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Expression of complement regulatory protein on porcine endogenous retrovirus (PERV) depends on molecular size

Aki Yamamoto [a], Chizuko Kobayashi [a], Saya Yamashita [a], Takayuki Miyazawa [b], Masaru Okabe [c], Masahiro Fukuzawa [a], Shuji Miyagawa [a,*]

[a] Division of Organ Transplantation, Department of Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
[b] Institute for Virus Research, Kyoto University, 53 Shogoin-Kawaracho, Sakyo-ku, Kyoto 606-8507, Japan
[c] Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan

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ABSTRACT

Expression of complement regulatory proteins (CRP) on pig cells is an effective means to avoid hyperacute rejection. However, pig endogenous retrovirus (PERV) from pig cells transfected with CRP may acquire resistance to human serum (HS). The present study investigated the size limitations of the transfected CRP that can be easily expressed and function on PERV particles. cDNAs of various sized DAF(CD55)s, including single-, double-, triple-, tetra-, as well as 2.1- and 2.2-DAF, were prepared. Pig endothelial cells (PEC) were transduced with the *Lac*Z gene, and were then infected with PERV-B to produce PEC(Z)/PB. The extent of complement-mediated lysis by the transfectant molecules on PEC(Z)/PB was then determined. HEK293 cells were incubated with PEC(Z)/PB culture supernatants in the presence of HS and the *Lac*Z pseudo-type assay was then carried out. Amelioration of complement-mediated lysis by the hybrid molecules was verified in each PEC(Z)/PB clone. All molecules appeared to effectively protect xenogeneic cells against complement-mediated lysis. While PERVs from the PEC(Z)/PB with both the single-DAF and double-DAF were resistant to HS, PERVs from the triple-DAF and tetra-DAF showed no significant increase in resistance. In addition, the PERVs from PEC(Z)/PB with 2.1-DAF and 2.2-DAF were less resistant than PEC with double-DAF. Resistance to HS was steadily attenuated with increasing size of the DAF molecule. The resistance to HS was disappeared by the anti-DAF blocking mAb, indicating that PERVs from the transfectants express DAF molecules on the surface of the PERV. The data clearly indicate that, to avoid the induction of resistance to HS in PERV particles, relatively large CRPs, such as triple-DAF and tetra-DAF or DAF with other large molecules, should be employed in the production of transgenic pigs.

1. Introduction

The lack of donor organs and the increasing demand for transplantable organs has revived interest in xenotransplantation. However, safe clinical xenotransplantation requires that PERV transmission to human patients be prevented. The pig genome contains at least 50 pro-viral copies of porcine endogenous retroviruses (PERVs) [1,2]. The ability of PERV to infect human cells in vitro and the production of a SCID mouse with a human PERV receptor have generated debate concerning the risk associated with xenotransplantation. The controversy persists in spite of the fact that no cases of PERV infections have been reported in humans [3–7].

Hyperacute rejection [8,9], which is a major obstacle to xenotransplantation, can be overcome by generating a pig strain that is resistant to the human immune system by transduction of the human complement regulatory protein (CRP) gene. However, these same genetic modifications may result in PERV particles from pig cells to be more resistant to normal human serum (HS), thus elevating the risk of PERV transmission to humans [10–12]. In general, HS efficiently lyses PERVs that are released from pig cells [3]. In contrast, most gamma-retroviruses produced in human cells are resistant to HS. Viral resistance to HS is thought to be controlled by envelope proteins and producer cell proteins [13–15]. In a previous study, we demonstrated that PERV from pig endothelial cells (PEC) transfected with decay-accelerating factor (DAF: CD55)[16,17] and CD59[10,18], showed increased resistance to HS compared with control PEC. Therefore, PERV from pigs transfected with human CRP may become resistant to HS in accordance with the acquired resistance of the graft to HS, because viral particles produced from the transgenic pig cell wall may express the human CRP.

In the present study, based on our hypothesis that the CRP molecules that can be expressed on the PERV molecules have a

Abbreviations: PERV, porcine endogenous retrovirus; CRP, complement regulatory protein; HS, human serum; PEC, pig endothelial cells; DAF, decay-accelerating factor; SCR, short consensus repeat; LDH, lactate dehydrogenase; BFU, blue focus-forming units.

* Corresponding author. Tel.: +81 6 6879 3062; fax: +81 6 6879 3069.
E-mail address: miyagawa@orgtrp.med.osaka-u.ac.jp (S. Miyagawa).

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limitation in the size, we investigated the size limitation and their ability to modulate resistance to HS.

2. Objective

To investigate the size limitations of the transfected CRP that can be easily expressed and function on PERV particle.

3. Materials and methods

3.1. Cell culture

A PEC line, MYP-30, and human embryonic kidney (HEK) 293 cells were maintained in Dulbecco's modified Eagle's medium (D-MEM) (Sigma Chemical Co., MO, USA) supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine and kanamycin/amphotericin. The cultures were maintained in a 5% CO_2/95% air atmosphere at 37 °C [16].

3.2. Preparation of PERV-producing cells

To determine PERV infectivity, we introduced a MFGnls*Lac* Z plasmid that encodes the *Lac*Z gene with the packaging signal of the murine leukemia virus (MuLV) under the control of the long terminal repeat to MuLV, into PEC by pseudo-infection, and prepared PEC(*Lac*Z). To establish PEC(*Lac*Z) that produce PERV-B, i.e., PEC(Z)/PB, PEC(*Lac*Z) was infected with PERV-B produced from HEK293 cells, which had been continually infected with PERV-B[19].

3.3. Construction of plasmids

We established cDNAs as follows: delta-short consensus repeat (SCR) single-DAF (1-DAF), containing of SCR2-3-4 with a FLAG-tag; double-DAF (2-DAF), consisting of SCR2-3-4-2-3-4 with a FLAG-tag; triple-DAF (3-DAF), consisting of SCR2-3-4-2-3-4-2-3-4 with a FLAG-tag; and, tetra-DAF (4-DAF), consisting of SCR2-3-4-2-3-4-2-3-4-2-3-4 with a FLAG-tag. In addition, 2.1-DAF, consisting of SCR2-2-3-4-2-3-4 with a FLAG-tag, and 2.2-DAF, consisting of SCR2-3-2-3-4-2-3-4 with a FLAG-tag, were also constructed (Fig. 1). These cDNAs were subcloned into the pCXN (chicken beta-actin promoter and CMV enhancer) [20]. All hybrid sequences were verified by means of an ABI 310 auto-sequencer (Perkin-Elmer Corporation, Norwalk, CT).

3.4. CRP transfection into PEC

The cDNAs (5 μg) were introduced into PEC by lipid-mediated DNA transfection with lipofectamine (LIPOFECTAMINE™ Reagent, GIBCO/BRL). Transfected PEC were maintained in complete medium for several days in an atmosphere of humidified 5% CO_2 at 37 °C. The cells were then transferred to complete medium containing 1.0 mg/ml G418 (GIBCO/BRL) for selection.

3.5. Flow cytometry

Transfected cells (1×10^6) were incubated with 1 μg of mouse mAb anti-FLAG-tag (M2) (Sigma, St Louis, MO, USA) or an isotype antibody control (mouse IgG1, DAKO A/S, Denmark) for 30 min at 4 °C and then were incubated with 1.25 μg of a secondary FITC-labeled rabbit anti-mouse IgG Ab (ICN, Costa Mesa, CA) for 30 min at 4 °C.

Stained cells were analyzed using an FACS Calibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). Naive MYP-30 cells were used as controls.

3.6. Western blotting

The protein content of transfectant and parental cell lysates was quantified by the BCA method (Pierce, Rockford, IL, USA), and 20 μg aliquots of the obtained proteins were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis under non-reducing conditions and were then transferred electrophoretically to a nitrocellulose membrane (Schleicher & Schuell). The membrane was blocked in phosphate buffered saline containing 0.05% Tween 20 for 1 h at 25 °C, and then incubated with the anti-DAF mAb D17 (binding to the S/T region, a gift from Prof. T. Seya, Hokkaido Univ.) for 1 h at 25 °C. After washing, the blots were incubated with horseradish peroxidase-conjugated anti-rabbit antibody and developed using Chemi-Lumi One (Nakalai tesque, Kyoto, Japan) [21].

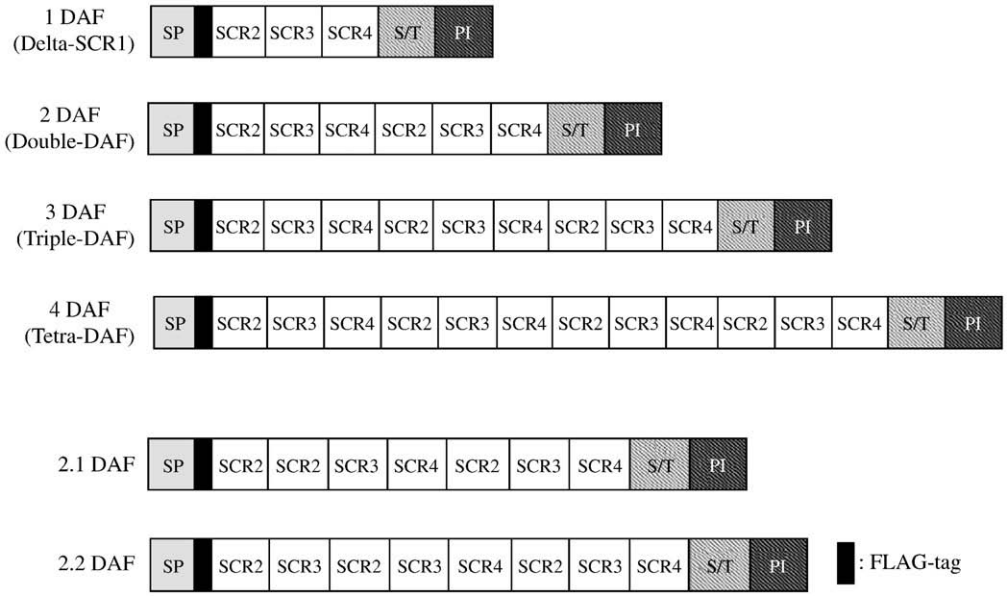

Fig. 1. Structure of the tandem forms of DAF. The following forms of DAF were constructed: delta-SCR1, consisting of SCR2-3-4 with a FLAG-tag (1-DAF); double-DAF, consisting of SCR2-3-4-2-3-4 with a FLAG-tag (2-DAF); triple-DAF, consisting of SCR2-3-4-2-3-4-2-3-4 with a FLAG-tag (3-DAF); and tetra-DAF, consisting of SCR2-3-4-2-3-4-2-3-4-2-3-4 with a FLAG-tag (4-DAF). In addition, 2.1-DAF, consisting of SCR2-2-3-4-2-3-4 with a FLAG-tag, and 2.2-DAF, consisting of SCR2-3-2-3-4-2-3-4 with a FLAG-tag, were also constructed. SP: signal peptide. S/T: serine/threonine rich region. PI: glycosyl phosphatidylinositol-anchor.