Impaired Cytokine Response in Male ICR Swiss Mice After Infection With D Variant of Encephalomyocarditis Virus

NANCY J. BIGLEY, ROY A. BLAY, AND RANDALL A. SMITH

SUMMARY

The basis for the resistance of the female and the susceptibility of the male ICR Swiss mouse to the diabetogenicity of the D variant of encephalomyocarditis virus (EMCV-D) is unknown. This pattern of disease resistance and susceptibility can be reversed if females are treated with testosterone and males are treated with estrogen before virus infection. As a possible explanation for this sex difference in disease development, differences in early antiviral host responses were explored. Cellular antiviral resistance mechanisms operative early in virus infection were evaluated in ICR Swiss mice of both sexes after intraperitoneal infection with virus. No differences were seen in splenic natural killer (NK) cell responses of male and female mice during the 1st wk of infection, during which only the males became diabetic. Depletion of NK cell activity with rabbit anti-asialo GM$_1$ serum did not render the infected ICR Swiss female susceptible to virus-induced diabetes. Treatment of ICR Swiss mice with type I carrageenan to compromise macrophage function rendered the female susceptible to diabetes after infection with EMCV-D but made only the male susceptible to diabetes by the usually avirulent interferon-inducing EMCV-B. Concanavalin A and recombinant interleukin 2, inducers of immune interferon, which in turn primes macrophages for activation and induces their expression of Ia antigens, protected the ICR Swiss male against the diabetogenic effects of EMCV-D. Interleukin 2 enhanced the male's capacity to exhibit an increase in the expression of Ia antigen by peritoneal exudate cells 1 day after infection with EMCV-D to a level seen in disease-resistant females. These results suggest that the more responsive macrophage system of the female is important in the innate resistance of the ICR Swiss female to virus-induced diabetes and, conversely, the less responsive macrophage system of the male contributes to his susceptibility to diabetes induction by EMCV-D and by EMCV-B after impairment of macrophage function. *Diabetes* 36:1408–13, 1987

From the Department of Microbiology and Immunology, Wright State University School of Medicine and College of Science and Mathematics, Dayton, Ohio. Address correspondence and reprint requests to Nancy J. Bigley, PhD, Department of Microbiology/Immunology, Wright State University, Dayton, OH 45435.

Current address of R.A.B.: Department of Pathology, University of Vermont, Burlington, VT 05404.

Received for publication 8 December 1986 and accepted in revised form 15 May 1987.

In animal models of insulin-dependent diabetes mellitus, differences are seen in disease susceptibility between strains and between sexes of a single strain (1). Resistance or susceptibility to the diabetogenic action of the D variant of encephalomyocarditis virus (EMCV-D) in ICR Swiss mice can be modulated by sex steroid hormone treatment before infection (2). Males can be protected from diabetes by estrogen injection and females can be rendered disease-susceptible by testosterone administration 48 h before virus infection (1). The role of the sex steroid hormones in the modulation of susceptibility or resistance to EMCV-D–induced disease in ICR Swiss mice is not understood. Pozzetto and Gresser (2) found that female Swiss mice produce greater amounts of virus-induced interferon (IFN) than males of the same strains early in infection with another strain of EMCV and suggested that this may be the basis for the greater susceptibility of males to picornavirus infection (2).

The objective of this study was to determine whether host cellular resistance mechanisms, operating early in virus infection, were responsible for the sex-dependent resistance or susceptibility to the diabetogenicity of EMCV-D in ICR Swiss mice. The results suggest that macrophage but not natural killer (NK) cell responses were responsible for this sex difference in disease susceptibility. Depletion of macrophage reticuloendothelial function with carrageenan caused females to develop diabetes after EMCV-D infection. Efforts were successful in protecting males against the diabetogenic effects of EMCV-D with immune modulators [concanavalin A and interleukin 2 (IL-2)] that induce production

of immune interferon (IFN-γ), a major macrophage-activation factor.

MATERIALS AND METHODS

Mice. ICR Swiss mice were purchased from Harlan Laboratories (Indianapolis, IN) and housed in groups of 10.

Virus. The EMCV-D and EMCV-B were obtained from J. Yoon (NIH, Bethesda, MD). The EMCV-D virus was passaged five times through L929 cells, twice through baby hamster kidney (BHK), and once again through L929 cells (1). The resulting virus stock is diabetogenic, produces large diffuse plaques in L929 cells, and does not induce IFN in vitro in L929 cells. The EMCV-B cultured in mouse L929 cells produces small discrete plaques and induces IFN in L929 cells. Stock virus was diluted in Hanks' balanced salt solution (HBSS) containing 2% calf serum. Mice were injected with 0.2 ml i.p. of diluted EMCV-D at a dose of 800 plaque-forming units (PFU). Mice were infected intraperitoneally with 20,000 PFU EMCV-B contained in 0.2 ml of HBSS containing 2% calf serum.

Nonfasting glucose tolerance test. Glucose was dissolved in distilled water and injected intraperitoneally into mice at a concentration of 2 mg/g body wt. The mice were bled from the supraorbital plexus 1 h later. Blood (serum) glucose (BG) was determined on a YSI-23A glucose analyzer (YSI, Yellow Springs, OH). Mice with BG at least 3SD above control means were considered to be diabetic. Values are given as means ± 1SD.

NK cell–mediated specific lysis of YAC-1 cells. Microcytotoxicity assay was performed with the methods of Bukowski et al. (3). Target cells at 5×10^6 cells/ml were labeled with 100 μCi of sodium chromate (^{51}Cr, New England Nuclear, Boston, MA) for 1 h at 37°C. Cells were then centrifuged, washed, and resuspended to 3×10^5 cells/ml. Effector cells in 0.2-ml aliquots at 3.75×10^6 cells/ml were delivered into each well of round-bottomed 96-well plates (no. 3799, Costar, Cambridge, MA). Then 50 μl of YAC-1 cells at 3×10^5 cells/ml were added to each well and the plates centrifuged at 50 × *g* for 5 min. Assays were performed in replicates of five at an effector/target ratio of 50:1. Spontaneous release was determined by adding medium (complete RPMI) to wells, and maximum release was determined by adding 1% Nonidet P-40 (Bethesda Research, Bethesda, MD). Plates were incubated in a humidified atmosphere of 37°C and 5% CO_2 for 4–5 h. Plates were then centrifuged at 200 × *g* for 5 min, and 0.1 ml of supernatant fluid was collected from each well for radiation counts in an Auto-Logic γ-counter (Abbott, Irving, TX). Data are expressed as percent specific release and calculated by the following formula (counts/min = cpm)

$$100 \times \frac{\text{cpm experimental} - \text{cpm spontaneous}}{\text{cpm Nonidet P-40} - \text{cpm spontaneous}}$$

Spontaneous release was <20% for YAC-1 target cells.

Target cells. The YAC-1 cell line is a Moloney leukemia virus-induced T-lymphocyte lymphoma originating from the A/Sn mouse strain. These cells were maintained in complete RPMI in 75-cm^2 flasks (Costar) and incubated at 37°C and 5% CO_2 in a humidified atmosphere.

Effector cells. Effector cells were taken from the spleens of normal and EMCV-D–infected ICR Swiss male and female mice. Spleens were forced through a 60-mesh wire sieve, washed three times in RPMI-1640 medium, and resuspended at a concentration of 3.75×10^6 cells/ml.

Isolation of peritoneal adherent cells. Resident peritoneal cells were harvested from mice by lavage of the peritoneal cavity with 10 ml of HBSS. Cells were washed and diluted to a concentration of $1–2 \times 10^6$ cells/ml in RPMI-1640. Aliquots of 0.2 ml were added to 6.4-mm culture wells (Cluster 96, Costar), and cells were allowed to adhere for 2 h at 37°C in 95% air/5% CO_2. After adherence, the nonadherent cells were removed by shaking the plates and washing three times with 0.1-ml volumes of warm HBSS without phenol red. This procedure resulted in the formation of uniform macrophage

TABLE 1
Effects of type I carrageenan on diabetogenesis of EMCV-B and EMCV-D in 9-wk-old ICR Swiss mice

Carrageenan	EMCV variant	Sex	Mean blood glucose ± SD 7 days after infection (mg/dl)	Diabetic mice/ total mice
Without		M	203 ± 31	0/6
With		M	102 ± 15	0/3
Without	D	M	426 ± 209	7/10
Without	B	M	220 ± 30	0/10
With	B	M		3/8*†
Without		F	198 ± 16	0/5
With		F	141 ± 9	0/5
Without	D	F	214 ± 64	1/10
With	D	F	483 ± 253	7/9‡
Without	B	F	163 ± 33	0/10
With	B	F	147 ± 27	0/6

Carrageenan dose, 2 mg i.p. 24 h before virus infection; EMCV-B dose, 20,000 plaque-forming units (PFU); EMCV-D dose, 800 PFU. EMCV, encephalomyocarditis virus.
*Blood glucose values 3.5 days after infection; diabetic animals were dead by day 7.
†Three of 8 males were severely ill on day 4; their mean blood glucose was 426 ± 382 mg/dl, with individual values of 512, 825, and 64 mg/dl. These mice died before day 7. Mean blood glucose of the 5 remaining mice on day 7 was 203 ± 108 mg/dl, with 1 diabetic animal (blood glucose 387 mg/dl).
‡EMCV-D–infected female mice were significantly less diabetic than EMCV-D–infected female mice receiving carrageenan ($P < .01$).

monolayers on the bottom of the wells. Monolayers were examined microscopically and demonstrated typical macrophage morphology—a ruffled cytoplasm, a moderately indented nucleus, a fine chromatin pattern, and cytoplasmic vacuolation. An additional sample was stained with Diff-Quik (Harleco, Gibbstown, NJ) to demonstrate their monocyte-macrophage nature (4), which was consistently >95%. Viability was determined by staining with acridine orange–ethidium bromide (AOEB) and examined with fluorescent microscopy; viability was usually >98%.

Peritoneal and spleen cell preparations. Peritoneal cells were harvested from mice immediately after killing by cervical dislocation by lavage of the peritoneal cavity with 10 ml of HBSS supplemented with 2% fetal calf serum and 1% penicillin-streptomycin. The peritoneal cells were washed after collection from the peritoneal cavity in cytotoxicity medium (RPMI-1640 plus 0.3% bovine serum albumin). Mononuclear spleen cells were isolated by perfusing spleens with 10 ml of RPMI-1640 in a syringe fitted with a 26-gauge needle. Erythrocytes were lysed in a single wash at 37°C with Tris-buffered ammonium chloride [9 parts 0.16 M NH_4Cl and 1 part 0.17 M Tris base, adjusted to pH 7.2 with 0.1 N HCl (5)]. The cells were then washed in cytotoxicity medium, counted in a Coulter counter (model D-2, Hialeah, FL), and viability determined by staining with AOEB (5); cell viability was usually ≥98%.

Cytotoxicity assay for cells expressing Ia antigens. Monoclonal antibodies anti-Ia^p and anti-I-E (Ia 7) (Cedarlane, Hornby, Ontario, Canada) reacted with peritoneal mononuclear cells and spleen cells from the outbred ICR Swiss mouse strain and were used to determine the percentage of cells expressing Ia antigen. The manufacturer's protocol for cytotoxicity was used. Final concentrations of 1:80 for anti-Ia^p and of 1:40 for anti-I-E were incubated with 1×10^6 cells suspended in cytotoxicity medium (RPMI-1640 plus 0.3% bovine serum albumin) for 60 min at 4°C. Samples were then centrifuged at 200 × *g* for 5 min, brought up to their original volumes with a 1:12 dilution of Cedarlane Low-Tox-M rabbit complement (C′), and incubated for 60 min at 37°C. All samples were then placed on ice, stained with AOEB, and the percent cytotoxicity was determined on a hemocytometer with a fluorescent microscope. Cells (1×10^6) incubated with the 1:12 dilution of C′ at 37°C for 60 min served as a control. The cytotoxicity index (C.I.) for each sample was determined by the formula

$$\text{C.I.} = \frac{\%\ \text{cytotoxicity (antibody + C')} - \%\ \text{cytotoxicity (C' alone)}}{100\% - \%\ \text{cytotoxicity (C' alone)}} \times 100$$

Biochemicals. Carrageenan (type I) (Sigma, St. Louis, MO) was diluted to a concentration of 10 mg/ml in sterile distilled water and injected intraperitoneally into mice in 0.2-ml volumes (6). Concanavalin A (Behring Diagnostic, La Jolla, CA) was solubilized in HBSS so that a concentration of 25 mg/kg was contained in 0.2 ml. Mice were injected intraperitoneally with 0.2 ml of concanavalin A (25 mg/kg body wt) (7). Human recombinant IL-2 in lyophilized form (provided by Dr. John Farrar, Hoffman-La Roche, Nutley, NJ) was solubilized in sterile, pyrogen-free water, and mice were injected intraperitoneally with 0.2-ml amounts of several IL-2 concentrations (see RESULTS).

Statistics. Student's *t* test was used for statistical evaluations.

RESULTS

Effects of type I carrageenan on diabetogenicity of EMCV-D and EMCV-B in ICR Swiss mice. The effectiveness of blockade of macrophage function was assessed by measuring the hydrogen peroxide–generating capacity of peritoneal adherent cells (macrophages) after stimulation with phorbol myristate acetate with the method of Pick and colleagues (8,9). The carrageenan treatment regimen depleted the hydrogen peroxide–generating ability of peritoneal macrophages from normal (uninfected) mice for at least 5 days. This was similar to the depletion of reticuloendothelial function described by Chaouat and Howard (6). Carrageenan appeared to diminish the hydrogen peroxide–generating capacity of macrophages from males more severely than that from females.

At 1 day after injection of carrageenan, the mean value

TABLE 2
Natural killer cell activity and diabetes induction in EMCV-D–infected 9-wk-old ICR Swiss mice

Sex	EMCV-D*	Days after infection	Mean blood glucose ± SD (mg/dl)	Diabetic mice/ total mice	Natural killer cell activity† (%)
M					19 ± 13
M	+	1			30 ± 6
M	+	3			42 ± 10
M	+	4	239 ± 108	5/11	48 ± 5
M	+	7	253 ± 125	2/8	42 ± 20
F					19 ± 11
F	+	1			34 ± 12
F	+	3			48 ± 4
F	+	4	151 ± 22	0/13	47 ± 4
F	+	7	179 ± 30	0/10	51 ± 18
F‡	+	1			3 ± 1
F‡	+	3			2 ± 2
F‡	+	4	164 ± 26	0/13	7 ± 3
F‡	+	7	191 ± 17	0/10	7 ± 1

*Dose, 800 plaque-forming units i.p. EMCV, encephalomyocarditis virus.
†Mean of triplicate determinations of YAC-1 cell killing.
‡Mice received 0.2 ml of 1:10 dilution of rabbit anti-asialo GM_1 12 h before and 72 h after infection.

TABLE 3
Modulation of virus-induced diabetes in 9-wk-old ICR Swiss males by concanavalin A

Treatment	Days after treatment	Mean blood glucose ± SD (mg/dl)	Diabetic mice/ total mice
Concanavalin A	7	160 ± 1	0/4*
	14	Not done	
EMCV-D	7	311 ± 57	6/11
	14	362 ± 72	4/9†
Concanavalin A/EMCV-D	7	193 ± 11	1/12‡
	14	179 ± 11	0/10§

Concanavalin A mice injected with 25 mg/kg i.p.; EMCV-D mice injected with 800 plaque-forming units (PFU) i.p.; concanavalin A/EMCV-D mice injected with 25 mg/kg i.p. concanavalin A immediately before 800 PFU i.p. EMCV-D. EMCV, encephalomyocarditis virus.
*In another experiment with 10 mice, blood glucose was 203 ± 27 7 days and 176 ± 20 17 days after injection of concanavalin A.
†Two animals died between days 7 and 14.
‡Mean blood glucose of diabetic mouse was 289 mg/dl ($P < .05$ compared with blood glucose of EMCV-D–infected group).
§Blood glucose of 2 mice in group not determined due to difficulty in obtaining blood sample ($P < .01$ compared with blood glucose of virus-infected group).

for males was 33% of that for hydrogen peroxide–generating values from normal males (75 vs. 229 nM H_2O_2/mg protein × 100). The mean value for females was ~60% that of normal (150 vs. 250 nM H_2O_2/mg protein × 100). The effects of reticuloendothelial blockade of macrophage function by type I carrageenan (6) on the diabetogenicity of the two EMCV variants were examined in ICR Swiss mice (Table 1). Seven of 10 males infected with EMCV-D were diabetic (mean BG 426 ± 66 mg/dl) 1 wk after infection. The effects of carrageenan pretreatment on EMCV-D infection in females and on EMCV-B infection in both males and females were determined. Only 1 of 10 females was diabetic (BG > 3SD above mean) as a result of EMCV-D infection. In females pretreated with carrageenan before infection with EMCV-D, 7 of 9 developed severe diabetes (mean BG 483 ± 89 mg/dl). The EMCV-B alone did not induce diabetes in either males or females. In carrageenan-pretreated females EMCV-B did not induce diabetes; 3 of 8 males pretreated with carrageenan infected with EMCV-B developed diabetes during the 1st wk of infection (Table 1).

NK cell responses of ICR Swiss mice after infection with EMCV-D. The NK cell responses of male and female ICR Swiss mice against YAC-1 target cells were monitored 1, 3, 4, and 7 days after intraperitoneal infection with EMCV-D (Table 2). Note that no difference in NK cell responses of the two sexes was evident. Also, a group of females was pretreated with rabbit anti-asialo GM_1 serum (Wako, Dallas, TX) 12 h before and 72 h after EMCV-D infection to deplete NK cell activity. As shown in Table 2, depletion of NK cell activity did not make the female ICR Swiss mice susceptible to the diabetogenicity of EMCV-D.

Effects of concanavalin A on pathogenesis of EMCV-D. Concanavalin A in a dose of 25 mg/kg i.p. (7) immediately before intraperitoneal infection with EMCV-D protected the males from development of severe diabetes over a 2-wk observation period. In Table 3, note that 6 of 11 virus-infected males were diabetic by 1 wk after infection, with mean BG >300 mg/dl, whereas 1 of 12 infected males pretreated with concanavalin A was diabetic at this time. Mean BG for 9-wk-old male ICR Swiss mice was 175 ± 24 mg/dl ($n = 115$). Concanavalin A alone did not induce hyperglycemia in ICR Swiss males. These results were confirmed (data not shown) in a second experiment.

Effects of IL-2 on EMCV-D–mediated disease. Nine-week-old ICR Swiss males were injected intraperitoneally with human recombinant IL-2 in several concentrations (100, 270, and 2000 U/mouse) immediately before infection with 800 PFU EMCV-D. Of the three concentrations used, only the 2000-U dose was effective in protecting most males against virus-induced diabetes when administered immediately before virus infection. In Table 4, note that this dose of IL-2 markedly diminished the incidence of diabetes seen at 1 and 2 wk after infection and also reduced the severity of diabetes (lower mean BG). The 2000-U dose was also given 12 h after infection, after one virus cycle. Although the data are not shown, this treatment also protected the males against severe diabetes (lower mean BG) and diminished the incidence of diabetes from 80 to 30% and from 70 to 40% at 1- and 2-wk observation times, respectively. These data indicate that IL-2 given immediately before virus infection was more effective in protecting most males from virus-induced diabetes than when administered 12 h after infection.

TABLE 4
Effect of interleukin 2 (IL-2) pretreatment on diabetogenicity of EMCV-D in 9-wk-old male ICR Swiss mice

Treatment	Days after treatment	Mean blood glucose ± SD (mg/dl)	Diabetic mice/ total mice
IL-2*	4	Not done	
	7	190 ± 37	0/8
	14	176 ± 50	0/8
EMCV-D†	4	383 ± 205	7/10
	7	390 ± 130	8/10
	14	330 ± 115	7/10
IL-2/EMCV-D‡	4	183 ± 74	1/10§
	7	245 ± 74	3/10§
	14	167 ± 61	1/10§

Mean blood glucose value of 161 normal males = 175 ± 30 mg/dl. EMCV, encephalomyocarditis virus.
*Injected with 2000 U i.p. human recombinant IL-2.
†Infected with 800 plaque-forming units (PFU) i.p. EMCV-D.
‡Injected with 2000 U i.p. human recombinant IL-2 immediately before infection with 800 PFU i.p. EMCV-D.
§$P < .01$ compared with EMCV-D–infected males at the same time point.

Modulation of Ia antigen expression in virus-infected male and female ICR Swiss mice. Peritoneal and spleen cells of ICR Swiss mice were examined for Ia antigen expression with anti-Ia^p and anti-I-E (Ia 7) monoclonal antibodies in cytotoxicity assays. With these monoclonal antibodies, similar percentages of Ia antigen–positive cells were seen in the resident peritoneal cell population (20–29%) and spleen cells (42–68%) of untreated male and female ICR Swiss mice. The percentage of Ia antigen–positive cells in the peritoneal cell population of this outbred mouse strain is considerably higher than that found in inbred mouse strains (10% Ia antigen–positive cells), but the percentage of Ia antigen–positive cells in the spleen is similar to the ~50% Ia antigen–positive cells found in the spleens of inbred mouse strains (10). Pooled peritoneal cell preparations from three mice were used in obtaining the values shown in Table 5. At 1 and 4 days after EMCV-D infection, ICR Swiss females showed an increase in the percentages of peritoneal cells expressing Ia antigen (as detected with anti-Ia^p monoclonal antibody), whereas infected males did not (Table 5). Males treated with 2000 U of human recombinant IL-2 immediately before EMCV-D infection showed a percentage of Ia antigen–positive cells equivalent to that of the virus-infected females 1 day but not 4 days after infection. At 4 days after infection with EMCV-D, the percentage of cells expressing I-E (Ia 7) antigen was significantly less ($P < .05$) in spleen cell preparations from infected males than from infected females (data not shown).

DISCUSSION

Early antiviral cellular host resistance mechanisms, i.e., macrophage and NK cell functions, were evaluated in male and female ICR Swiss mice infected with EMCV-D in an effort to discern whether either contributed to the differential sex-dependent susceptibility or resistance to the diabetogenic effects of EMCV-D. Because carrageenan impairs both macrophage and NK cell activities (6,11), the effects of type I carrageenan on the diabetogenesis of EMCV-D in females and EMCV-B in male and female ICR Swiss mice were first determined. The carrageenan treatment regimen of Chaouat and Howard (6), which effectively depletes mice of reticuloendothelial activity for at least 5 days, was used. Type I carrageenan injected intraperitoneally 1 day before infection with EMCV-D made the usually resistant ICR Swiss females as susceptible to virus-induced diabetes as the diabetes-susceptible ICR Swiss males (Table 1). Carrageenan pretreatment affected the ICR Swiss male, but not the female, to the extent that the nonpathogenic EMCV-B variant caused diabetes in males. Peritoneal macrophage preparations from males were more severely depleted of hydrogen peroxide–generating potential by 1 day after carrageenan injection than those from carrageenan-treated females. The only other instance in which EMCV-B exerted diabetogenicity in male mice occurred when mice were treated with antibody to murine fibroblast IFN 1 h before infection (12).

TABLE 5
Percent Ia antigen–positive cells in peritoneal washings of 9-wk-old ICR Swiss mice after intraperitoneal infection with 800 plaque-forming units (PFU) EMCV-D

	Ia-positive cells (%)*	
Sex	1 day	4 days
M	24 ± 1	28 ± 13
F	71 ± 5‡	56 ± 8§
M†	71 ± 8‡	29 ± 8

Cedarlane anti-Ia^p monoclonal defining a "new" public antigen. Reacts with Ia from $Ia^{p,k,b,q,r,s}$ haplotypes; reactivity against b haplotype has been localized to A^b subregion. Triplicate assays of each sample (2–3 mouse cell pools) were done. EMCV, encephalomyocarditis virus.
*Values (mean ± SD) for percent Ia antigen–positive cells in peritoneum of uninfected mice: males, 23 ± 21%; females, 26 ± 21%.
†2000 U human recombinant IL-2 injected intraperitoneally immediately before virus.
‡$P < .01$ compared with value for infected males.
§$P < .05$ compared with value for infected males.

The diabetogenic (D) and nondiabetogenic (B) variants of EMCV were isolated from a stock of the diabetogenic EMCV-M by Yoon et al. (15). Because EMCV-D does not induce IFN in mouse L929 cell cultures, whereas EMCV-B induces IFN in vivo as well as in vitro, the diabetogenicity of the D variant has been attributed to both its deficiency in inducing IFN and its tropism for pancreatic β-cells (15). Cohen et al. (16) showed that the IFN-inducing property of the nondiabetogenic EMCV-B was responsible for the greater sensitivity of EMCV-B to exogenous IFN as a result of augmented local IFN induction (16). The EMCV-D–mediated diabetes can be prevented by exogenous IFN in susceptible SJL (12) and SWR/J males (17) but not in susceptible ICR Swiss (17) or DBA/2J males (D.J. Giron, personal communication).

No differences in the splenic NK cell responses of EMCV-D–infected males and females were seen during the 1st wk of infection with YAC-1 cells as target cells (Table 2). Depletion of NK cell activity in female ICR Swiss mice with rabbit anti-asialo GM_1 serum did not make the females susceptible to diabetes induction by the diabetogenic EMCV-D (Table 2). Because no differences were seen in NK cell activity between males and females after infection with EMCV-D, any effects that carrageenan exerted in rendering the infected female susceptible to virus-induced diabetes were likely to be the result of its action on macrophages.

Receptors for EMCV are present on small numbers of either resting or activated macrophages but only activated lymphocytes (18). These cells are also involved in the induction of immune responses and are major producers of virus-induced IFN (IFN-α/β) and immune IFN-γ (19). Females exhibit greater macrophage activity and are generally better responders immunologically than are males (20–22). Estrogens enhance murine macrophage expression of Ia antigens in vitro (23) and in vivo (24) and induce the expression in rat uterus of 2′,5′-oligoadenylate synthetase, an enzyme implicated in the activation of the IFN system (25).

The effects of immunomodulators that trigger the production of IFN-γ, i.e., concanavalin A and IL-2 (7,26,27), on the pathogenesis of EMCV-D in diabetes-prone male ICR Swiss mice were then examined. Estrogen pretreatment prevents diabetes from developing in the diabetes-susceptible ICR Swiss (1) and DBA/2J males (D.J. Giron, personal communication). The observations that estrogens both enhance macrophage reticuloendothelial activity and induce macrophage expression of Ia antigens in mice (20,22) suggest that females may also be more competent producers of IFN-γ

as well as virus-induced IFN-α/β as shown by Pozzetto and Gresser (2). Male ICR Swiss mice treated with concanavalin A immediately before infection with EMCV-D were protected from virus-induced disease (Table 3). This observation suggested that deficits in the production of concanavalin A–induced cytokines, e.g., IL-2 and IFN-γ (26,28–33), occurred in disease-susceptible males after EMCV-D infection. Administration of 2000 U human recombinant IL-2 intraperitoneally immediately before intraperitoneal injection of EMCV-D was effective in reducing both the incidence and severity of diabetes in ICR Swiss males (Table 4).

Additional evidence that the cytokine cascade response of immune induction is compromised in male but not in female ICR Swiss mice after EMCV-D infection has been provided by determining the percentage of Ia antigen–positive cells in the peritoneal injection site and spleen 1 and 4 days after infection (Table 5). At these times, the percentages of cells expressing either the I-A or I-E glycoproteins (Ia antigens) in cytotoxicity assays were decreased in males, but not in females, or 1 day after infection in males pretreated with 2000 U of human recombinant IL-2. Collectively, these findings agree with the dogma that Ia antigen–bearing cells increase early in the induction of immune responses (10,31) and suggest that females are more competent in the production of IFN-γ, the major inducer of Ia antigen expression in vivo, in response to EMCV-D. The disease susceptibility of the ICR Swiss male may result from EMCV-D–induced defect in macrophage stimulation of T-lymphocyte production of IL-2 and IFN-γ.

ACKNOWLEDGMENTS

We thank Dr. David J. Giron of Wright State University and Dr. Howard M. Johnson of the University of Florida, Gainesville, for their interest and helpful suggestions.

This study was supported in part by a grant-in-aid to N.J.B. from the Miami Valley Chapter/Ohio Affiliate of the American Heart Association.

REFERENCES

1. Giron DJ, Patterson RR: Effect of steroid hormones on virus-induced diabetes mellitus. *Infect Immun* 37:820–22, 1982
2. Pozzetto B, Gresser I: Role of sex and early interferon production in the susceptibility of mice to encephalomyocarditis. *Virus* 66:701–709, 1985
3. Bukowski JF, Biron BA, Welsh RM: Elevated natural killer cell-mediated cytotoxicity, plasma interferon and tumor rejection in mice persistently infected with lymphocytic choriomeningitis virus. *J Immunol* 131:991–96, 1983
4. Boraschi D, Ghezzi P, Sclimona M, Tagliabue A: Interferon-beta-induced reduction of superoxide by macrophages. *Immunology* 45:621–28, 1982
5. Mishell BB, Shiigi SM (Eds.): *Selected Methods in Cellular Immunology*. San Francisco, CA, Freeman, 1980
6. Chaouat G, Howard JG: Influence of reticuloendothelial blockage on the induction of tolerance and immunity by polysaccharides. *Immunology* 30:221–27, 1976
7. Cleveland PR, Claman HN: T cell signals: tolerance to DNFB is converted to sensitization by a separate nonspecific signal. *J Immunol* 124:474–80, 1980
8. Pick E, Mizel D: Rapid microassays for the measurement of superoxide and H_2O_2 production by macrophages in culture using an automatic enzyme immunoassay reader. *J Immunol Methods* 46:211–26, 1981
9. Pick E, Keisari Y: A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. *J Immunol Methods* 38:161–70, 1980
10. Unanue ER: Antigen-presenting function of the macrophage. *Annu Rev Immunol* 2:395–428, 1984
11. Herberman RB, Djeu JY, Kay HD, Ortaldo JR, Riccardi C, Bonnard GD, Holden HT, Fagnani R, Santoni A, Puccetti P: Natural killer cells: characteristics and regulation of activity. *Immunol Rev* 44:43–70, 1979
12. Yoon J-W, Cha C-Y, Jordan GW: The role of interferon in virus induced diabetes. *J Infect Dis* 147:155–259, 1983
13. Boraschi D, Niederhuber JE: Regulation of macrophage suppression and cytotoxicity by interferon: role of Ia-bearing macrophages. *J Immunol* 129:1854–58, 1982
14. Boraschi D, Censini S, Bartalini M, Tagliabue A: Regulation of arachidonic acid metabolism in macrophages by immune and nonimmune interferons. *J Immunol* 135:502–505, 1985
15. Yoon J-W, McClintock PR, Onodera T, Notkins AL: Virus-induced diabetes mellitus. XVIII. Inhibition by a nondiabetogenic variant of encephalomyocarditis virus. *J Exp Med* 152:878–92, 1980
16. Cohen SH, Bolton V, Jordan GW: Relationship of interferon-inducing particle phenotype to encephalomyocarditis virus-induced diabetes mellitus. *Infect Immun* 42:605–11, 1983
17. Giron DJ, Cohen SJ, Lyons SP, Wharton CH, Cerutis DR: Inhibition of virus-induced diabetes mellitus by interferon is influenced by the host strain. *Proc Soc Exp Biol Med* 173:328–31, 1983
18. Morishima T, McClintock PR, Billups LC, Notkins AL: Expression and modulation of virus receptors on lymphoid and myeloid cells: relationship to infectivity. *Virology* 116:605–18, 1982
19. Oppenheim JJ, Ruscetti FW, Steeg PS: Interleukins and interferons. In *Basic and Clinical Immunology*. 5th ed. Stites DP, Stobo JD, Fudenberg HH, Welks JV, Eds. Los Altos, CA, Lange, 1984, p. 86–103
20. Vernon-Roberts B: The effects of steroid hormones on macrophage activity. *Int Rev Cytol* 25:131–159, 1967
21. Nothdurft W, Fleming K: Quantitative study of sex difference in RE phagocytosis. *Adv Exp Med Biol* 15:95–110, 1971
22. Arend WP, Mannik M: The macrophage receptor for IgG: number and affinity of binding sites. *J Immunol* 110:1455–63, 1973
23. Flynn A: Induction of class II major histocompatibility complex (MHC) antigen (Ia) expression on murine cells with sex steroids (Abstract). *Fed Proc* 45:1138, 1986
24. Flynn A: Expression of Ia and the production of interleukin-1 by peritoneal exudate macrophages activated in vivo by steroids. *Life Sci* 38:2455–60, 1986
25. Tan S-P, Hache RJG, DeeLey RG: Estrogen memory effect in human hepatocytes during repeated cell divisions without hormone. *Science* 234:1234–36, 1986
26. Torres BA, Farrar WL, Johnson HM: Interleukin-2 regulates immune interferon (IFN gamma) production by normal and suppresson cell cultures. *J Immunol* 128:2217–19, 1982
27. Malkovsky M, Medawar PB: Is immunological tolerance (non-responsiveness) a consequence of interleukin-2 deficit during the recognition of antigen? *Immunol Today* 5:340–43, 1984
28. Steeg PS, Moore RN, Johnson HM, Oppenhein JJ: Regulation of murine Ia antigen expression by a lymphokine with immune interferon activity. *J Exp Med* 156:1780–93, 1982
29. Steeg PS, Johnson HM, Oppenheim JJ: Regulation of murine Ia antigen expression by an immune interferon-like lymphokine: inhibitory effect of endotoxin. *J Immunol* 128:2402–406, 1982
30. Epstein LB: The special significance of interferon gamma. In *Interferon. Interferons and the Immune System*. Vol. 2. Vilcek J, DeMaeyer E, Eds. Amsterdam, Elsevier, 1984, p. 186–200
31. Unanue ER: The regulatory role of macrophages in antigenic stimulation. Pt. 2. Symbiotic relationship between lymphocytes and macrophages. *Adv Immunol* 31:1–136, 1981
32. Beller DI, Kiely J-M, Unanue ER: Regulation of macrophage populations. I. Preferential induction of Ia-rich peritoneal exudates by immunological stimuli. *J Immunol* 124:1426–32, 1985
33. Nathan EF, Murray HW, Wiebe ME, Rubin BY: Identification of interferon γ as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J Exp Med* 158:670–89, 1983
34. Hugin A, Cerny A, Wrann M, Hengartner H, Zinkernagel RM: Effect of cyclosporin A on immunity to *Listeria monocytogenes*. *Infect Immun* 52:12–17, 1986