

# Painting Qa-2 onto *Ped slow* Preimplantation Embryos Increases the Rate of Cleavage

ABIGAIL S. McELHINNY, GINGER E. EXLEY, AND CAROL M. WARNER

*McElhinny AS, Exley GE, Warner CM. Painting Qa-2 onto Ped slow preimplantation embryos increases the rate of cleavage. AJRI 2000; 44:52–58 © Munksgaard, Copenhagen*

**PROBLEM:** Qa-2 protein, the *Ped* gene product, is linked to the cell surface by a glycosylphosphatidylinositol (GPI) anchor. Some GPI-linked proteins can be spontaneously incorporated into the membranes of cells via a technique called “protein painting.” We investigated whether Qa-2 could be painted onto T cells and embryos and whether the painted protein would be functional.

**METHOD OF STUDY:** Incorporation of Qa-2 into the membranes of T cells and embryos was measured by FACScan and Immuno-PCR, respectively. Function of Qa-2 was measured by cell proliferation.

**RESULTS:** Qa-2 was incorporated by T cells and embryos and was functional.

**CONCLUSION:** GPI-linked Qa-2 protein “painted” onto both T cells and preimplantation embryos is functional, as shown by increased proliferation of T cells after cross-linking with anti-Qa-2 antibody, and increased rate of cleavage division of the embryos.

Key words:

Cleavage, Immuno-PCR, MHC, *Ped* gene, protein painting, T-cell activation

ABIGAIL S. McELHINNY  
Department of Cell Biology and  
Anatomy, University of Arizona,  
Tucson, Arizona

GINGER E. EXLEY  
CAROL M. WARNER  
Department of Biology,  
Northeastern University, Boston,  
Massachusetts

Address reprint requests to  
Carol M. Warner, Northeastern  
University, Biology Department,  
414 Mugar, 360 Huntington  
Ave., Boston, MA 02115.

E-mail address: cmw@neu.edu

Submitted November 8, 1999;  
accepted November 20, 1999.

## INTRODUCTION

Many years of research have shown that the product of the *Ped* gene, Qa-2, controls the rate of embryonic cleavage during the preimplantation stages in the mouse.<sup>1</sup> Mice lacking functional Qa-2 produce preimplantation embryos that cleave slowly. Although these *Ped slow* strains are able to reproduce, the litters are smaller and the pups are underweight both at birth and at weaning.<sup>2</sup>

Qa-2 is a class Ib major histocompatibility complex (MHC) protein. While structurally similar to class Ia MHC proteins that present antigens to T cells, class Ib proteins have different or unknown functions and are encoded by genes in a separate region of the MHC. Two congenic strains of mice that differ only at the Qa-2 locus are B6.K1, which carries a deletion of the Qa-2 locus and is *Ped slow*, and B6.K2, which is Qa-2 positive and *Ped fast*.<sup>3,4</sup>

Qa-2 is different from other class Ia and class Ib proteins because it is linked to the cell surface by a glycosylphosphatidylinositol (GPI) anchor

instead of a transmembrane domain. GPI-linked proteins have been found in all eukaryotes and perform a wide variety of functions, including antigenic functions, enzymatic digestion, cell adhesion, and ligand-mediated signal transduction.<sup>5,6</sup> The GPI anchor is added post-translationally and penetrates only the outer leaflet of the cell membrane. Nonetheless, many GPI-linked proteins are able to transduce signals across the cell membrane by interacting with accessory signal transduction proteins. Qa-2 is expressed on lymphocytes in the adult and can trigger T-cell activation when cross-linked with antibodies.<sup>7,8</sup>

Within the past decade, it has been shown that GPI-anchored proteins can be spontaneously and stably incorporated into the membranes of recipient cells.<sup>9,10</sup> In many cases, the exogenous GPI-linked protein is functional and can confer a new phenotype to the recipient cell. The purpose of these experiments was to determine whether exogenously added GPI-linked Qa-2 protein could confer the *Ped fast* phenotype to *Ped slow* recipient embryos, enhancing the rate of preimplantation cleavage.

## MATERIALS AND METHODS

### *Mice*

The congenic B6.K1 (Qa-2 negative) and B6.K2 (Qa-2 positive) strains were originally obtained from L. Flaherty (Wadsworth Center, Albany, NY) and maintained in our laboratory. B6.129 mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and bred in our laboratory. Mice were provided with food (NIH31M) and water *ad libitum* and kept on a light/dark cycle in which the lights came on at 4 am and went off at 6 pm Eastern standard time.

### *Preparation of Qa-2 Protein Paint*

For Experiment A, lymphocytes were isolated from B6.K1 and B6.K2 mice, two spleens per sample. The cell density was adjusted to  $5 \times 10^7$  cells/mL in lysis buffer (50 mM NaCl, 2% NP-40). A mammalian protease inhibitor cocktail (Sigma, St Louis, MO) was then added at 100  $\mu$ L per mL of lysate. The suspension was vortexed vigorously for several seconds and incubated on ice for 1–2 hr, with intermittent vortexing to ensure lysis. The lysates were centrifuged at  $1500 \times g$  for 10 min at 4°C, then at  $10,000 \times g$  for 15 min at 4°C to remove non-soluble material. Lysates were stored at  $-20^\circ\text{C}$  prior to concentration. For concentration and simultaneous detergent removal, 2 mL of lysate was centrifuged

through each Centricon-10 column (Millipore, Bedford, MA) at  $5000 \times g$  for 1 hr. The centrifugation step was repeated five times, and the retentate was diluted with PBS to a final volume of 2 mL with each spin, in order to ensure removal of the detergent. The final retentate was aliquoted and stored at  $-20^\circ\text{C}$ .

For Experiment B, splenic lymphocytes from B6.K1, B6.K2, and B6.129 mice (two spleens per sample) were pelleted by centrifugation at  $300 \times g$  and resuspended by pipetting up and down in PBS containing 1 mM EDTA and 1 mM EGTA (PBSEE) at a cell density of  $1-2 \times 10^7$  cells/mL. The tubes were kept on ice and 40  $\mu$ L of a  $25 \times$  stock of Complete™ Protease Inhibitor Cocktail, EDTA-free (Boehringer Mannheim GmbH, Mannheim, Germany) were added per mL of the cell suspension. A total of 20  $\mu$ L of 5% saponin were added per mL of suspension to a final concentration of 0.1%. The samples were mixed well and incubated for 15 min on ice. Next, 1  $\mu$ L of NP-40 was added per mL of lysate to a final concentration of 0.1%. The samples were mixed well and incubated for 45 min on ice. The lysates were centrifuged at  $1500 \times g$  for 10 min. The supernatants were centrifuged at  $10,000 \times g$  for 15 min. The supernatants were flash frozen in liquid nitrogen and stored at  $-20^\circ\text{C}$  prior to concentration. For concentration and detergent removal, each mL of lysate was added to a pre-rinsed Centricon-10 column and diluted with 1 mL of PBSEE. Complete™ Protease Inhibitor Cocktail (45  $\mu$ L) was added to each column and the columns were centrifuged at  $5000 \times g$  in a fixed angle rotor for 1 hr. The volume of the retentate was brought to 2 mL with another 45  $\mu$ L of  $25 \times$  protease inhibitors and PBSEE and spun for another hr. This step was repeated again, then the retentates were transferred to fresh pre-rinsed Centricon-10 columns. The retentates were diluted to 2 mL in PBS and centrifuged for 1 hr. This step was repeated once. The final retentates were then aliquoted, flash frozen in liquid nitrogen, and stored at  $-20^\circ\text{C}$ .

### *Painting of Splenocytes and Purified T cells*

Purified splenocytes were isolated by Ficoll-Hypaque centrifugation. T cells were isolated by nylon wool chromatography of the splenocytes. Purified T cells were washed three times in PBS to remove residual FCS (Experiment A). In both Experiment A and Experiment B, the cells were diluted to a concentration of  $2.5 \times 10^6$ /mL in PBS, and 100  $\mu$ L of the suspension was added to each FACScan tube. A total of 50  $\mu$ L of crude splenic lysate (diluted in PBS) was then added to each tube. The tubes were incubated at

37°C, 5% CO<sub>2</sub> for 1 hr. The cells were then washed two (Experiment B) or three (Experiment A) times in 300 µL PBSAZ (PBS containing 1% bovine serum albumen [BSA] and 0.1% sodium azide) per wash. The supernatant was discarded and the cells were resuspended in 50 µL PBSAZ per tube. For immunostaining, 50 µL of monoclonal antibody to the GPI-linked protein Qa-2 (1-12-1, 2.5–5 µg/mL)<sup>11</sup> was then added to each tube and the tubes were incubated for 45–60 min at 4°C. The cells were washed with 300 µL PBSAZ, then resuspended in 50 µL PBSAZ. A total of 50 µL of FITC-conjugated secondary antibody (goat anti-mouse IgG Fc; ICN, Aurora, OH) diluted to 1/400 in PBS was added to each tube and the tubes were incubated for 30–60 min at 4°C. The cells were washed again with 300 µL PBSAZ as described above, then resuspended in 100 µL PBSAZ. The cells were fixed by adding 200 µL PBSAZ containing 3% formaldehyde or paraformaldehyde. Samples were stored at 4°C and analyzed on a FACScan flow cytometer using LYSIS software (Becton-Dickinson, Rutherford, NJ).

#### *Activation of Painted T Cells*

After incubation in crude lysate as previously described, painted T cells were washed and resuspended in RPMI 1640 complete medium. The samples were transferred to the wells of a 96-well plate (time 0). After 0, 2, or 8 hr of incubation at 37°C in 5% CO<sub>2</sub>, half of each sample was treated with monoclonal antibody to the GPI-linked protein Qa-2 (1-12-1, 5 µg/mL), followed by secondary antibody (50 µg/mL rabbit anti-mouse IgG; ICN) and PMA (5 ng/mL; Sigma, St Louis, MO) in RPMI 1640. The other half of each sample was non-specifically activated with a 1/100 dilution of PHA (Gibco BRL). The cells were incubated for 48 hr at 37°C in 5% CO<sub>2</sub>. After the first 42 hr of incubation, 125 µL of an 8 µCi/mL solution of [<sup>3</sup>H-TdR] (specific activity = 20 Ci/mmol; NEN-Dupont, Wilmington, DE) was added to each sample. After the final 6 hr of incubation, the cells were harvested onto filtermats (Skattron, Lier, Norway) using a Titertek cell harvester connected to a carboy of distilled water. The filters were dried under vacuum at 65°C for 30 min, then counted in a liquid scintillation counter in toluene fluor.

#### *Painting of Embryos*

Female mice were superovulated with 5 IU eCG (Sigma, St Louis, MO) at the ninth hr of the light cycle, followed by 10 IU hCG (Sigma, St Louis, MO) 48 hr later, prior to mating. Preimplantation

embryos were collected in Whitten-Biggers (WB) medium under gas (5% CO<sub>2</sub>, 5% O<sub>2</sub>, 90% N<sub>2</sub>). The embryos were washed three times in WB with no BSA. Embryos were then incubated in crude lysate diluted in WB, no BSA, at room temperature for 45 min. The embryos were then washed three times in WB, and either cultured in microdrops of WB under oil at 37°C, 7% CO<sub>2</sub> for 24–48 hr, or placed into PBSAZ for Immuno-PCR (I-PCR) analysis.

#### *Immuno-PCR*

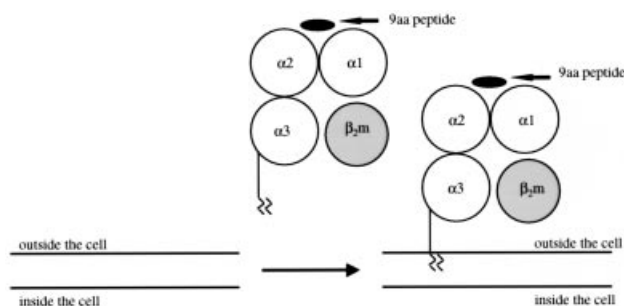
Preimplantation embryos were painted as described above, then assessed for incorporation of Qa-2 by I-PCR as described in McElhinny and Warner.<sup>12</sup> Painted embryos were washed once in PBSAZ and the zonae pellucidae were removed by incubation in Acid Tyrode's solution (pH 2.5) for 1–2 min. The embryos were washed three times in PBS plus 0.1% sodium azide (PBSZ), then incubated for 1–2 hr in 50–500 µg/mL of the monoclonal antibody 1-12-1 at 4°C. The embryos were washed five times in PBST (PBSZ plus 0.02% Tween<sup>®</sup> 20) and five times in PBSZ, then incubated for 45–60 min at 4°C in a 1 × 10<sup>-15</sup> M solution of a chimeric protein–DNA complex formed by the conjugation of a biotinylated fragment of pUC19 to streptavidin-protein A.<sup>13,14</sup> The embryos were then washed five times in PBST and five times in PBSZ, then transferred to PCR tubes in a minimal volume. Pipettes were changed with each wash. PCR was carried out for 30 cycles using Perkin-Elmer reagents (Branchburg, NJ) in 3 mM MgCl<sub>2</sub>, 1 × PCR buffer, 0.2 mM dNTPs, 2.5 U Taq polymerase, and 0.2 µM each primer (forward = 5'-CAGGCAACTATGGATGAACGA-3'; reverse = 5'-GGCGAAACCCGACAGGACTAT-3'). PCR products were analyzed by electrophoresis through 6% polyacrylamide mini-gels followed by ethidium bromide staining.

#### *Embryonic Cell Counts*

Embryos were incubated in 200 µL drops of WB containing 5 µg/mL bisbenzamide (Sigma, St Louis, MO) at 37°C for 1 hr in 5% CO<sub>2</sub>. The embryos were washed twice in WB, then mounted in 10 µL WB on a microscope slide. After applying a coverslip with slight pressure, the embryos were immediately observed under UV light with an Olympus microscope at 200 × and stained nuclei were counted.

#### *Statistical Analysis*

The difference in mean cell number was assessed by using Student's *t*-test.



*Fig. 1.* Model for painting Qa-2 onto the cell surface. Qa-2, like other Class I MHC proteins, is composed of  $\alpha 1$ ,  $\alpha 2$ , and  $\alpha 3$  domains non-covalently bound to  $\beta_2$ -microglobulin. Peptide is bound to a cleft between  $\alpha 1$  and  $\alpha 2$ . In contrast to other Class I proteins, Qa-2 has a GPI linkage instead of a transmembrane domain, by which it is able to insert into the outer leaflet of the cell membrane on recipient cells.

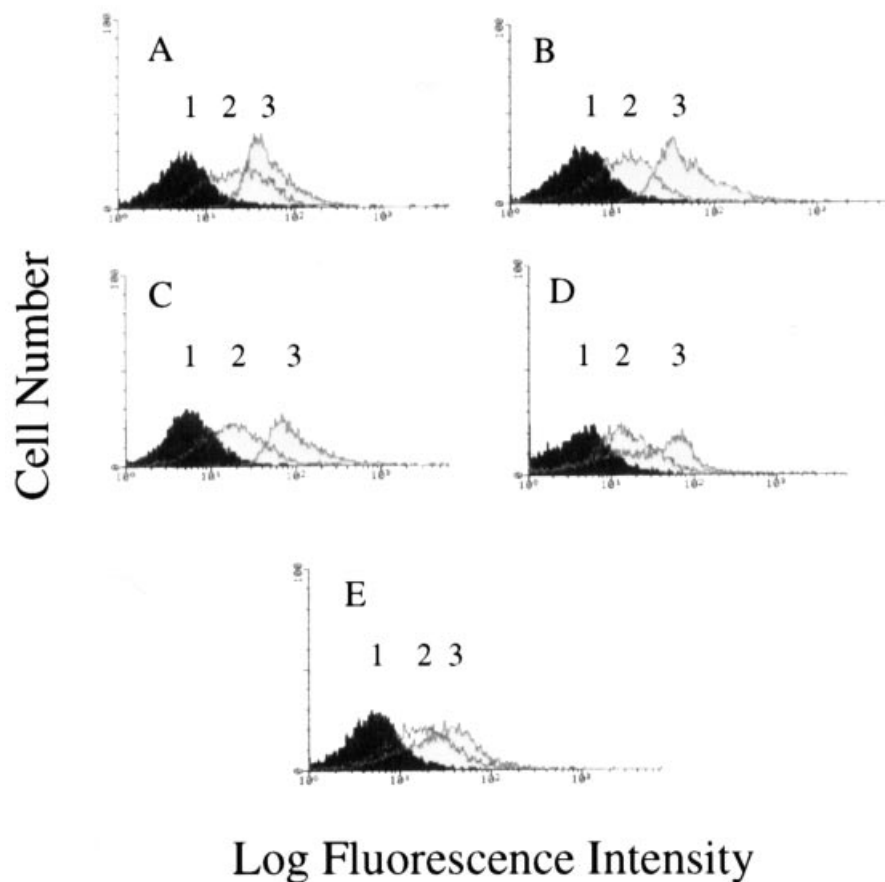
## RESULTS

### *Experiment A*

Initial experiments were performed on purified T cells to conserve embryonic material. We first tested whether exogenous Qa-2 could be detected by FACScan analysis on the surface of painted B6.K1 (Qa-2-

negative) T cells. When B6.K1 T cells were painted with an NP-40 crude lysate from B6.K2, exogenous Qa-2 incorporation could be detected at final dilutions of 1/3-1/3000 (Figs. 1 and 2). We also confirmed that the painting process was inhibited by the presence of BSA or by incubation at 4°C (data not shown) as has been reported by others.<sup>10</sup> We next tested whether the incorporated Qa-2 was functional by cross-linking Qa-2 with a specific monoclonal antibody in the presence of PMA and assaying for incorporation of <sup>3</sup>H-TdR as a measure of cellular proliferation (Fig. 3). Half of the cells in each treatment group were non-specifically activated with PHA to show that the cells were capable of activation. When cross-linking with monoclonal antibody was performed immediately after painting (0 hr) or 2 hr after painting, no <sup>3</sup>H-TdR incorporation could be detected, although the cells could be activated by adding PHA. When cross-linking was performed 8 hr after treatment with a 1/10 dilution of B6.K2 lysate, <sup>3</sup>H-TdR incorporation could be detected in the absence of PHA.

We next tested the effects of the crude NP-40 lysate on eight-cell embryos. Some embryos were processed for I-PCR immediately after painting to confirm the presence of exogenous Qa-2 on the surface of B6.K2



*Fig. 2.* Incorporation of Qa-2 onto the cell surface of Qa-2-negative T cells: Experiment A. B6.K1 T cells were painted with different dilutions of NP-40 lysates and analyzed by immunostaining and FACScan. Peak 1: Cells painted with PBS only, autofluorescence; Peak 2: Cells painted with B6.K1 lysate as a negative control; Peak 3: Cells painted with B6.K2 lysate. Panel A: final concentration of lysate = 1/3; Panel B: final concentration of lysate = 1/6; Panel C: final concentration of lysate = 1/30; Panel D: final concentration of lysate = 1/300; Panel E: final concentration of lysate = 1/3000.

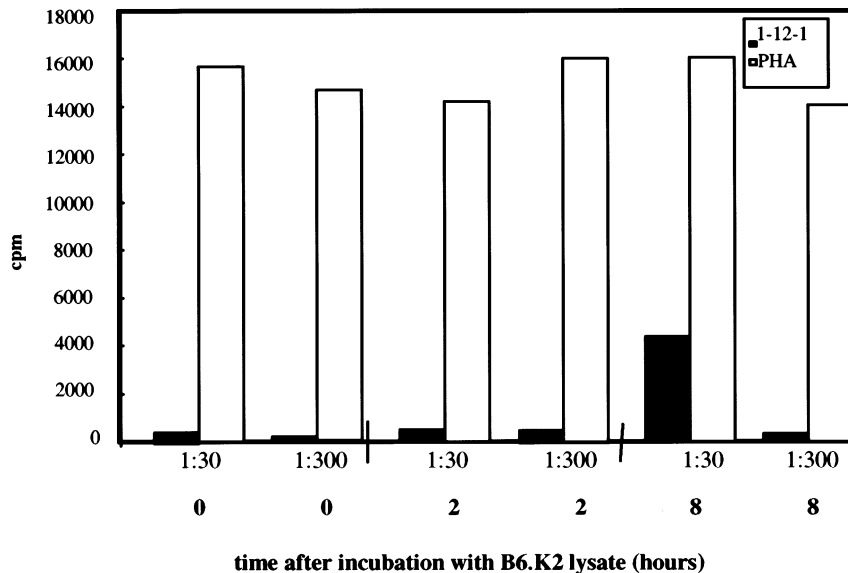


Fig. 3. At 0, 2, or 8 hr after painting T cells with 1/30 and 1/300 dilutions of B6.K2 splenic lysate, half of each sample was cross-linked with antibody to Qa-2 and half was non-specifically activated with PHA. Activation of cellular proliferation was assessed by measuring the incorporation of <sup>3</sup>H-TdR.

painted embryos. The results are shown in Fig. 4. Qa-2 was detected on the surface of B6.K1 embryos painted with B6.K2 lysate but not on B6.K1 embryos painted with B6.K1 lysate or left untreated. Eight-cell B6.K1 embryos were painted and cultured for 24 or 48 hr before staining with bis-benzamide for cell counts. The lysate was toxic to the embryos at high concentrations (diluted 1/20) but non-toxic at 1/100. The results are summarized in Table I. Although the embryos survived after treatment with a 1/100 dilution of the B6.K2 lysate, no significant increase in the cleavage rate relative to untreated embryos or embryos treated with B6.K1 lysate was detected after 24 or 48 hr of culture. However, treatment with a 1/200 dilution of B6.K2 lysate resulted in a significant increase in cleavage rate relative to untreated and B6.K1-painted embryos after 48 hr of culture.

#### Experiment B

A limiting factor to the effectiveness of painting embryos with crude lysate is the toxicity of residual NP-40 in the lysate. NP-40 has a very low critical micelle concentration (cmc, 0.30 mM) above which it forms micelles (88 kDa) that are larger than Qa-2 itself (40 kDa), making it difficult to remove by methods based on pore size of the filtration membrane.<sup>15</sup> It seemed that it would be advantageous to extract Qa-2 using less detergent. We found that Qa-2 could be extracted from splenic lymphocytes by pre-treatment with 0.1% saponin and extraction with 0.1% NP-40. In addition, we found that higher levels of Qa-2 could consistently be extracted from B6.129 splenocytes than from B6.K2 splenocytes. Extracted Qa-2 could be detected on painted splenocytes at dilutions of 1/18

to 1/30 (Fig. 5). However, all lysates were toxic to embryos at 1/30. A B6.K1 lysate was non-toxic at 1/100 (90% of painted eight-cell embryos matured to the blastocyst stage), but the B6.129 lysate tested was still toxic at this dilution (data not shown).

## DISCUSSION

In Experiment A, we showed that Qa-2 can be successfully extracted from B6.K2 splenocytes, and that it can be incorporated into the membranes of recipient cells from crude NP-40 lysates and retain its original function. However, in both recipient T cells and recipient embryos, functionality was not apparent immediately after the painting reaction. Instead, an equilibration period was required before Qa-2 function could be detected. This result is consistent with studies on other painted GPI-linked proteins that also required an equilibration period before functionality could be detected.<sup>16,17</sup> It may be that this period is

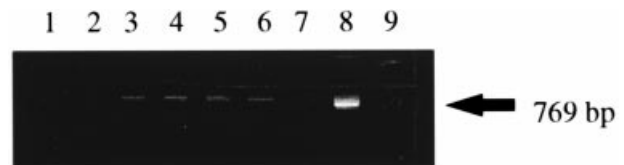


Fig. 4. Incorporation of Qa-2 onto the surface of *Ped slow* preimplantation embryos. B6.K1 eight-cell embryos were painted with 1/100 dilutions of B6.K1 or B6.K2 lysate. Levels of exogenous Qa-2 on the embryonic surface were determined by I-PCR just after painting. Lane 1: 5 embryos + B6.K1 lysate; Lane 2: 3 embryos + B6.K1 lysate; Lane 3: 2 embryos + B6.K2 lysate; Lane 4: 3 embryos + B6.K2 lysate; Lanes 5-6: single embryos + B6.K2 lysate; Lane 7: nothing; Lane 8: chimeric protein/DNA complex only; Lane 9: PBSAZ only.

TABLE I. Increased Rate of Cleavage of Preimplantation Embryos Painted with Crude NP-40 Lysate, Experiment A: Effect of Increasing Concentrations of B6.K2 Lysate on 8-cell B6.K1 Embryos Painted and Cultured to the Blastocyst Stage

Hr in culture	Untreated	Cell number per embryo at increasing concentrations of lysate (mean $\pm$ SEM)			
		1/100 B6.K1	1/200 B6.K2	1/100 B6.K2	1/20 B6.K2
24 (n = 219)	17.4 $\pm$ 4.5	17.1 $\pm$ 2.9	17.5 $\pm$ 4.7	17.9 $\pm$ 4.1	9.4 $\pm$ 2.0
48 (n = 118)	20.9 $\pm$ 5.7	20.9 $\pm$ 6.5	25.5 $\pm$ 6.4*	23.4 $\pm$ 5.6#	N.D.

\* Significantly different from embryos painted with a 1/100 dilution of B6.K1 lysate as a negative control at  $P < 0.05$ . # Significantly different from embryos painted with a 1/100 dilution of B6.K1 lysate as a negative control at  $P = 0.11$ .

required for exogenous Qa-2 to encounter and interact with other proteins in the membrane that are needed to transduce proliferative signals. The identities of these hypothetical proteins are unknown, but other GPI-linked proteins have been found to associate with the  $\alpha$  subunit of G proteins<sup>18</sup> and intracellular src tyrosine kinase activity.<sup>19</sup> Experiments are underway in our laboratory to try to identify signal transduction molecules that associate with Qa-2 in preimplantation embryos.

Although Qa-2 was incorporated onto T cells at a dilution of 1/30 in Experiment A and could mediate activation of the cells, the same lysate was toxic to

embryos at 1/20 and had no significant effect on cleavage at 1/100, presumably due to toxicity and inhibition by residual detergent, although we cannot rule out the possibility that the GPI-linked proteins themselves could have a toxic or inhibitory effect. Although we are working on purifying Qa-2 to homogeneity, in the meantime it seems that it would be advantageous if less detergent could be used. Therefore, in Experiment B we used a much lower concentration of NP-40 (0.1 vs. 2%) combined with pre-treatment with saponin. Qa-2 has been solubilized using as little as 0.5% NP-40.<sup>20,21</sup> Draberova et al.<sup>22</sup> found that pre-treatment with saponin, which se-

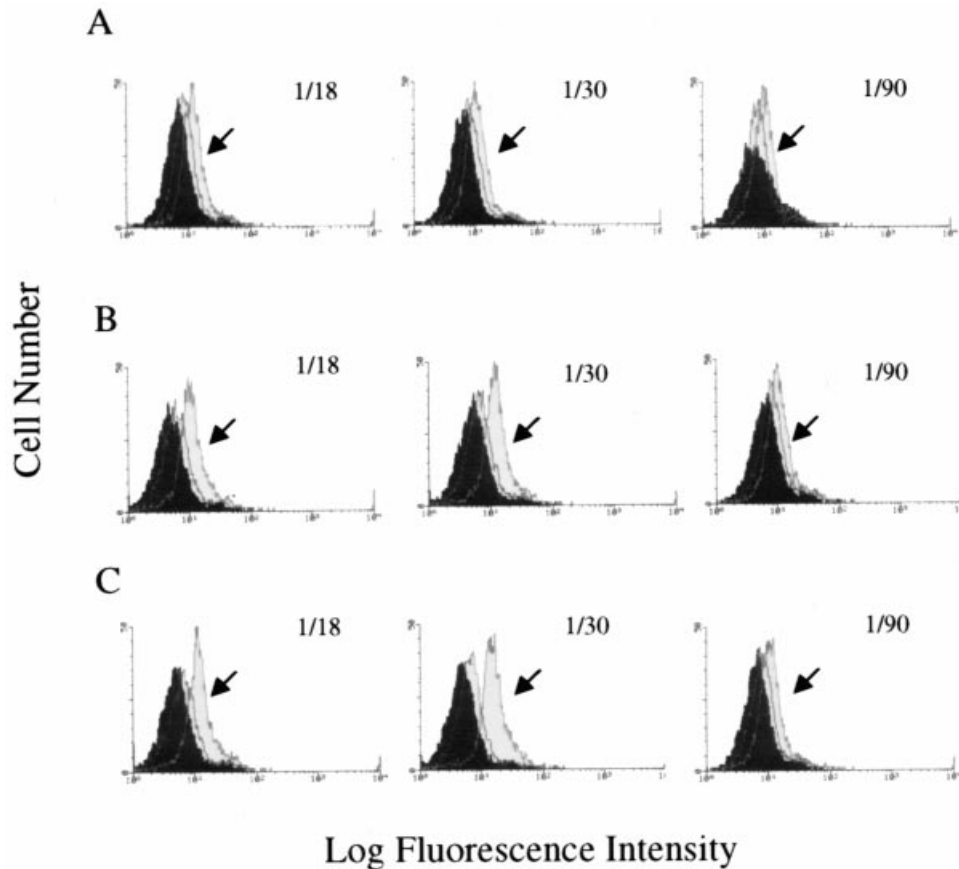


Fig. 5. Incorporation of Qa-2 onto the cell surface of Qa-2-negative splenocytes: Experiment B. B6.K1 splenocytes were painted with 1/18, 1/30, and 1/90 dilutions of saponin/NP-40 lysates and analyzed by immunostaining and FAC-Scan. Solid peak: cells painted with lysate, autofluorescence; Middle peak: cells painted with lysate, secondary antibody only (negative control); Outer peak (designated by arrow): cells painted with lysate, treated with primary and secondary antibody. Panel A: B6.K1 cells painted with B6.K1 lysate; Panel B: B6.K1 cells painted with B6.K2 lysate; Panel C: B6.K1 cells painted with B6.129 lysate.

questers cholesterol, greatly increased the NP-40 solubility of the GPI-linked protein Thy-1. This would not be unexpected in light of recent work that suggests that GPI-linked proteins localize to lipid "rafts" in the cell membrane that are enriched with cholesterol (reviewed in Jacobson and Dietrich<sup>23</sup>).

Although we found that Qa-2 could be extracted with 0.1% NP-40 and 0.1% saponin, toxicity to embryos was still observed at 1/100 dilutions. Because working with crude lysates is non-quantitative and does not eliminate other GPI-linked proteins from the lysate, we are working on purifying Qa-2 to homogeneity, which should eliminate any toxic effects due to residual detergent or other proteins.

The rate of preimplantation cleavage is of great interest to practitioners at *in vitro* fertilization (IVF) clinics. When human eggs are fertilized *in vitro*, they are cultured for several days before introduction into the uterus. There are not many criteria by which to visually judge the health of a preimplantation embryo, but experience has shown that the greatest number of live births result from introduction of those embryos that are cleaving most rapidly.<sup>24</sup> It will be of interest to see whether the cleavage rate of the slower embryos can be enhanced by painting on Qa-2 or its putative human equivalent, and whether the painted embryos result in more successful births.

#### Acknowledgments

This work was supported by NIH grant HD31505.

#### REFERENCES

1. Warner CM, McElhinny A, Wu L, Cieluch C, Ke X, Cao W, Tang C, Exley GE: Role of the *Ped* gene and apoptosis genes in control of preimplantation development. *J Assist Reprod Genet* 1998; 15:331–337.
2. Warner CM, Brownell MS, Rothschild MF: Analysis of litter size and weight in mice differing in *Ped* gene phenotype and the Q region of the H-2 complex. *J Reprod Immunol* 1991; 19:303–313.
3. Warner CM, Gollnick SO, Goldbard SB: Linkage of the *Ped* gene to the mouse major histocompatibility complex (MHC). *Biol Reprod* 1987a; 36:606–610.
4. Warner CM, Gollnick SO, Flaherty L, Goldbard SB: Analysis of Qa-2 antigen expression by preimplantation mouse embryos: Possible relationship to the *Ped* gene product. *Biol Reprod* 1987b; 36:611–616.
5. Low MG: Glycosyl-phosphatidylinositol: A versatile anchor for cell surface proteins. *FASEB J* 1989; 3:1600–1608.
6. Brown D, Waneck GL: Glycosyl-phosphatidylinositol-anchored membrane proteins. *J Am Soc Nephrol* 1992; 3:895–906.

7. Cook RG, Leone B, Leone JW, Widacki SM, Zavell PJ: Characterization of T cell proliferative responses induced by anti-Qa-2 monoclonal antibodies. *Cell Immunol* 1992; 144:367–381.
8. Hahn AB, Tian H, Wiegand G, Soloski MJ: Signals delivered via the Qa-2 molecule can synergize with limiting anti-CD3-induced signals to cause T lymphocyte activation. *Immunol Invest* 1992; 21:203–217.
9. Ilangumaran S, Robinson PJ, Hoessli DC: Transfer of exogenous glycosylphosphatidylinositol (GPI)-linked molecules to plasma membranes. *Trends Cell Biol* 1996; 6:163–167.
10. Medof ME, Nagarajan S, Tykocinski ML: Cell-surface engineering with GPI-anchored proteins. *FASEB J* 1996; 10:574–586.
11. Sharrow S, Arn JS, Stroynowski I, Hood L, Sachs D: Epitope clusters of Qa-2 antigens defined by a panel of new monoclonal antibodies. *J Immunol* 1989; 142:3495–3502.
12. McElhinny A, Warner CM: Detection of cell surface protein on single preimplantation mouse embryos by Immuno-PCR. *BioTechniques* 1997; 23:660–662.
13. Sano T, Cantor CR: A streptavidin-protein A chimera that allows one-step production of a variety of specific antibody conjugates. *Biotechnology* 1991; 9:1378–1381.
14. Sano T, Smith CL, Cantor CR: Immuno-PCR: Very sensitive antigen detection by means of specific antibody-DNA conjugates. *Science* 1992; 258:120–122.
15. Neugebauer JM: Detergents: An overview. *Methods Enzymol* 1990; 182:239–253.
16. Zhang F, Schmidt WG, Hou Y, Williams AF, Jacobson K: Spontaneous incorporation of the glycosyl-phosphatidylinositol-linked protein Thy-1 into cell membranes. *Proc Natl Acad Sci* 1992; 89:5231–5235.
17. Morgan BP, van den Berg CW, Davies EV, Hallett MB, Horejsi V: Cross-linking of CD59 and of other glycosyl phosphatidylinositol-anchored molecules on neutrophils triggers cell activation via tyrosine kinase. *Eur J Immunol* 1993; 23:2841–2850.
18. Solomon KR, Rudd CE, Finberg RW: The association between glycosylphosphatidylinositol-anchored proteins and heterotrimeric G protein subunits in lymphocytes. *Proc Natl Acad Sci* 1996; 93:6053–6058.
19. Brown D: The tyrosine kinase connection: How GPI-anchored proteins activate T cells. *Curr Opin Immunol* 1993; 5:349–354.
20. Michaelson J, Flaherty L, Bushkin Y, Yudkowitz H: Further biochemical data on Qa-2. *Immunogenet* 1981; 14:129–140.
21. Soloski MJ, Vitetta ES: Immunochemical purification and analysis of Qa and TL antigens. *Methods Enzymol* 1984; 108:549–558.
22. Draberova L, Amoui M, Draber P: Thy-1-mediated activation of rat mast cells: The role of Thy-1 membrane microdomains. *Immunology* 1996; 87:141–148.
23. Jacobson K, Dietrich C: Looking at lipid rafts? *Trends Cell Biol* 1999; 9:87–91.
24. Levy T, Goldman JA, Dicker D, Ashkenazi J, Feldberg D: Very early pregnancy wastage in *in vitro* fertilization and embryo transfer (IVF-ET). *J Vitro Fert Embryo Transfer* 1991; 8:250–253.