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*J Immunol* 1998; 160:3014-3022; ;
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Functional Differences Among Multiple Isoforms of Guinea Pig Decay-Accelerating Factor

Guixian Wang,*† Mayumi Nonaka,† Changqing He,† Noriko Okada,† Izumi Nakashima,* and Hidechika Okada‡

Decay-accelerating factor (DAF, CD55) is a membrane inhibitor that protects host cells from the autologous C-mediated attack. The guinea pig homologue of DAF consists of multiple isoforms generated by alternative splicing from a single copy gene. These isoforms are mainly comprised of a glycosylphosphatidylinositol (GPI)-anchored form and a transmembrane form (TM) that is not present in human DAF. Both forms occur in at least three variations that differ in the length of the Ser/Thr-rich region (termed ST-a, ST-ab, and ST-abc). We have transfected cDNAs of the six major isoforms into Chinese hamster ovary cells, and their functional differences were evaluated in inhibition of C-mediated cytosis and C3 deposition, using the transfectants expressing DAF at the same level on cell membranes. The degree of inhibition in both the classical and alternative pathways differed according to the length of the ST region in the order of abc > ab > a in both GPI and TM forms. When GPI and TM forms were compared, those with the ab or abc variation exhibited almost the same activity, whereas a-TM was less efficient than a-GPI. Although several isoforms are expressed constitutively in most of tissues, spermatozoa preferentially express the abc-GPI isoform, suggesting that this isoform offers effective protection to spermatozoa in the female genital tract. The Journal of Immunology, 1998, 160: 3014–3022.

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Received for publication July 15, 1997. Accepted for publication November 24, 1997.

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1 This work was supported by grants-in-aid from Ministry of Education, Science, Sports, and Culture, and from Ministry of Health and Welfare of Japan.

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3 Abbreviations used in this paper: DAF, decay-accelerating factor; CHO, Chinese hamster ovary; GPI, glycosylphosphatidylinositol; GPS, guinea pig serum; GVB, gelatin veronal-buffered saline; GVB2, gelatin veronal-buffered saline containing Ca2+ and Mg2+; MCP, membrane cofactor protein; PI, propidium iodide; SCR, short consensus repeat; SEC, secreted form; ST, serine/threonine-rich; TCL, putative form containing a transmembrane domain followed by a longer cytoplasmic domain; TCS, transmembrane form with a shorter cytoplasmic domain, referred to as TM in this work; TM, transmembrane form.

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In this study, we first investigated tissue distribution profiles of guinea pig DAF isoforms by Northern and Western blotting analyses, as well as by RT-PCR, using an expanded variety of guinea pig tissues. Second, to evaluate the functional differences among the DAF isoforms, we transfected the six major isoforms into Chinese hamster ovary (CHO) cells and measured their inhibition of C-mediated cytolytic and C3 deposition. The differences in tissue distribution and inhibitory activity observed suggested that the variability among isoforms of guinea pig DAF is of biologic significance.

Materials and Methods

Northern blotting

Total RNA was isolated from various tissues of adult guinea pigs (Std: Hartley, Japan SLC, Shizuoka, Japan) using the guanidine thiocyanate/CsCl method. Ten micrograms of total RNA were denatured with glyoxal and DMSO, as described (23), electrophoresed on a 1% agarose gel, followed by staining with ethidium bromide, and transferred to a Hybond-N nylon membrane (Amersham Japan, Tokyo). An approximately 0.9-kb fragment of guinea pig DAF cDNA corresponding to the SCR1-SCR4 region was labeled with \( \left[ {\text{32P}} \right]\text{dCTP} \) using the Megaprime DNA labeling kit (Amersham Japan) and analyzed by FACSCalibur (Becton Dickinson, Malvern, PA) was added and cells were kept on ice for 30 min. Finally, cells were suspended in sheath solution (FacsCalibur) and analyzed by FACSCalibur (Becton Dickinson, San Jose, CA). For detection of the CHO cells transfected with vector alone, PCR analysis was conducted using isolated DNA.

Reverse-transcriptase PCR

The following oligonucleotides were synthesized and used as primers: P1, 5'-GACACTTACGAATATAAG-3'; P2, 5'-TGGGAAACAGACCCGTATACCA 3'; P3, 5'-ATCGGTTCTGGTCCAG-3'; and P4, 5'-CAGCTAGCCATGATT-3' (Fig. 1A). cDNAs were prepared from various tissue RNAs using the cDNA Synthesis System (Amersham Japan). PCR amplification was performed at 95°C for 3 min, followed by 20 to 30 cycles of 95°C for 0.5 min; 42°C for 0.5 min; 72°C for 1 min; and 72°C for 5 min. The PCR products were analyzed on a 2% agarose gel or a 6% polyacrylamide gel.

Transfection

For the a-GPI, a-TM, and ab-TM isoforms of guinea pig DAF, full-length cDNAs of the isolated clones, GD18 (Gb6-GPITM type), GD10 (Gb6-TCS), and GD19 (Gb6-TCS), respectively, were cloned into the expression vector pCDM8 (Invitrogen Corp., San Diego, CA) as described previously (22). To construct a-GPI, abc-GPI, and abc-TM isoforms, clones GD10, GD18, and GD21 (Gb6-TCS) in pCDM8 were digested to two fragments with BglII, which is located in the SCR3 and S/T-d regions of the inserted DAF cDNA, and the DNA fragment containing the ST-a or ST-abc region was ligated with the fragment containing the GPI-anchor or the transmembrane region as well as the vector region. These plasmids or vector alone as a control were cotransfected with the neomycin-resistant plasmid, pSVneo (24), into CHO-K1 cells by the calcium phosphate-DNA precipitate method. CHO cells were cultured in Ham’s F12 medium, containing 0% FCS and antibiotics, and transfectants were selected using 400 \( \mu \text{g/m} \)l of Geneticin (Life Technologies, Grand Island, NY).

For detection of guinea pig DAF on the surfaces of the CHO cells, the transfected cells were removed from tissue culture plates with PBS containing 0.02% EDTA (EDTA-PBS). After washing, 1 \( \times \) 10\(^5\) cells were incubated on ice for 30 min with anti-guinea pig DAF mAb MCA44 (21). After washing twice with PBS, FITC-conjugated sheep anti-mouse IgG (Cooper Biomedica, Malvern, PA) was added and cells were kept on ice for an additional 30 min. Finaly, cells were suspended in sheath solution (Fujisawa Pharm., Osaka, Japan) and analyzed by FACS Calibur (Becton Dickinson, San Jose, CA). For detection of the CHO cells transfected with vector alone, PCR analysis was conducted using isolated DNA.

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Preparation of tissue lysates for Western blotting

Tissues and epididymal spermatozoa freshly obtained from a 14-week-old guinea pig were homogenized in lysis solution containing 0.1% Triton X-100, 1 mM PMSF, 5 mM EDTA, 10 mM iodoacetamide, 5 mM aprotinin, 10 μg/ml leupeptin, and 10 μg/ml pepstatin, and kept on ice for 30 min. After centrifugation at 15,000 × g for 10 min at 4°C, the supernatants were immunoprecipitated with MCA44. Aliquots of the redissolved precipitates were subjected to 8% SDS-PAGE. Transfected CHO cells lysed similarly were applied to SDS-PAGE without immunoprecipitation. Samples were electrophoretically transferred at 18 V onto a nitrocellulose membrane (Bio-Rad, Richmond, CA) at 4°C overnight in Tris-glycine buffer containing 20% methanol. After blocking at room temperature overnight with 0.05% Tween-20/PBS containing 2% (v/v) FBS, the membrane was incubated with 10 μg/ml of MCA44 for 1 h. After washing, it was treated with peroxidase-conjugated goat anti-mouse IgG (Jackson Immunoresearch Laboratories, West Grove, PA) for 1 h at room temperature, and bands were detected using a Konica Immunostain kit (Konica, Tokyo, Japan).

51Cr release cytotoxicity assay

A quantity amounting to 1 × 10⁶ transfected CHO cells was collected by EDTA-PBS and incubated with 100 μl of Na₂⁵¹CrO₄ for 60 min at 37°C. After washing twice with PBS, 2 × 10⁶ labeled cells were placed in wells of 96-well U-bottom plates and incubated with 50 μl of 1/100 rabbit anti-CHO antisera on ice for 30 min. Further incubation with 100 μl of various concentrations of guinea pig serum (GPS) diluted in GVB for 30 min with 100 μl of various concentrations of guinea pig serum (GPS) diluted in GVB was conducted for 60 min at 37°C, after which the plates were centrifuged at 1500 rpm for 5 min, and radioactivity in the supernatants was determined with an autogamma counter. Untreated CHO cells were used to measure the spontaneous release of 51Cr (control cpm), and cells treated with 5% Triton X-100 were used to determine the maximum release (max cpm) for each isoform. Cytotoxicity (%) was calculated as follows: [(sample cpm – control cpm)/(max cpm – control cpm)] × 100. Assays were performed in triplicate and at least three times.

C3 deposition assay

A quantity amounting to 1 × 10⁶ transfected CHO cells was treated on ice for 30 min with 100 μl of various concentrations of anti-CHO antisera. After washing twice, the cells were incubated with 10% GPS in GVB for 1 h at 37°C. Cells were washed with GVB containing 10 mM EDTA (EDTA-GVB) and then with PBS containing 1% NaN₃, after which they were incubated with FITC-conjugated goat anti-guinea pig C3 (Organon Teknika Corporation, Durham, NC) on ice for 30 min and washed three times. Finally, cells were stained with 20 μl of propidium iodide (PI) (0.5 μg/ml) for 3 min and then suspended in sheath solution. Flow-cytometric analysis was performed on a FACScalibur. PI-positive cells were excluded when fluorescence intensity was calculated.

For analysis of inhibition of alternative pathway-mediated C activation, 1 × 10⁶ transfected CHO cells in 200 μl of PBS were treated with 4 μl of neuraminidase (1 U/ml) for 30 min at 37°C. After washing twice with PBS, the cells were incubated with anti-CHO antiserum at 0°C for 30 min, and 10% GPS in 2 mM Mg·EGTA-GVB was then added. After a further incubation for 30 min at 37°C, cells were washed twice with EDTA-GVB and treated with FITC-conjugated goat anti-guinea pig C3, followed by PI staining. Only PI-negative cells were analyzed by FACScalibur.

Results

Tissue distribution of guinea pig DAF transcripts

Northern blotting analysis using 0.9-kb guinea pig DAF cDNA corresponding to the SCR1-SCR4 region as a probe revealed two bands of 2.4 to 2.5 kb and 1.6 to 1.8 kb in all tissues tested with several differences in pattern (Fig. 2). The previous study indicated that these bands correspond to two species with different lengths of the 3′-untranslated region (22). Among the various tissues examined, a significantly high expression of DAF was observed in placenta and lung. The different sizes observed among the smaller transcripts seemed to correspond to the isoforms detected in each tissue by RT-PCR, as described below (Fig. 3).

Figure 3 shows the tissue distribution of the expressed isoforms and their relative amounts, as determined by RT-PCR analysis. Figure 3A shows the PCR products obtained with the region between the SCR4 and G/G′/G″ regions (Fig. 1A), indicating six major isoforms expressed in each tissue. Figure 3B shows the PCR products obtained with the region between the SCR4 and ST-d regions, which include the ST-a, ab, and abc isoforms, and Figure 3C shows the PCR products obtained with the region between the ST-d and G/G′/G″ regions, which include the GPI and TCS (TM) isoforms. Multiple isoforms were noted in most tissues, but their relative amounts were different. A strong predominance of GPI-anchored isoforms was observed in intestine and testis, while TM isoforms were predominant in lung, bladder, ovary, and fetal lung. RT-PCR analysis was performed using RNA from at least three guinea pigs, excluding tissues from placenta, ovary, and fetal organs, and similar results were obtained, although the precise ratios differed slightly. These results were in agreement with the transcript sizes observed with Northern blotting analysis. That is, the transcripts of 1.6 to 1.8 kb in intestine and testis apparently have a smaller size than those detected in lung and fetal lung (Fig. 2). It is noteworthy that the abc isoform was predominant in testis, but not in all other tissues. Since a predominance of the abc-GPI isoform is more characteristic of mature guinea pigs than of immature ones (data not shown), the abc-GPI isoform was expected to be expressed in the spermatozoa.

Transfection of guinea pig DAF isoforms into CHO cells

For transfection, we used cDNAs of the six major isoforms of guinea pig DAF, a-GPI, ab-GPI, abc-GPI, a-TCS, ab-TCS, and abc-TCS. A previous investigation indicated that the GPI and TCS isoforms are expressed on cell membranes (22). The TCL isoform contains the same hydrophobic sequence as the transmembrane domain of the TCS isoform (Fig. 1A). However, a preliminary experiment showed that the TCL isoform was virtually undetected on transfected CHO cell membranes, although it was present in the medium (data not shown). Therefore, TCS will be referred to hereafter in this work as TM. Schematic diagrams of these isoforms are shown in Figure 1B.
These isoforms were stably transfected into CHO cells with a neomycin-resistant plasmid. The level of expression of DAF isoforms on transfectant CHO cells was determined by flow cytometry, and the sizes of the expressed proteins were confirmed by Western blotting of the cell lysates using anti-guinea pig DAF mAb MCA44 (21).

Western blotting of the transfected CHO cells and the tissue lysates

Figure 4A shows results of Western blotting analysis of the transfected CHO cells detected by MCA44. Compared with 44Ag (left lane), which is guinea pig DAF purified from erythrocytes using mAb MCA44 (21), the a, ab, and abc isoforms corresponded to three bands of 55, 70, and 88 kDa, respectively. A difference in size between the GPI and TM forms was not detected in this analysis.

Figure 4B shows results of Western blotting of tissue lysates from various guinea pig organs. Each tissue displayed detectable levels of at least three bands of guinea pig DAF, except for spermatozoa, in agreement with the results of RT-PCR (Fig. 3). Spermatozoa showed only one band, which obviously corresponded to the abc-GPI isoform seen with RT-PCR (Fig. 3). This was confirmed by the fact that normal adult testis (testis 1) showed a strong band in the same position as in the spermatozoa, while testis 2 from the guinea pig with few spermatozoa in its epididymis did not (Fig. 4B). Spermatozoa DAF is smaller than the abc isoforms of other tissues. This may be due to a difference in glycosylation, as described for human spermatozoa DAF (25). However, this point remains to be confirmed.

Cytotoxicity assay

To investigate differences in the capacity of these isoforms to inhibit complement, the CHO transfectants of each isoform were compared with respect to their susceptibility to classical pathway-mediated cytolysis, as measured by $^{51}$Cr release. CHO cells were sensitized for complement activation by incubating with 1/100 rabbit polyclonal anti-CHO antiserum, and guinea pig serum diluted in GVB2 was used as a source of C. As a control, CHO cells transfected with the vector alone were treated similarly.
expressing four different levels of DAF were first compared for their susceptibility to cytosis. Figure 5 shows the percentage of cytotoxicity of four transfectants expressing the abc-TM isofom at different levels (A), and their extent of DAF expression was detected by flow cytometry (B). Increased DAF expression on cell membranes paralleled a decreased susceptibility to C-mediated cytoprosis. Similar results were obtained using transfectants of other isoforms (data not shown). These results indicated that the inhibition observed was caused by guinea pig DAF expressed on the cell membranes.

We then analyzed functional differences among CHO cells transfected with each of the six isoforms: a-GPI, ab-GPI, abc-GPI, a-TM, ab-TM, and abc-TM. For comparison, we used CHO transfectants with the same levels of DAF expression, as determined by flow-cytometric analysis (Fig. 6). As shown in Figure 7, all isoforms on CHO cells inhibited classical C-mediated cytolysis, although the length of the ST region affected the activity of both GPI and TM forms. In other words, ST-abc isoforms inhibited cytolysis most effectively, while the ST-a isoforms exhibited the least inhibitory capacity. When the GPI (Fig. 7A) and TM (Fig. 7B) forms were compared, the ab and abc isoforms exhibited an almost identical efficiency, while a significant difference was observed between the ST-a isoforms of GPI- and TM-DAF.

**C3 deposition assay**

For further elucidation of the inhibitory effect of these isoforms, we investigated inhibition of C3 deposition using the same transfectant cells. After incubation of the anti-CHO-sensitized transfec-tant cells with 10% GPS in GVB would, the extent of classical pathway-mediated C3 deposition on PI-negative cell membranes was analyzed by flow cytometry using FITC-conjugated anti-guinea pig C3. As shown in Figure 8, the transfectant CHO cells avoided C3 deposition more successfully than did control CHO cells. Differences among the isoforms were similar to those observed in the cytotoxicity assay, that is, the ST-abc isoforms showed the highest efficiency, while the ST-a isoforms exhibited the lowest in both the GPI and TM forms (Fig. 8, A and B). The observed differences were more significant than those seen in the cytotoxic assay. In addition, both forms of DAF were almost equivalent in terms of the efficiency of their abc isoforms, and there was only a slight difference in the ab isoforms, while the a-TM isoform displayed significantly lower inhibition in CHO cells than did the a-GPI isoform (Fig. 8C).

**Inhibition of alternative pathway-mediated C3 deposition**

To assay alternative pathway-mediated C activation, transfected CHO cells were treated with neuraminidase, and 10% GPS in 2 mM Mg* · EGTA-GVB was used as a source of C. As shown in Figure 9, results were similar to those obtained in the classical pathway-mediated C assay, although the differences among isoforms were smaller. That is, all transfectants showed inhibitory activity, but the degree of inhibition in both the GPI and TM forms differed according to the length of the ST region in the order of abc > ab > a (Fig. 9, A and B). When the GPI and TM forms were compared, the a-TM isoform had significantly less effect than the a-GPI isoform, while differences between the ab or abc isoforms were not significant (Fig. 9C). Even using the transfectants poorly expressing the abc isoforms at the same level as abc-TM2 in Figure 5, no difference was observed between the GPI and TM forms of DAF (data not shown).

**Discussion**

DAF shows structural variability that is dependent on species. Human DAF has two isoforms generated by alternative splicing; one is a GPI-anchored form and the other is a secreted form that is produced in an amount one-tenth that of the GPI form (9). Guinea pig DAF exists as multiple isoforms that are generated by alternative splicing and include GPI-anchored, TM, and secreted forms with ST regions of various lengths (22). On the other hand, mouse DAF consists of two isoforms, a GPI-anchored form and a TM form, that are produced from two separate genes (26). In human and mouse DAF, no variations in the ST region have been found.
In this study, we analyzed the tissue distribution and functional differences of the multiple isoforms of guinea pig DAF. Guinea pig DAF is distributed in a wide variety of tissues, as it is in human (27, 28), as seen with Northern blotting (Fig. 2). The reason for the significantly high expression of guinea pig DAF in placenta and lung remains unknown. It was shown that mouse DAF is expressed preferentially in lung and testis (26), whereas in guinea pig, DAF expression in testis is not as significant. The existence of another complement membrane inhibitor such as MCP seems to contribute to DAF tissue distribution. Human DAF and MCP are distributed in many tissues (27–29). However, guinea pig MCP is expressed preferentially in testis (30), while DAF has a much broader distribution in this species. Although mouse DAF is expressed preferentially in tissues such as lung and testis, Crry, which is the structural homologue of human CR1, but with DAF and MCP activities, is widely distributed (31, 32). From the above findings, it is suggested that these proteins protect most of the body tissues from C-mediated cytolysis.

**FIGURE 7.** \(^{51}\)Cr release assay mediated by the classical pathway. A, Results for the GPI isoforms. B, Results for the TM forms. Transfectant CHO cells and control CHO cells (vector only) were labeled and incubated with anti-CHO Ab (1/100) and various dilutions of 10% GPS in GVB. Cytotoxicity was measured by \(^{51}\)Cr release, as described in Materials and Methods. The experiments were performed several times in triplicate, and error bars represent SD.

**FIGURE 8.** C3 deposition assay mediated by the classical pathway. A, Results for the GPI isoforms. B, Results for the TM isoforms. C, Comparison of the GPI and TM isoforms using ST regions of the same length based on the data of A and B. Transfectant CHO cells and control CHO cells (vector only) were taken from tissue culture plates and incubated with various dilutions of anti-CHO Ab and 10% GPS in GVB. The cells were then incubated with FITC-conjugated goat anti-GP C3. C3 deposition on CHO cells was analyzed by flow cytometry. Only PI-negative cells selected by gating were used for calculations. Data are representative of three separate experiments.
We next showed, using RT-PCR, that the multiple isoforms of guinea pig DAF are expressed in most of the tissues examined, although the relative amounts of the various isoforms differed from tissue to tissue. The GPI-anchored form was obviously predominant in tissues such as intestine and testis, while a predominance of the TM form was observed in lung, bladder, ovary, and as well as in fetal lung. Although significant expression of total DAF was observed in placenta and lung by Northern blotting, these tissues exhibited different patterns of isoform distribution. It is noteworthy that the TM form of DAF in mouse is expressed preferentially in testis (26, 33), while most of DAF expressed in guinea pig testis are the GPI forms. Since mouse testis also expresses the GPI form, two isoforms of mouse DAF might be distributed differently in this tissue and involved in the different roles. By means of Western blotting analysis, wide distribution of DAF was confirmed at the protein level. Although we could not distinguish the GPI and TM forms with this method, three bands corresponding to the a, ab, and abc isoforms were detected in most tissues. In addition, spermatozoa was shown to express DAF preferentially of the abc isoform.

To investigate the functional differences among the isoforms of guinea pig DAF, we transfected the six major isoforms, a-GPI, ab-GPI, abc-GPI, a-TM, ab-TM, and abc-TM, into CHO cells and measured their inhibitory effects against classical pathway-mediated C3 deposition as well as alternative pathway-mediated C3 deposition. With respect to human DAF, the difference in efficiency between the GPI and TM forms (34) and the effect of the length of the ST region on cytotoxicity (35) have already been investigated using artificial isoforms. In this study, however, we used the naturally occurring isoforms that were detected in most of the guinea pig tissues as major products. In all experiments in which the GPI and TM forms were compared, no significant differences were detected when we used the ST-ab or ST-abc transfectants, but when the ST-a isoforms were used, the TM form had much less of an inhibitory effect than the GPI form. Lublin and Coyne (34) reported that GPI-anchored human DAF and transmembrane rDAF are equally efficient in protecting against cytolysis. It is presumed that no difference was detected in their experiments since the ST region of human DAF is approximately twice the size of the guinea pig ST-a region. With a very short ST region as in ST-a, the GPI-anchored form seems to be advantageous in protecting against C-mediated cytolysis compared with the TM form, probably because of its mobility. Since the ST region has many putative O-glycosylation sites, sugars might affect lateral mobility. However, the biologic implication of the preferential expression of the GPI or TM form in some tissues remains to be elucidated.

On the other hand, the length of the ST region of guinea pig DAF significantly affected the inhibitory effects. Coyne et al. (35) demonstrated that the ST region of DAF plays a role as a spacer, by using a mutant human DAF lacking an ST region and a fusion construct with four SCRs together with the C-terminal region of another transmembrane protein. Our study indicates that a longer ST region appears advantageous in protection against C-mediated cytolysis. Similar results were obtained with human MCP (36). Human MCP also has multiple isoforms similar to guinea pig DAF, including ST regions of three different lengths, termed ABC, BC, and C, which are produced by alternative splicing of three exons of A, B, and C, and possess two types of cytoplasmic tails, termed CYT1 and CYT2 (reviewed in Ref. 37). Liszewski and Atkinson (36) have reported that the BC isoform displays a higher degree of inhibition classical pathway-mediated cytolysis than does the C type, independent of the difference in the cytoplasmic.

**FIGURE 9.** C3 deposition assay mediated by the alternative pathway. A, Results for the GPI isoforms. B, Results for the TM isoforms. C, Comparison of the GPI and TM isoforms using ST regions of the same length based on the data of A and B. Neuraminidase-treated transfectant CHO cells were incubated with various dilutions of anti-CHO Ab and 10% GPS in 2 mM Mg·EGTA-GVB. The cells were then incubated with FITC-conjugated goat anti-GP C3. C3 deposition on CHO cells was analyzed by flow cytometry. Only PI-negative cells selected by gating were used for calculations. Data are representative of three separate experiments.
The ST-abc isofoms provide the highest degree of cell protection. However, except for spermatozoa, the ST-abc isofom is not predominant in most guinea pig tissues. As we discussed in the previous paper (22), variability in the structure of the ST region on membranes might also be important for protection since it would facilitate the action of C inhibitors by restricting C activation, which can occur at any membrane site. Since the inhibitory effects depend on the amount of DAF expressed (Fig. 5), a low level of expression of the abc isoform may be sufficient for protection against C attack. In spermatozoa, the abc-GPI isoform of DAF may be expressed preferentially to provide protection in the female genital tract, where intense protection is needed. In this respect, it is interesting that guinea pig MCP, which is expressed preferentially in testis, has only one domain of the ST region corresponding to the Ser/Thr/Pro-rich C domain of human MCP (30). In guinea pig spermatozoa, DAF may be the major player in protecting against the C system, and MCP may be involved in other systems such as sperm-egg recognition, as suggested in humans (38).

Our findings also indicated that for models of xenotransplantation using guinea pig DAF, there should be as much expression of the abc isoform as possible on the surface of the graft to minimize the incidence of hyperacute rejection caused by C. The precise mechanisms of protection afforded by DAF and other C membrane inhibitors against autologous C attack remain unresolved. Recently, we have reported that the intron following the exon encoding the ST-abc region is composed of the same repetitive sequence as the ST-abc region in all DAF genes of the species tested to date. Interestingly, the intron sequences showed the possibility that DAF isoform with a longer ST region might be expressed since it had no stop codon when they were presumably translated in the same reading frame as the franking exons (39). Further investigations using mutant forms of DAF such as those with longer ST regions would be useful for elucidating the mechanisms responsible for this protection.

Acknowledgments

We thank Dr. William Campbell for helpful discussion and Ms. Catherine Campbell for English editing of this manuscript.

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