2000) 579–586.

[15] I. Vallee, J.M. Guillamin, G. Thibault, Y. Gruel, Y. Lebranchu, P. Bardos, H. Watier, Human T lymphocyte proliferative response to resting porcine endothelial cells results from an HLA-restricted, IL-10-sensitive, indirect presentation pathway but also depends on endothelial-specific costimulatory factors, J. Immunol. 161 (1998) 1652–1658.

[16] I. Vallee, S.W. Tait, P.P. Powell, African swine fever virus infection of porcine aortic endothelial cells leads to inhibition of inflammatory responses, activation of the thrombotic state, and apoptosis, J. Virol. 75 (2001) 10372–10382.

[17] I. Vallee, H. Watier, G. Thibault, H. Salmon, Y. Gruel, Y. Lebranchu, P. Bardos, Evidence of noninvolvement of swine MHC class II in the in vitro proliferative response of human lymphocytes to porcine endothelial cells, Transplantation 59 (1995) 897–901.

[18] A.N. Vaughan, P. Malde, N.J. Rogers, I.M. Jackson, R.I. Lechler, A. Dorling, Porcine CTLA4-Ig lacks a MYPPP motif, binds inefficiently to human B7 and specifically suppresses human CD4+ T cell responses costimulated by pig but not human B7, J. Immunol. 165 (2000) 3175–3181.

[19] M. Wada, S. Amae, N. Sano, T. Ishii, H. Sasaki, K. Nishi, M. Nio, Y. Hiyashi, R. Ohi, Expression of the co-stimulatory molecule CD80 (B7-1) in a porcine intestinal graft, Transpl. Proc. 34 (2002) 1042–1044.

[20] J.H. Wang, A. Smolyar, K. Tan, J.H. Liu, M. Kim, Z.Y. Sun, G. Wagner, E.L. Reinhzer, Structure of a heterophilic adhesion complex between the human CD2 and CD38 (LFA-3) counter-receptors, Cell 97 (1999) 791–803.

[21] H. Watier, I. Vallee, G. Thibault, A.C. Lalmanach, M. Lacord, Y. Gruel, Y. Lebranchu, H. Salmon, P. Bardos, Effect of human inflammatory cytokines on porcine endothelial cell MHC molecule expression: unique role for TNF-alpha in MHC class-II induction, Transpl. Proc. 26 (1994) 1152–1155.

[22] Y.W. Wong, A.F. Williams, S.F. Kingsmore, M.F. Seldin, Structure, expression, and genetic linkage of the mouse BCM1 (OX45 or Blast-1) antigen. Evidence for genetic duplication giving rise to the BCM1 region on mouse chromosome 1 and the CD2/LFA3 region on mouse chromosome 3, J. Exp. Med. 171 (1990) 2115–2130.