BIOLOGY OF REPRODUCTION 36, 611–616 (1987)

Analysis of Qa-2 Antigen Expression by Preimplantation Mouse Embryos: Possible Relationship to the Preimplantation-Embryo-Development (*Ped*) Gene Product[1]

CAROL M. WARNER,[2,3] SANDRA O. GOLLNICK,[3]
LORRAINE FLAHERTY,[4] and SIMON B. GOLDBARD[3]

Department of Biochemistry and Biophysics[3]
Iowa State University
Ames, Iowa 50011
and
Wadsworth Center for Laboratories and Research[4]
New York State Department of Health
Albany, New York 12201

ABSTRACT

The preimplantation-embryo-development (Ped) gene, a gene that controls the cleavage rate of preimplantation mouse embryos, maps to the Qa-2 subregion of the mouse major histocompatibility complex (MHC). A highly sensitive enzyme-linked immunosorbent assay (ELISA) procedure was used to detect Qa-2 antigens on mouse embryos. The use of a monoclonal antibody specific for Qa-2 antigens showed that Qa-2 antigens were present on oocytes, 2-cell, 8-cell, and blastocyst-stage embryos, with the greatest expression found on blastocysts. Expression of Qa-2 antigens by the embryos correlated completely with Ped gene phenotype. Those embryos expressing the fast Ped allele showed the presence of Qa-2 antigens (Qa-2^a mice), whereas those embryos expressing the slow Ped allele showed the absence of Qa-2 antigens (Qa-2^b mice). It is hypothesized that the Qa-2 antigen may be the Ped gene product.

INTRODUCTION

The preimplantation-embryo-development (*Ped*) gene, linked to the mouse major histocompatibility complex (MHC), the *H-2* complex, influences the rate of cleavage division of preimplantation mouse embryos (reviewed in Warner, 1986). Three classes of proteins are encoded in the mouse MHC. Class I molecules are membrane-bound glycoproteins, all of which have a similar structure, a 40,000- to 45,000-dalton heavy chain and a 12,000-dalton light chain. The main types of Class I molecules are the H-2, Q, and TL antigens. The H-2 antigens are crucial for self vs. nonself recognition and the regulation of cell-cell interactions in the immune response (Dorf, 1981). H-2 antigens are highly polymorphic and widely distributed on all tissues and organs of the mouse (Klein, 1975), including preimplantation embryos (Searle et al., 1976; Krco and Goldberg, 1977; Webb et al., 1977; Cozad and Warner, 1981; Sawicki et al., 1981; Cozad and Warner, 1982; Goldbard et al., 1984; Warner and Spannaus, 1984; Goldbard et al., 1985; Warner et al., 1985a,b).

The Class I antigens encoded in the *Q/TL* region of the mouse MHC have no known function. Serological and biochemical studies have shown at least four distinct Class I protein products encoded in the *Q/TL* region, which have been designated Qa-1, Qa-2, Q10, and Tla (Flaherty, 1981; Devlin et al., 1985; Soloski et al., 1986). The Q and TL antigens are much less polymorphic and much less widely distributed than the H-2 antigens (Kincade et al., 1980; Flaherty, 1981).

The analysis of congenic strains has suggested that the *Ped* gene is located in the *Qa-2* subregion of the mouse MHC (Warner, 1986). This has led us to hypothesize that the *Ped* gene product may be the Qa-2 protein. The purpose of the present paper is to report data on Qa-2 antigen expression on preimplantation mouse embryos. The use of a highly sensitive enzyme-linked immunosorbent assay (ELISA) technique has allowed us to detect Qa-2 antigens on

Accepted September 23, 1986.
Received July 9, 1986.
[1] This work was supported by NIH grants HD 13748 and AI 12603.
[2] Reprint requests.

oocytes and all stages of preimplantation embryos. The presence of Qa-2 antigens on the embryos is consistent with the hypothesis that the *Ped* gene product is the Qa-2 protein.

MATERIALS AND METHODS

Mice

CF1 mice (outbred) were obtained from Charles River, Wilmington, DE. The C57BL/10J (B10), C57BL/6J (B6), and B10.BR mice were purchased from the Jackson Labs, Bar Harbor, ME. The B6.K1 and B6.K2 mice were developed and bred by L. Flaherty. These two strains are congenic and differ only at the Qa-2 subregion of the mouse MHC. The *Qa-2* genotype of the inbred strains is as follows: C57BL/10J ($Qa\text{-}2^a$), C57BL/6J ($Qa\text{-}2^a$), B10.BR ($Qa\text{-}2^b$), B6.K1 ($Qa\text{-}2^b$), B6.K2 ($Qa\text{-}2^a$). Mice were superovulated and embryos collected as described previously (Warner et al., 1986).

Antisera and Monoclonal Antibodies

Polyclonal antiserum to Qa-2 antigens was prepared as described previously (Flaherty et al., 1975). Monoclonal antibody to Qa-2 antigens, designated D3.262, was generated by somatic cell fusion according to the method of Galfre et al. (1977). The D3.262 monoclonal antibody is directed to $Qa\text{-}2^a$. Specificity of this monoclonal antibody has been confirmed by the immunoprecipitation studies of Sherman et al. (1984) and the cytotoxic T cell studies of Forman et al. (1982). The monoclonal antibody does not crossreact with any known H-2K or H-2D allele, and its reactivity against cells from adult mice correlates completely with the strain distribution of Qa-2.

Normal mouse serum was used as a negative control for the studies with the polyclonal anti-Qa-2 antiserum. Because D3.262 is an immunoglobulin M (IgM) antibody, an unrelated anti-sheep, red blood cell-IgM monoclonal antibody was used as a negative control in the experiments utilizing D3.262. The anti-sheep, red blood cell-monoclonal antibody, N-S.2.1, was purchased from the American Type Culture Collection, Rockville, MD.

Enzyme-Linked Immunosorbent Assay (ELISA) Procedure

The use of the ELISA procedure to detect surface antigens on preimplantation mouse embryos has been described previously (Goldbard et al., 1984; Warner et al., 1986). In the studies using the polyclonal antiserum, both the polyclonal antiserum and normal mouse serum were used at a 1:10 dilution. The second antibody, rabbit anti-mouse IgG, was used at a 1:100 dilution of an affinity-purified (γ- and L-chain-specific) stock solution of 1 mg/ml (Cappel Laboratories, Cochranville, PA). Protein-A-β-galactosidase (Zymed, Burlingame, CA) was used at a 1:50 dilution and substrate, o-nitro-phenyl-β-D-galactopyranoside (Sigma, St. Louis, MO), at 4 mg/ml.

In the studies using the monoclonal antibody, both D3.262 (anti-Qa-2) and N-S.2.1 (negative control) were used at a 1:10 dilution of ascites fluid. The second antibody, rabbit anti-mouse IgM, was used at a 1:100 dilution of an affinity-purified (μ-chain-specific) stock solution of 1 mg/ml (Cappel Laboratories). Protein A-β-galactosidase was used at a 1:50 dilution and substrate at 4 mg/ml.

RESULTS

The first set of experiments was designed to detect Qa-2 antigens on mouse blastocyst stage embryos. The results in Figures 1 and 2 show that both polyclonal conventional antiserum (Fig. 1) and mono-

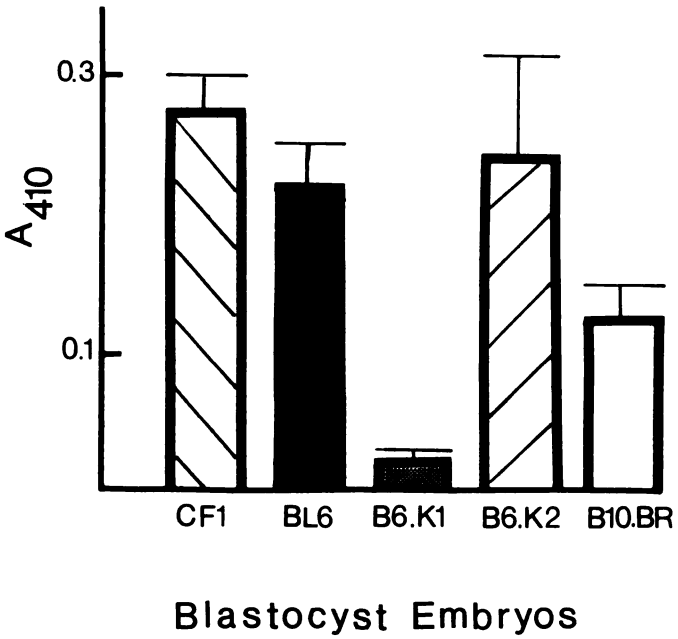

FIG. 1. Reactivity of anti-Qa-2 polyclonal antiserum with blastocyst-stage embryos. Results of the ELISA are expressed as A_{410} per 5 embryos and are adjusted by subtraction of the A_{410} of embryos treated with normal mouse serum. A minimum of 20 embryos was used from each strain *(CF1, BL6, B6.K1, B6.K2, B10.BR)* and the standard deviations are shown as *error bars*.

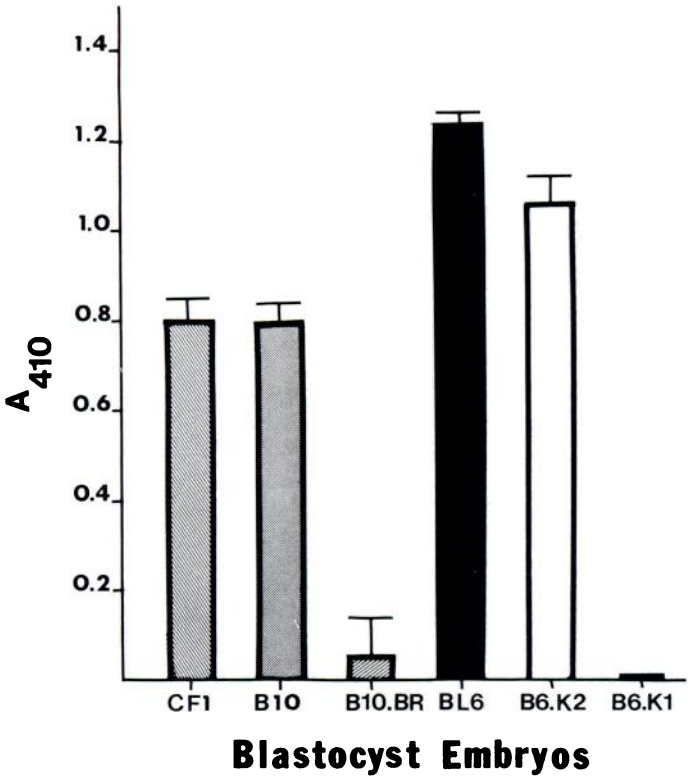

FIG. 2. Reactivity of D3.262 (anti-Qa-2) monoclonal antibody with blastocyst-stage embryos. Results of the ELISA are reported at A_{410} per 5 embryos and are adjusted by subtraction of A_{410} of embryos treated with the control antibody, N-S.2.1. A minimum of 20 embryos was used from each strain *(CF1, B10, B10.BR, BL6, B6.K2, B6.K1)* and the standard deviations are shown as *error bars*.

clonal antibody (Fig. 2) are able to detect Qa-2 antigens on mouse blastocysts. The results are exactly as predicted from the known Qa-2 genotypes of the mice tested. The higher than expected value seen for the B10.BR strain in Figure 1 may be due to different background genes in B10 mice as opposed to B6 mice. Interestingly, the outbred strain, CF1, shows the same profile as the strains expressing Qa-2^a. Thus, Qa-2^a must be the predominant allele in this mouse population. The expression of Qa-2 antigens on CF1 embryos allowed us to undertake the next set of experiments, the development study, which required the large number of embryos readily available from the outbred CF1 mice.

The results of the ELISA procedure on oocytes, and 2-cell, 8-cell, and blastocyst-stage embryos from CF1 mice, are shown in Table 1. Each experiment contained a common point, the oocyte point, and results were normalized to this point in Experiment 1 to accommodate day-to-day variation well known to occur in ELISA tests. The data in Table 1 were pooled, with the results graphed in Figure 3. It is seen that Qa-2 antigen expression increases markedly in blastocyst-stage embryos.

DISCUSSION

The experiments reported in this paper show that Qa-2 antigens are present on preimplantation mouse embryos. Those strains possessing the *Qa-2^a* allele express a protein product on the embryos, whereas those strains possessing the *Qa-2^b* allele do not. Thus, gene expression by the embryos is the same as for adult cells. The absence of a protein product in *Qa-2^b* mice may represent a deletion at the DNA level (Flaherty et al., 1985).

The amount of Qa-2 antigen per embryo increases dramatically at the blastocyst stage of development. However, the number of cells per embryo also increases at this stage of development (see Fig. 3). Therefore, as shown in Table 2, the amount of Qa-2 antigen per cell has been calculated. On a per-cell basis, blastocyst embryos have more Qa-2 antigens than earlier stages of development. Also shown in Table 2 is a calculation of the amount of Qa-2 antigen per "outside" cell. The reason is that blastocysts consist of an outside trophectoderm, clearly accessible to the ELISA reagents, and an inner cell mass, which may not be accessible to the ELISA reagents. Calculated on an "outside"-cell basis, the blastocyst cells still appear to have more Qa-2 antigens than the cells of the earlier stages of development.

The level of Qa-2 antigen expression on the mouse blastocysts appears to be about eight times higher than the previously reported (Goldbard et al., 1985) level of expression of H-2 antigens on mouse blastocysts. The absorbance value at 410 nm (A_{410}) per blastocyst is 0.550 for Qa-2 antigens, as reported in this paper, but only 0.070, as reported previously for H-2 antigens (Goldbard et al., 1985). One difference in the assays used to detect Qa-2 vs. H-2 antigens was that that anti-Qa-2 monoclonal antibody was an IgM antibody, whereas the anti-H-2 monoclonal antibody was an IgG antibody. It remains to be determined whether the amounts of antigen on the embryo cell surface are truly different, or merely a reflection of the different valence and affinity of the antibodies used.

The suggested location of the *Ped* gene in the *Qa-2* subregion of the mouse MHC (Warner, 1986), coupled with the data on Qa-2 expression reported in this paper, make it attractive to hypothesize that the *Ped*

TABLE 1. The detection of Qa-2 antigens on CF1 embryos by the ELISA procedure.

Experiment	Stage	Antibody	No. of embryos/well	A_{410} Well	A_{410} Embryo	Normalized[a] A_{410}/embryo
1	oocyte	N-S.2.1	42	0.12	0.003	0.003
			42	0.13	0.003	0.003
		D3.262	42	0.61	0.015	0.015
			42	0.70	0.017	0.017
	2-cell	N-S.2.1	35	0.44	0.012	0.012
			35	0.20	0.006	0.006
		D3.262	35	1.13	0.032	0.032
			35	1.10	0.031	0.031
	8-cell	N-S.2.1	10	0.46	0.046	0.046
			10	0.26	0.026	0.026
		D3.262	10	0.98	0.098	0.098
			10	1.01	0.101	0.101
2	oocyte	N-S.2.1	42	0.04	0.001	0.003
			42	0.05	0.001	0.003
		DS.262	42	0.28	0.007	0.018
	blastocyst	N-S.2.1	5	0.26	0.052	0.133
			5	0.32	0.064	0.164
		D3.262	5	1.32	0.264	0.676
			5	1.35	0.270	0.691
3	oocyte	N-S.2.1	40	0.08	0.002	0.006
		D3.262	40	0.24	0.006	0.017
	8-cell	N-S.2.1	10	0.07	0.007	0.020
			10	0.07	0.007	0.020
		D3.262	10	0.31	0.031	0.090
			10	0.35	0.035	0.102
	blastocyst	N-S.2.1	5	0.24	0.048	0.140
			5	1.34	0.268	0.780
		D3.262	5	1.01	0.202	0.588

[a]Experiments were normalized to the oocyte point in Experiment 1 by multiplying the values of Experiment 2 by 2.56 and of Experiment 3 by 2.91.

gene product is the Qa-2 protein. The data in this paper show that embryos expressing Qa-2 antigens (Qa-2^a mice) have a fast rate of cell division. On the other hand, embryos from mice having the absence of Qa-2 antigens (Qa-2^b mice) show slow cleavage rates.

It is, however, possible that the Qa-2 antigen, instead of being the *Ped* gene product, controls the expression of the *Ped* gene product. In adult cells, the *Qa-2* gene appears to control the expression of other antigens encoded in the *Q/TL* region (Michaelson et al., 1981). Expression of the Qa-3, Qa-5, and Qa-6 antigens is dependent on Qa-2 expression. In light of this, it is possible that the *Qa-2* subregion controls an undefined region of the genome that contains the *Ped* gene, and that expression of the *Ped* gene product is dependent on Qa-2 expression.

Thus, there are two different models for the relationship of Qa-antigens to the *Ped* gene product. In the first model, the Qa-2 antigen is proposed to be the actual *Ped* gene product, and as such would be directly involved in the control of the rate of cell

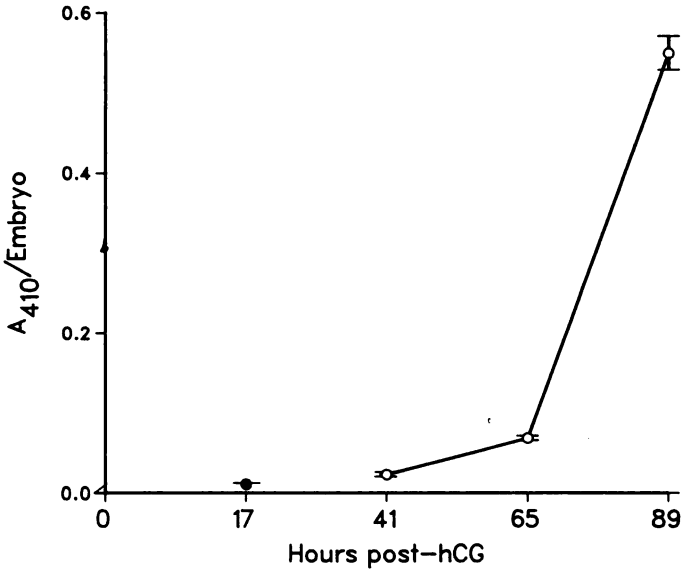

FIG. 3. Expression of Qa-2 antigens by oocytes (•) and preimplantation mouse embryos (○). Results of the ELISA are expressed as A_{410} per embryo and are adjusted by subtraction of the A_{410} of embryos treated with the control antibody, N-S.2.1. A minimum of 20 embryos was used for each time point and the standard errors of the means are shown.

TABLE 2. Summary of Qa-2 antigen expression during development.

Time post-hCG (h)	Mean cell no./embryo (SE)[a]	No. of "outside" blastomeres	ELISA results: Mean A_{410} per Embryo	Cell	"Outside" cell
17 (oocyte)	1.0 (0.0)	1	0.011	0.011	0.011
41 (2-cell)	2.0 (0.0)	2	0.023	0.011	0.011
65 (8-cell)	6.8 (0.3)	6.8	0.069	0.010	0.010
89 (blastocyst)	39.3 (1.8)	19[b]	0.550	0.014	0.029

[a]Determined by the Tarkowski method (Tarkowski, 1966). A minimum of 30 embryos were scored for each point.

[b]Extrapolated from Handyside (1981).

division. The second model proposes that the Qa-2 molecule acts as a control molecule, regulating the expression of the *Ped* gene product. The *Ped* gene would then be located outside the *Qa-2* subregion, but elsewhere in the MHC. The complete elucidation of the structure of the mouse MHC, at the DNA level, should help to distinguish these two hypotheses. However, mapping of the *Ped* gene to the *Qa-2* subregion, based on the analysis of congenic strains, favors the first hypothesis.

Finally, the possible relationship of the *Ped* gene to the *T/t* complex merits some discussion. The *T/t* complex, located 13.5 cm to the centromeric side of the *H-2* complex, influences early embryo development and sperm cell function in the mouse (Artz and Bennett, 1975; Silver et al., 1984). Some mutations in the *T/t* complex cause death of preimplantation and postimplantation embryos, whereas others cause male sterility. Recent evidence has shown that some "*t*" haplotypes contain inversions, so that several *t*-lethal genes are located within the MHC (Shen et al., 1983). For instance, the t^{w5} lethal is very close to the *H-2K* region, t^{t2} and t^{w32} are in the *Q* region, and t^{w18} and t^{Lub1} are close to the *H-2D* region, although their exact position relative to the *Q/TL* region is not defined. Wild type chromosomes may have the same inverted organization. These findings raise the intriguing possibility that the *Ped* gene and the *t*-alleles may somehow be related.

In conclusion, this paper shows that Qa-2 antigens are expressed on all mouse embryos expressing the *fast Ped* gene phenotype. It is suggested that the control of cleavage rate in embryos may be a direct function of the presence of Qa-2 antigens. Furthermore, mapping of the *Ped* gene to the *Qa-2* subregion of the mouse MHC suggests that the Qa-2 protein may be the *Ped* gene product.

REFERENCES

Artz K, Bennett D, 1975. Analogies between embryonic (T/t) antigens and adult major histocompatibility (H-2) antigens. Nature 256: 545–47

Cozad KM, Warner CM, 1981. Specificity of H-2 antigens expressed on mouse blastocysts. J Exp Zool 218:313–20

Cozad KM, Warner CM, 1982. Detection of H-2 antigens on 8-cell mouse embryos. J Exp Zool 221:213–17

Devlin JJ, Lew AM, Flavell R, Coligan J, 1985. Secretion of a soluble class I molecule encoded by the Q10 gene of the C57BL/10 mouse. EMBO J 4:369–74

Dorf ME (ed.), 1981. The Role of the Major Histocompatibility Complex in Immunobiology. New York: Garland, 406 pp

Ey PL, Prowse SJ, Jenkins CR, 1978. Isolation of pure IgG, IgG 2a and IgG 2b immunoglobulins from mouse serum using protein A-sepharose. Immunochemistry 15:429–36

Flaherty L, 1981. Tla-region antigens. In: Dorf M (ed.), The Role of the Major Histocompatibility Complex in Immunology. New York: Garland, pp. 33–57

Flaherty L, Karl M, Dibase K, 1985. The *Qa* series of antigens. In: Pernes B, Vogel HJ (eds.), The Cell Biology of the Major Histocompatibility Complex. London: Academic Press, pp. 97–106

Flaherty L, Zimmerman D, Hansen T, 1975. Further serological analysis of the Qa antigens: analysis of an anti-H-2.28 serum. Immunogenetics 6:245–51

Forman J, Trial J, Tonkonogy S, Flaherty L, 1982. The Qa-2 subregion controls the H-2-unrestricted cytotoxic T cells. J Exp Med 155: 749–67

Galfre G, Howe S, Milstein C, Butcher GW, Howard JC, 1977. Antibodies to major histocompatibility antigens produced by hybrid cell lines. Nature 266:550–52

Goldbard SB, Gollnick SO, Warner CM, 1984. A highly sensitive method for the detection of cell surface antigens on preimplantation mouse embryos. J Immunol Methods 68:137–46

Goldbard SB, Gollnick SO, Warner CM, 1985. Synthesis of H-2 antigens by preimplantation mouse embryos. Biol Reprod 33:30–36

Kincade PW, Flaherty L, Lee G, Watanabe T, Michaelson J, 1980. Qa antigen expression on functional lymphoid, myeloid, and stem cells in adult mice. J Immunol 124:2879–85

Klein J, 1975. Biology of the Mouse Histocompatibility-2 Complex. New York: Springer-Verlag, pp. 330–334

Krco CJ, Goldberg EH, 1977. Major histocompatibility antigens on preimplantation mouse embryos. Transplant Proc 9:1367–70

Michaelson J, Flaherty L, Bushkin Y, Yudkowitz H, 1981. Further biochemical data on Qa-2. Immunogenetics 14:129–40

Sawicki JA, Magnuson T, Epstein CJ, 1981. Evidence of the paternal genome in the 2-cell mouse embryo. Nature 294:450–51

Searle RF, Sellens MH, Elson J, Jenkinson EJ, Billington WD, 1976. Detection of alloantigens during preimplantation development and early trophoblast differentiation in the mouse by immunoperoxidase. J Exp Med 143:348–59

Shen H-S, Flaherty L, Artz K, Bennett D, Ravetch J, 1983. Inversions in the *H-2* complex and *t*-haplotypes in mice. Nature 306:380–83

Sherman DH, Kranz DM, Eisen H, 1984. Expression of structurally diverse Qa-2-encoded molecules on the surface of cloned cytotoxic T lymphocytes. J Exp Med 160:1421–30

Silver LM, Garrels JI, Lehrach H, 1984. Molecular studies of mouse chromosome 17 and the *t* complex. In: Setlow JK, Hollaender A (eds.), Genetic Engineering Principles and Methods, Vol. 6. New York: Plenum Press, pp. 141–156

Soloski MJ, Vernachio J, Einhorn G, Lattimore A, 1986. Qa gene expression: biosynthesis and secretion of Qa-2 molecules in activated T cells. Proc Natl Acad Sci USA 83:2949–53

Warner CM, 1986. Genetic manipulation of the major histocompatibility complex (MHC). J Anim Sci 63:279–87

Warner CM, Gollnick SO, Goldbard SB, 1986. Linkage of the preimplantation-embryo-developed (*Ped*) gene to the mouse major histocompatibility complex (MHC). Biol Reprod 36:606–10

Warner CM, Meyer TE, Gollnick SO, Goldbard SB, 1985a. Ontogeny of class I MHC antigens in preimplantation mouse embryos. In: Streilein JW, Black S, Voellmy RW (eds.), Advances in Gene Technology: Molecular Biology of the Immune System (Proceedings of the 17th Miami Winter Symposium). New York: Academic Press, pp. 337–38

Warner CM, Ostlie NS, Spannaus DJ, 1985b. Effect of absorbed and nonabsorbed anti-H-2 antiserum on mouse blastocysts. J Exp Zool 233:261–67

Warner CM, Spannaus DJ, 1984. Demonstration of H-2 antigens on preimplantation mouse embryos using conventional antisera and monoclonal antibody. J Exp Zool 230:37–52

Webb CG, Gall E, Edelman GM, 1977. Synthesis and distribution of H-2 antigens in preimplantation mouse embryos. J Exp Med 146:923–32