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Original Contributions

Intrauterine Latent Herpes Simplex Virus Infection:

I. Spontaneous Abortion

JAMES A. ROBB, MD,* KURT BENIRSCHKE, MD,† AND ROBERT BARMEYER, MD‡

Herpes simplex virus (HSV, probably type 2) antigen was detected in nonpregnant and pregnant endometria, placentae, umbilical cords, and neonatal tissues (companion paper) by avidin–biotin complex immunohistochemical studies. HSV cytologic abnormalities were not detected in any of the 380 cases examined: included were specimens from therapeutic and spontaneous abortions (200 cases) and endometrial curettage (180 cases). The presence of inflammation was not correlated with HSV positivity. Endometrial HSV positivity was significantly correlated with normal late secretory phase (40 per cent of specimens positive), abnormal secretory phase (67 per cent positive), and therapeutic (33 per cent positive) versus spontaneous (26 per cent positive) abortions. Placental HSV positivity was significantly correlated with spontaneous (39 per cent positive) versus therapeutic (14 per cent positive) abortions and with blighted ova (67 per cent positive). No significant correlation was found between HSV positivity and a clinical history of oral or genital HSV infection in either the patient or the male partner. The data support the concept of a subclinical latent intrauterine endometrial HSV infection that is hormonally regulated and can produce transplacental infection of the embryo or fetus, with variable consequences. HUM PATHOL 17:1196–1209, 1986.

Acute intrauterine herpes simplex virus (HSV) infection, although rare,[1] has been increasing in frequency.[2] Our study was prompted by the following case of acute intrauterine HSV infection. A full-term infant was delivered alive with acute HSV infection of the skin and died a few days later of systemic infection. The presence of acute HSV infection in the skin and brain was confirmed by morphologic HSV-specific cytologic studies, culture, and transmission electron microscopy. No evidence of HSV infection was found in routine studies of the placenta and umbilical cord by the same techniques. Transplacental infection, however, must have occurred, because the infection was not acquired from the birth canal during delivery. A newly developed and highly sensitive glucose oxidase–avidin–biotin immunohistochemical technique for formalin-fixed tissues[3] demonstrated HSV-specific antigen in phagocytic cells of the placental subamnionic chorion, in Hofbauer cells in chorionic villi, and in the subamnionic mesenchyme of the umbilical cord. No inflammation or HSV cytologic abnormalities were present in these areas.

Spontaneous and therapeutic abortion material, endometrial curettage tissue, full-term placentae and umbilical cords, and stillborn and liveborn neonatal tissues were studied by HSV-specific immunohistochemical methods to evaluate the prevalence and distribution of these HSV antigens in cases in which HSV infection was not suspected or detectable as HSV cytologic abnormalities by light microscopy, virus particles by transmission electron microscopy, or infectious HSV by direct culture. The presence and tissue distribution of the HSV antigens were then correlated with the finding for normal and pathologic conditions. The data concerning the full-term neonates are presented in the companion paper.[4] The data concerning the detection of persistent intrauterine HSV infection and its correlation with pathologic states in abortion and curettage material are presented below.

MATERIALS AND METHODS

All tissues were routine surgical (products of conception, endometrial curettage, placentae, and umbilical cords) or autopsy (neonates and infants) specimens fixed in 10 per cent buffered formalin or Bouin's solution (placentae and cords only) and embedded in paraffin. The products of conception and endometrial tissues had been collected from 1981 to 1984 at the Green Hospital of Scripps Clinic. The placentae, umbilical cords, and neonatal or infant tissues had been collected from 1981 to 1984 at the University of California San Diego Medical Center. Some neonatal cases had been submitted from other hospitals for consultation. Serial 4-μm sections were attached to glass slides with either 1 per cent Elmer's glue in deionized water or chromic acid/poly-D-lysine (50 per cent chromic acid in deionized water for 30 minutes at room temperature, followed by rinsing and 0.01 per cent poly-D-lysine [Sigma, St. Louis,

Received from the Departments of *Pathology and ‡Obstetrics, Green Hospital of Scripps Clinic, La Jolla, and the †Department of Pathology, University of California San Diego Medical Center, San Diego, California. Accepted for publication April 22, 1986. Presented in part in poster format at the 1984 International Academy of Pathology annual meeting (Lab Invest 50:50A, 1984). Supported by Scripps Clinic General Research Center Clinfo Computer–NIH Division of Research Resources grant RR-00833 for data analysis and institutional overhead for facilities.

Address correspondence and reprint requests to Dr. Robb: Department of Pathology, Green Hospital of Scripps Clinic, La Jolla, CA 92037.

0046-8177/86 $0.00 + .25

Missouri] in deionized water for 30 minutes at room temperature, followed by rinsing). The paraffin sections were not dried in an oven, but were left at room temperature for at least two hours before routine removal of paraffin and immunohistochemical staining.

All of the following steps were done at room temperature in a humidified box. The tissue sections were treated with 0.25 per cent crude porcine trypsin (Sigma, catalog no. T-8128) in phosphate-buffered saline (PBS, pH 7.4) for 60 minutes and rinsed with PBS by a jet-dribble technique. Primary polyclonal rabbit anti-HSV type 1 and type 2 IgG (Dako, Santa Barbara, California; lots 059A and 1188, respectively) and nonimmune rabbit IgG (Cappell Labs, West Chester, Pennsylvania) were incubated for 22 to 24 hours at 1:2,000 (HSV types 1 and 2) and at 10 μg/ml (nonimmune rabbit IgG) in PBS+ (PBS with 0.2 per cent sodium azide [Sigma] and 0.1 per cent bovine serum albumin [Calbiochem, La Jolla, California; type V]). The tissues were again rinsed with PBS, and biotinylated goat antirabbit IgG (3.25 μg/ml PBS+; Vector Labs, Burlingame, California), biotinylated horse antimouse IgG (30 μg/ml, Vector), or biotinylated goat anti-guinea pig IgG (7.5 μg/ml, Vector), depending on the species of primary antibody (see table 2), was added for 30 minutes. After PBS rinsing, a glucose oxidase–avidin–biotin complex (ABC–GO, Vector) was added for 30 minutes at room temperature. The staining reaction was identical to the previously described glucose oxidase–avidin–biotin (GAB) technique originally used in this study.[3] Tetranitroblue tetrazolium (TNBT) formazan formation (Vector) was used to detect sites of antibody binding. This "colorization reaction" was identical to that originally used in this study.[3] After a deionized water rinse, aqueous counterstaining was accomplished with 0.25 per cent metanil yellow in deionized water for 1 minute, deionized water rinse, and 0.1 per cent nuclear fast red for 5 minutes; a 0.45-μm filter (Gelman Acrodisc, Gelman Sciences, Ann Arbor, Michigan) was used at the time of addition to remove microcrystals. Aquamount (Lerner Labs, New Haven, Connecticut) was used for coverslipping. No loss of stain occurred during a four-year period.

For statistical analyses a Clinfo software program was used in the Scripps Clinic General Clinical Research Center. All data were nonparametric, except for the patient age data, which were normally distributed and analyzed by the Anderson-Darling *t*-test. The nonparametric data were analyzed with the Wilcoxon nonpaired rank sum test.

RESULTS

Types of HSV 2 Staining Detected

The rabbit anti-HSV 2 antibody staining in placental tissues was cytoplasmic and occurred in individual cells in the subamnionic chorion (fig. 1) and in single Hofbauer cells in chorionic villi (fig. 2). Umbilical cord staining occurred in single cells in the subamnionic mesenchyme (fig. 3), perivascular mesenchyme (fig. 4) and, rarely, vascular smooth muscle (fig. 4). No nuclear, amnionic, trophoblastic, or endothelial staining was detected in placental tissues. Apical and/or diffuse cytoplasmic staining occurred focally in the endometrial epithelium in both products of conception and nonpregnant endometrium, without specific nuclear staining (fig. 5). Single choriodecidual cells were positive, as in the placentae (fig. 6). Endothelial staining was rare (three of 380 cases) and was found only in choriodecidual tissue, where it was both cytoplasmic and nuclear (fig. 6).

Acute HSV endometrial epithelial infections, although rare, have been documented.[5,6] Altered endometrial epithelial nuclei with features suggestive of HSV infection, but without viral particles by transmission electron microscopy, were found in 7 per cent of a group of 200 cases in which endometrium was associated with trophoblastic tissue.[7] Endothelial cells can be infected by HSV, with subsequent altered function and death.[8,9] Histologically occult cytomegalovirus (CMV) infection has been detected in human tissue by immunohistochemical methods[10] and by in situ DNA hybridization.[11]

Either the detected antigens or their detecting antibodies were at a low concentration, because the screening anti-HSV 2 antibody titer difference between acute and chronic or latent infection was about 100-fold (acute—1:20,000 for two hours; chronic or latent—1:2,000 for 20 hours). The pattern of embryonic and neonatal staining is described in the companion paper.[4]

Antibody Specificity

Other virus-infected tissues. Table 1 demonstrates the specificity of the anti-HSV antibodies when tested against culture-proven acute HSV 1 infection in the skin (fig. 7), brain, and esophagus, and against HSV 2 infection in the liver (acute) and adrenal gland (subacute, i.e., resolving intrauterine infection, several weeks after acute infection; fig. 7). Anti-HSV 1 and HSV 2 antibodies strongly cross-react in tissues acutely infected with HSV 1 and HSV 2 (see table 1 for end-point titers). The antibodies were absolutely negative in other tissues with culture-proven and/or immunohistochemically proven infections with the other three herpetoviruses (herpes zoster virus, CMV, and Epstein-Barr virus) and other miscellaneous viruses (hepatitis A and B, papillomavirus, and echovirus). A placenta with culture-proven listeria infection was also negative. The virus used as antigen for antibody production was purified from culture medium containing fetal bovine serum. For that reason, the antibodies were tested against tissues containing both human and bovine alpha-fetoprotein and were found to be negative (table 1). Furthermore, adsorption of the anti-HSV 2 IgG with fresh fetal bovine liver powder (acetone-extracted) did not decrease the specific staining in target tissues.

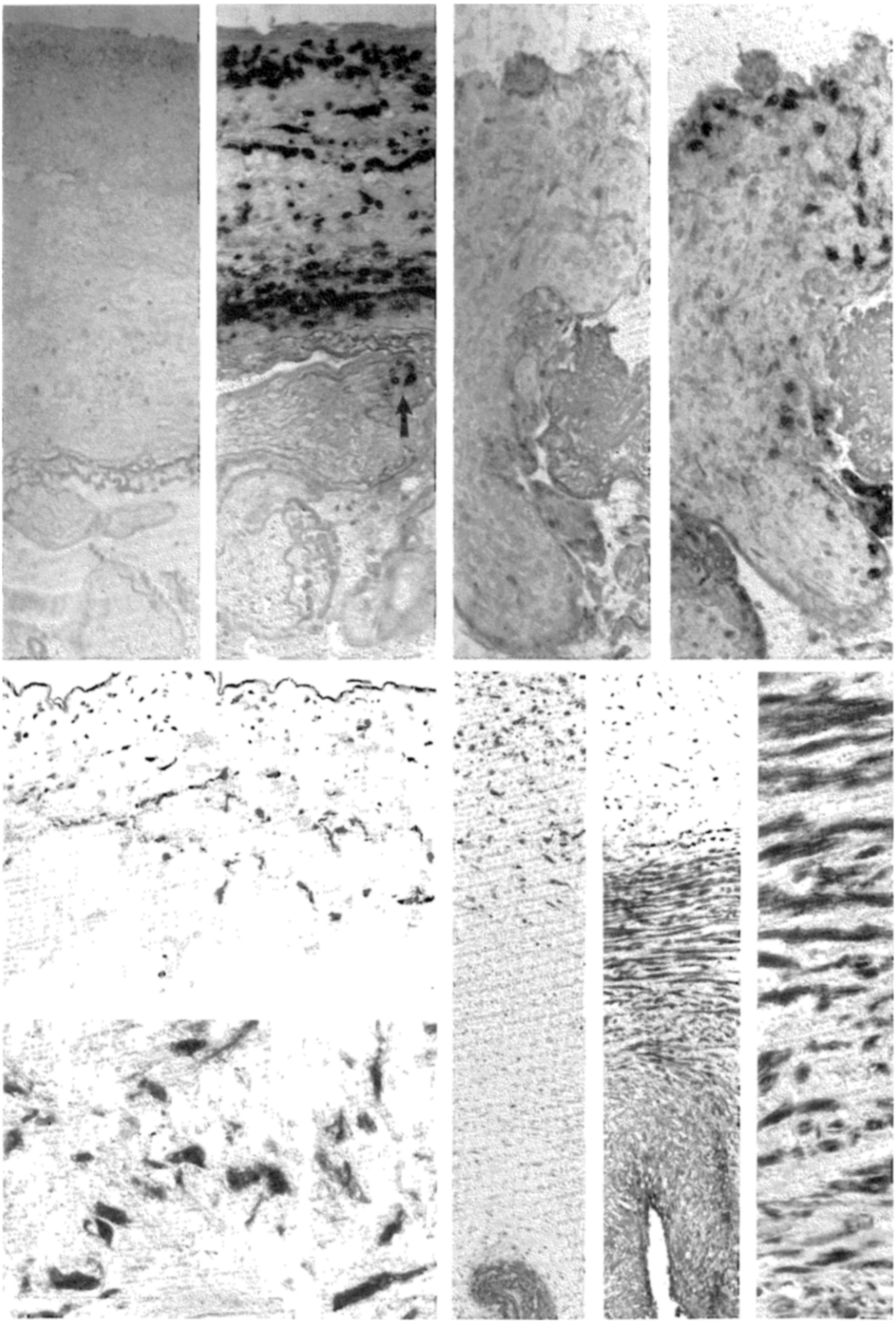

FIGURE 1 (*top left*). Full-term placenta stained with nonimmune rabbit or rabbit anti-HSV 1 IgG (*left*) and rabbit anti-HSV 2 IgG (*right*). Notice the lack of staining in the amnion (upper right) with strong individual cell staining in the subamnionic chorion and villus Hofbauer cells (*arrow*). (Original magnification, ×100.)

FIGURE 2 (*top right*). Full-term chorionic villus stained with nonimmune rabbit or rabbit anti-HSV 1 IgG (*left*) and rabbit anti-HSV 2 IgG (*right*). Strongly positive single Hofbauer cells are visible (*left*). (Original magnification, ×400.)

FIGURE 3 (*bottom left*). Full-term umbilical cord stained with rabbit anti-HSV 2 IgG showing strong individual cell staining in the subamnionic mesenchyme. (Original magnification: *left,* ×40; *right,* ×400.)

FIGURE 4 (*bottom right*). Rabbit anti-HSV 2 IgG staining of full-term umbilical cord perivascular mesenchyme (*left*) with negative vascular smooth muscle. Rare staining of vascular smooth muscle by one control rabbit anti-HSV 1 serum (Richman serum, *middle* and *right*). The perivascular mesenchyme did not stain. *Left* and *middle,* ×100; *right,* ×400.

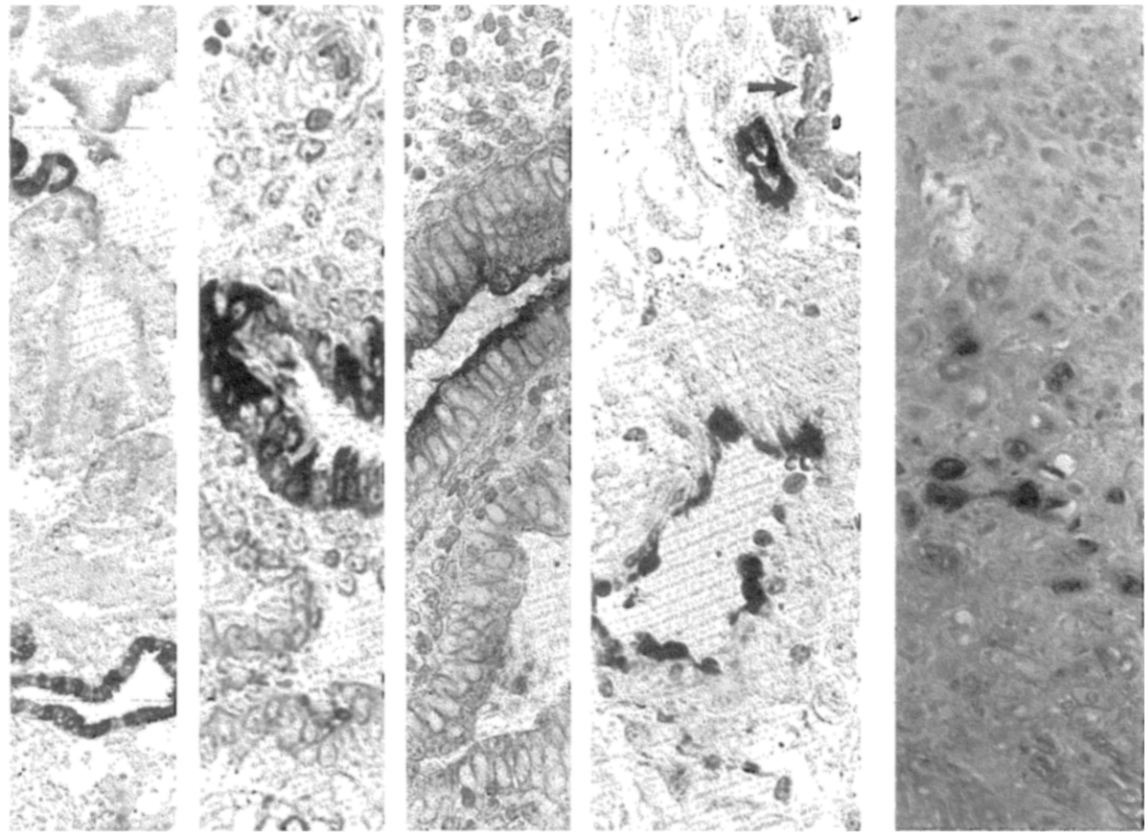

FIGURE 5 (*left*). Rabbit anti-HSV 2 IgG staining of endometrial glandular epithelium. The adjacent glands are negative. The diffuse cytoplasmic pattern (*left* and *middle*) predominates over the apical pattern (*right*), with both patterns occurring in a few cases. (Original magnification: *left*, ×100; *middle* and *right*, ×400.)

FIGURE 6 (*right*). Rabbit anti-HSV 2 IgG staining of endothelial nuclei focally in capillaries of choriodecidua (*left*); the endometrial epithelium is negative (*arrow*). *Right*, individual cell staining in choriodecidua. (Original magnification, ×400.)

TABLE 1. Herpes Simplex Virus (HSV) Antibody Specificity: Other Tissues

Infectious Agent	Tissue	Clinical Diagnosis	Detection Method
Negative tissues			
Varicella-Zoster	Skin	Acute shingles	Culture
Epstein-Barr	Spleen (patient 1)	Acute infectious mononucleosis	Serum IgM
Epstein-Barr	Tonsil (patient 2)	Acute infectious mononucleosis	
Cytomegalovirus	Lung (3 patients)	Bone marrow transplant/leukemia	Culture/immunohistochemistry
Cytomegalovirus	Placenta (3 patients)	Intrauterine infection	Culture/immunohistochemistry/cytopathology
Cytomegalovirus	Stomach	AIDS	Culture/immunohistochemistry/cytopathology
Hepatitis A	Liver (3 patients)	Acute hepatitis	Serum IgM
Hepatitis B	Liver	Acute hepatitis	Serum markers/immunohistochemistry
Hepatitis B	Liver	Chronic active hepatitis	Serum markers/immunohistochemistry
Echovirus	Brain	Acute encephalitis	Immunohistochemistry
Human papilloma	Skin	Verruca vulgaris	Immunohistochemistry
Human papilloma	Cervix	Condyloma	Immunohistochemistry
Listeria	Placenta	Necrotizing placentitis	Culture
No agent	Endodermal sinus tumor	Alpha-fetoprotein-positive	Immunohistochemistry
No agent	Hepatoma	Alpha-fetoprotein-positive	Immunohistochemistry
No agent	Fetal bovine liver	Alpha-fetoprotein-positive	Immunohistochemistry
Positive tissues			
HSV 1*	Brain (patient 1)	Acute encephalitis	Culture
HSV 1*	Skin (patient 2)	Recurrent cold sore	Culture/cytopathology
HSV 1*	Esophagus (patient 3)	Acute esophagitis	Cytopathology
HSV 2*	Liver (patient 4)	Adult systemic HSV 2 infection	Culture/cytopathology
HSV 2*	Adrenal (patient 5)	Intrauterine subacute infection	Culture/cytopathology

* HSV 1 and HSV 2 antibody titers against the HSV 1 acutely infected skin were 1:16,000 and 1:8,000, respectively; the titers against the HSV 2 subacutely infected adrenal gland were 1:4,000 and 1:16,000, respectively.

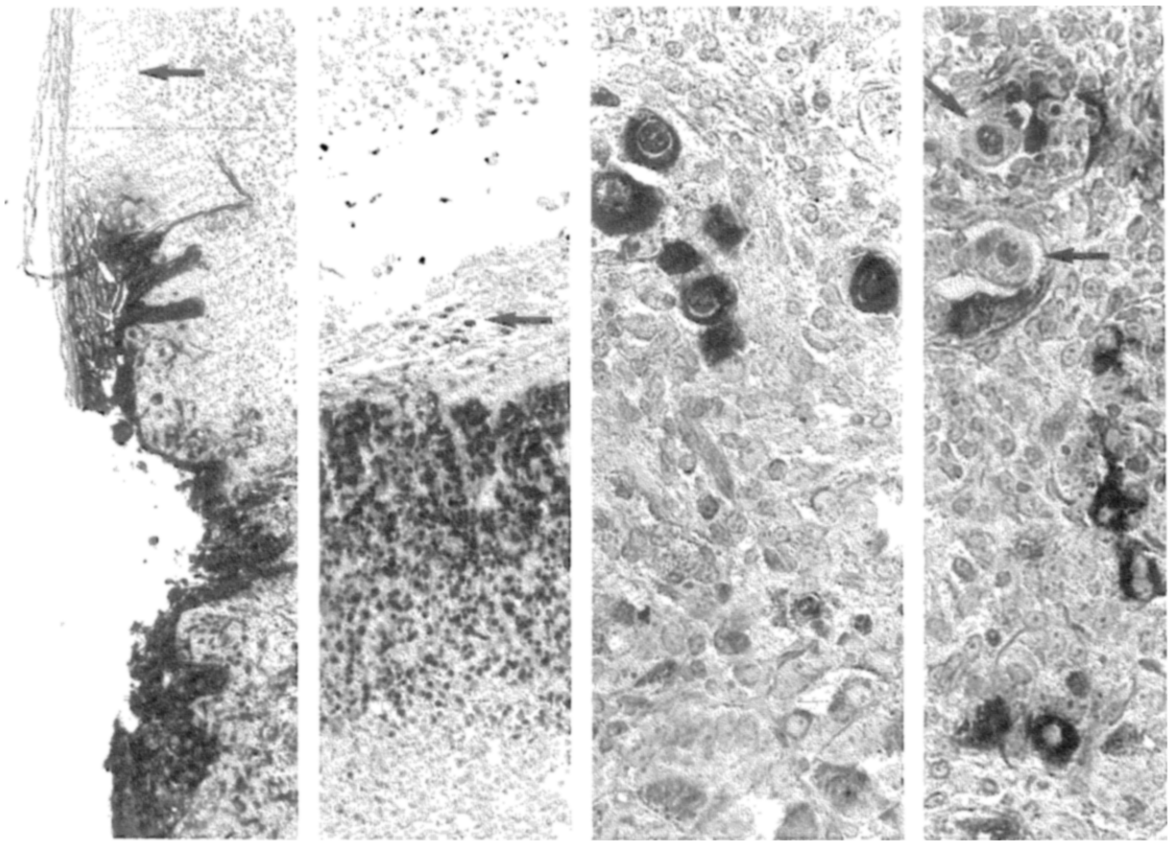

FIGURE 7 (*left*). Rabbit anti-HSV 2 IgG staining of acute HSV 1 infection in skin (*left*; negative epidermis is designated by arrow) and resolving or subacute (no viral cytologic abnormalities) HSV 2 infection in neonatal adrenal gland (*right*; positive macrophages in adjacent connective tissue are shown by the arrow). (Original magnification, ×100.)

FIGURE 8 (*right*). Goat anti-cytomegalovirus (CMV) serum (*left*) and rabbit anti-HSV 2 IgG (*right*) staining of gastric biopsy specimen from a patient with AIDS and acute CMV infection of the gastrointestinal tract. The anti-CMV serum stains only cells with cytologic evidence of CMV (*left* versus arrow in right photograph), and anti-HSV 2 IgG stains only cells without cytologic evidence of CMV (*right*). (Original magnification, ×100.)

Other antiviral antibodies. Table 2 lists the staining results for various HSV-specific and nonspecific antibodies with the various target tissues. All of the UCSD/DR rabbit antisera and the Yale/GDH guinea pig antisera were coded samples at the time of tissue staining and evaluation. The following findings strongly support our hypothesis that the staining detected in the target and patient tissues with the Dako rabbit anti-HSV 2 IgG (lot 1188, our screening antibody) is specific for HSV and, probably, for type 2 infection in the products of conception, placentae, umbilical cords, and endometrial curettage specimens:

1. All preinfection, noninfected, and non-HSV sera were negative with all target tissues, with one exception. One of 11 UCSD/DR HSV 1 antisera produced 4+ staining of the vascular smooth muscle of the umbilical cord target (fig. 4). The meaning of this finding is not known, although histologically occult CMV infection has been detected in human smooth muscle by immunohistochemical methods.[10]

2. All HSV-infected animal sera were positive with at least one target tissue. There was wide individual variation, however, when the same target was used with different sera and different targets were used with the same sera. This phenomenon of variation in individual animal antibody expression to individual virus antigens is to be expected, both in humans[12] and in syngeneic mouse littermates,[13] as observed when cloned virus was used for "identical infection." One example of the specificity of the rabbit anti-HSV 2 antibody is shown in figure 8. This gastric biopsy specimen from a patient with the acquired immunodeficiency syndrome shows CMV-positive cells (goat anti-CMV, Polysciences, 1:200 with trypsin pretreatment), which are negative for HSV 2 antigen, while adjacent gastrothelial cells show latent HSV 2 infection.

3. All of the NIH/MZ mouse anti-HSV monoclonal antibodies were positive in at least three targets. These antisera stain cells infected acutely with both HSV 1 and HSV 2 in cell culture.

4. The NIH/MZ mouse monoclonal antibody ICP4 recognizes an immediate/early (alpha) antigen in a nuclear location in both HSV 1 and 2 acute infections in cell culture. Scattered amnionic nuclei in the placental target were unequivocally positive with this antibody (fig. 9), an indication of latent infection in the amnion. Intranuclear staining by ICP4 was also detected in chorionic nuclei in one case (fig. 9).

TABLE 2. Herpes Simplex Virus (HSV) Antibody Specificity: Other Antibodies

Animal	Virus	Source	Infection Route	Lot	Dilution	Pool/ Individual	HSV 1, Skin	HSV 2, Adrenal	Placenta		Cord			Endometrium
									SAC	VH	SAM	PVM	VSM	
Rabbit	HSV 1	Dako	HISV:SubQ	059A	20/2,000	Pool	4/4	4/4	0/0	0/0	0/0	0/0	0/0	0/0
	HSV 1	Dako	HISV:SubQ	013A	100/2,000	Pool	4/4	4/4	0/0	0/0	0/0	0/0	0/0	0/0
	HSV 2*	Dako	HISV:SubQ	1188	2,000	Pool	4	4	4	4	4	4	4	4
	HSV 2*	Dako	HISV:SubQ	AA	20	Individual	ND	1	0	ND	0	0	0	0
	HSV 2*	Dako	HISV:SubQ	BB	20	Individual	ND	3	0	ND	0	0	0	0
	HSV 2*	Dako	HISV:SubQ	CC	20	Individual	ND	2	2	ND	0	0	0	0
	HSV 2*	Dako	HISV:SubQ	DD	20	Individual	ND	4	0	ND	0	0	0	0
	HSV 2*	Dako	HISV:SubQ	EE	20	Individual	ND	2	2	ND	0	0	0	0
	HSV 2*	Dako	HISV:SubQ	FF	20	Individual	ND	3	4	ND	0	0	0	0
	HSV 2*	Dako	HISV:SubQ	012C	100/2,000	Pool	4/4	4/4	4/1	0/0	0/0	0/0	0/0	0/0
Rabbit	HSV 2	UCSD:DR	Corneal	Pre	25	Individual	0	0	0	0	0	0	0	0
	HSV 2	UCSD:DR	Corneal	Post	25	Individual	3	4	4	ND	0	0	0	0
	CMV	UCSD:DR	Corneal	Pre	25	Individual	0	0	0	ND	0	0	0	0
	CMV	UCSD:DR	Corneal	Post	25	Individual	0	0	0	ND	0	0	0	0
	HSV 1	UCSD:DR	Corneal	11 Pre	25	Individual	0	0	0	ND	0	0	1/11 = 4	0
	HSV 1	UCSD:DR	200 PFU	A	25	Individual	0	3	3	ND	0	3	4	0
	HSV 1	UCSD:DR	Ref. 49	27	25	Individual	1	0	0	ND	0	0	0	0
	HSV 1	UCSD:DR	Ref. 49	28	25	Individual	1	3	0	ND	0	0	0	0
	HSV 1	UCSD:DR	Ref. 49	31	25	Individual	0	1	3	ND	0	0	0	0
	HSV 1	UCSD:DR	Ref. 49	33	25	Individual	1	3	3	ND	0	0	0	0
	HSV 1	UCSD:DR	Ref. 49	34	25	Individual	3	2	3	ND	0	0	0	0
	HSV 1	UCSD:DR	Ref. 49	35	25	Individual	1	3	3	ND	0	0	0	0
	HSV 1	UCSD:DR	Ref. 49	36	25	Individual	1	2	0	ND	0	0	0	0
	HSV 1	UCSD:DR	Ref. 49	38	25	Individual	1	3	0	ND	0	0	0	0
	HSV 1	UCSD:DR	Ref. 49	40	25	Individual	1	3	0	ND	0	0	0	0
	HSV 1	UCSD:DR	Ref. 49	41	25	Individual	3	2	4	ND	0	0	0	0
Guinea pig	HSV 2	Yale:GDH	Vaginal 10^4 PFU	7:-inf	200	Individual	0	5/7:0 2/7:NSB @1:1000	6:0 1:NSB	ND	6:0 1:NSB	7:0	6:0 1:NSB	7:0
		Neut Tit	Ref. 50,51											
	HSV 2	1:80	Ref. 50,51	1	200	Individual	3	4	0	3	0	0	0	0
	HSV 2	1:160	Ref. 50,51	3	200	Individual	2	4	2	0	0	0	0	0
	HSV 2	1:80	Ref. 50,51	4	200	Individual	2	4	0	2	0	0	0	0
	HSV 2	1:160	Ref. 50,51	6	200	Individual	4	4	2	0	2	3	0	0
	HSV 2	1:80	Ref. 50,51	365	200	Individual	3	4	2	4	0	1	0	2
	HSV 2	1:80	Ref. 50,51	393	200	Individual	2	4	2	0	0	0	0	0
	HSV 2	1:40	Ref. 50,51	389	200	Individual	4	4	2	2	3	3	1	0
Mouse	HSV 1	NIH:MZ	Monoclonal	ICP4	40	Individual	0	1	3AN	3CC	0	0	4C?N	2C
	HSV 1	NIH:MZ	Ref. 19	ICP6	40	Individual	1	2	2	2	0	0	4	?Rare
	HSV 1	NIH:MZ	Ref. 19	gD	40	Individual	1	2	4	3	2	2	4	0
	HSV 1	NIH:MZ	Ref. 19	gC	40	Individual	2	Macro	3	2	2	0	4	2
	HSV 2	NIH:MZ	Ref. 19	gC	40	Individual	1	Macro	3	3	0	0	4	0
	HSV 1	NIH:MZ	Ref. 19	ICP8	40	Individual	0	1	4	2	3	2	4	0
	HSV 1	NIH:MZ	Ref. 19	gA/gB	40	Individual	1	1	1	2	0	0	2	2
	HSV 1	NIH:MZ	Ref. 19	ICP5	40	Individual	0	1	1AN	3	0	0	1	?Rare

Negative control antibodies (negative in all above target tissues): Rabbit—normal nonimmune IgG, anti-fetuin, anti-fetal bovine serum, anti-callous keratin, anti-carcinoembryonic antigen, and anti-EBV. Antibovine alpha-fetoprotein was positive only in phagocytic cells of SAC, a subset of which were HSV-2-positive by dual labeling experiments. Guinea pig—normal nonimmune IgG, anti-insulin, and anti-varicella/zoster. Mouse—MOPC-21 and anticytokeratins 35BH11 and AE1. Goat—normal nonimmune IgG and anti-CMV.

ABBREVIATIONS: AN, amniotic nuclear staining; C, cytoplasmic staining; CC, chorionic cell cytoplasmic staining; HISV, hyperimmunization with solubilized virions; Macro, adrenal adventitial macrophages; N, nuclear staining; ND, not done due to scarcity of target tissue; NSB, nonspecific background staining; Post, postinfection; Pre, preinfection; PVM, perivascular mesenchymal cells; SAC, subamnionic chorion cells; SAM, subamnionic mesenchymal cells; VH, chorionic villus Hofbauer cells; VSM, vascular smooth muscle cells; Neut Tit, neutralization titer.

* Lot of anti-HSV 2 IgG used to screen all patient tissues.

Staining grade of 1 to 4: Positive cell number and intensity of stain were compared with the staining observed in tissues stained by the lot 1188 rabbit anti-HSV 2 IgG as follows: 4+, 75–100%; 3+, 50–75%; 2+, 25–50%; 1+, less than 25%.

Endothelial nuclei were positive in three of 380 abortion cases (fig. 6); the rabbit anti-HSV 2 antibody stained specimens in all three cases, while staining with the mouse anti-ICP4 antibody was observed in only two cases. Weak intranuclear staining of amnionic cells in the placental target cells was observed with the mouse anti-ICP5 antibody, which detects nuclear antigen in HSV 1 and 2 acute infection in cell culture.

5. The Dako HSV 2 individual sera were from rabbits that had not had the extensive repeat immunizations undergone by the rabbits in the pool of the screening antibody (lot 1188). They were, therefore, of much lower titer. The "new" lot (no. 012C) of

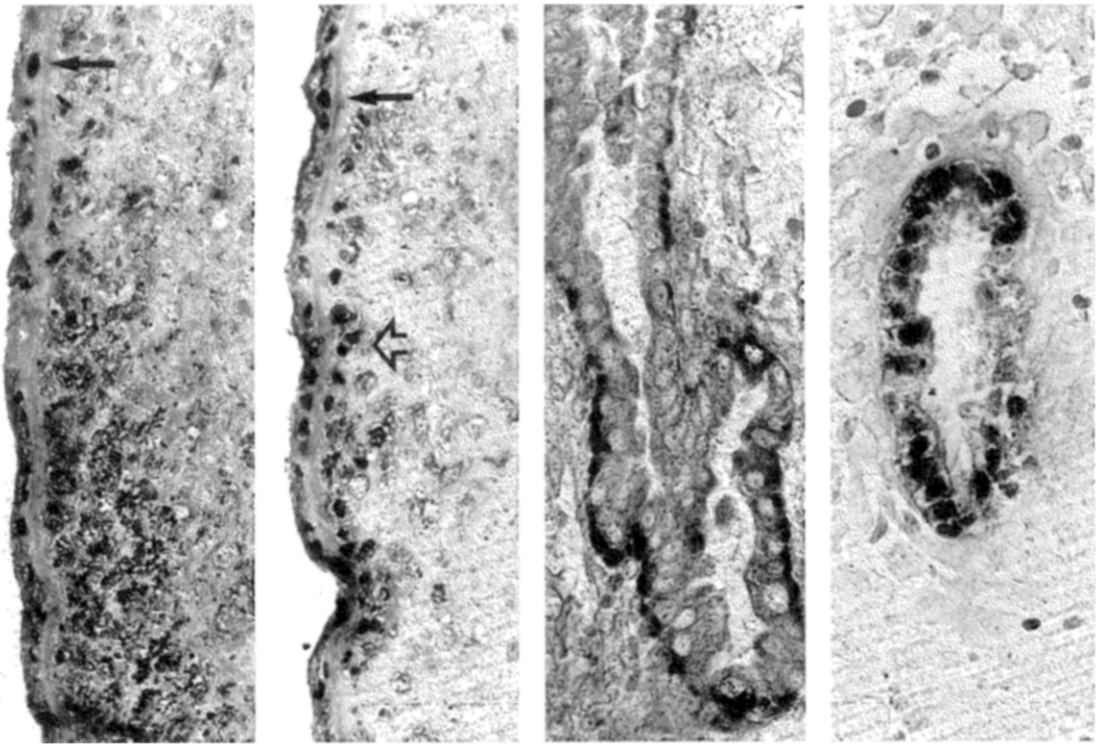

FIGURE 9 (*left*). Mouse monoclonal ICP4 cell culture medium stains amnionic nuclei in full-term placenta (*left*, serial sections of case shown in fig. 1) and both amnionic (*closed arrow*) and chorionic (*open arrow*) nuclei in a second different case (*right*). (Original magnification, ×400.)

FIGURE 10 (*right*). False-positive staining of endometrial gland epithelium: basal cytoplasmic (*left*) and nuclear (*right*). (Original magnification, ×400.)

Dako HSV 2 pooled antibody was intermediate both in the number of repeat immunizations and in titer between the individual antisera and the screening antibody (unpublished data, A. Ingild, Dako).

Adsorption experiments. Two identical adsorption experiments were performed four months apart with coded samples of noninfected frozen cells, or cells infected with CMV or HSV 1 or HSV 2. The thawed cell lysates were mixed with the screening HSV 1 and HSV 2 antibodies for 30 minutes at room temperature and centrifuged, and the supernatant fluid was tested on the target tissues. In the first experiment, the anti-HSV 2 antibody was easily identified by decreased staining, compared with that produced by the nonadsorbed antibody assayed at the same time in serial sections of target tissues. In the second experiment, no reduction in any of the adsorbed antibodies was detected. The reason for the discrepancy is not known, although adsorption experiments such as this can be difficult to replicate and interpret, because of the frozen, then thawed, nonfixed antigens and complement receptors.

False-positive results. Only two false-positive staining patterns were identified, both involving the endometrial epithelium. The first, and more frequent (20 of 380 cases), was a basal cytoplasmic granular staining pattern, without the usual diffuse and/or apical staining (fig. 10). This pattern occurred with several of the HSV-specific and nonspecific antibodies and was independent of the secondary antibody, ABC complex, and colorizing solution. The mechanism of staining is not known. The second, and quite rare (two of 380 cases), false-positive pattern was confined to endometrial nuclei in a focal pattern (fig. 10). This intranuclear staining was independent of the primary and secondary antibodies, the enzymes attached to the ABC complex (peroxidase, alkaline phosphatase, and glucose oxidase), and the various colorizing solutions. It was dependent only on the ABC complex itself. The mechanism remains unknown, because neither biotin or avidin preincubations blocked the staining.

Clinical/Pathologic Abortion Correlations

Table 3 lists the statistical correlations between the observed types of staining, inflammation, gestational history, and clinical histories of HSV infection in both the patient and the male partner. The patient's gestational and HSV infection history was taken at the time of the abortion (nine of 190 patients had clinical histories of genital herpes infection). The information concerning the partner's HSV infection history was obtained from a confidential questionnaire sent to the patient; 44 per cent of the questionnaires were returned, and 7 per cent of the remaining 56 per cent of the patients were lost to follow-up study and could not be reached. Although

44 per cent is a good response rate, we have HSV infection histories for only 75 of 200 (oral infections) and 65 of 200 (genital infections) fathers, and these histories were derived from the mothers.

No viral cytologic abnormalities were detected in any of the 200 specimens. Material from spontaneous abortions was more inflamed than that from therapeutic abortions (92 versus 66 per cent, $P < 0.001$), but there was no difference between the two groups with regard to acute versus chronic choriodeciduitis ($P = 0.565$). Although total HSV positivity (endometrial epithelial positivity and/or placental subamnionic chorion positivity and/or chorionic villus positivity) was similar in the material from spontaneous and therapeutic abortions (48 and 41 per cent, $P = 0.386$), placental subamnionic chorion positivity and chorionic villus positivity were significantly more frequent in spontaneous abortion specimens (39 and 14 per cent) than in those from therapeutic abortions (13 and 0.0 per cent, $P < 0.001$ and $P < 0.001$, respectively), while endometrial epithelial positivity was more frequent in therapeutic than in spontaneous abortion material (33 versus 26 per cent, $P = 0.230$), but not significantly so. Placental subamnionic chorion positivity was dependent on endometrial epithelial positivity (endometrium positive in 40 per cent and negative in 20 per cent, $P = 0.004$), and chorionic villus positivity was dependent on placental subamnionic chorion positivity (chorion positive in 17 per cent and negative in 1.8 per cent, $P = 0.001$), but not on endometrial epithelial positivity (endometrium positive in 4.4 per cent and negative in 7.2 per cent, $P = 0.526$). Blighted ova were associated with spontaneous abortions (material from 78 per cent of spontaneous abortions versus 27 per cent of therapeutic abortions was HSV-positive, $P = 0.001$), acute and chronic choriodeciduitis (more commonly with chronic inflammation—73 per cent of patients with inflammation versus 28 per cent of normal subjects were HSV-positive, $P = 0.001$), placental subamnionic chorion positivity (chorion positive in 67 per cent and negative in 44 per cent, $P = 0.008$), and, possibly, with chorionic villus positivity (villi positive in 70 per cent and negative in 49 per cent, $P = 0.207$, trend) and total HSV positivity (56 per cent positive versus 46 per cent negative, $P = 0.201$), but were not associated with endometrial epithelial positivity (51 per cent positive versus 51 per cent negative, $P = 0.820$). Multiparity was significantly associated with chronic choriodeciduitis (79 per cent with choriodeciduitis versus 58 per cent without choriodeciduitis were HSV-positive, $P = 0.034$) and was possibly associated with total HSV positivity ($P = 0.143$), endometrial epithelial positivity ($P = 0.179$), placental subamnionic chorion positivity ($P = 0.084$), and inflammation ($P = 0.189$).

The presence of genital HSV positivity in the partner was associated only with genital HSV positivity in the patient (50 per cent of the patients with histories of HSV positivity had partners with histories of HSV positivity, whereas 6.7 per cent of patients without histories of HSV infection had partners with histories of HSV positivity, $P = 0.001$). The presence of oral or genital HSV infection in the patient and/or the partner was not associated with any of the HSV-positive staining patterns ($P = 0.522$ and $P = 0.918$, respectively). Recurrent spontaneous abortion may possibly (trend) be associated with endometrial epithelial positivity (endometrium positive in 30 per cent and negative in 18 per cent, $P = 0.143$) and chorionic villus positivity (villi positive in 50 per cent and negative in 23 per cent, $P = 0.092$). Recurrent spontaneous abortion was not associated with total HSV positivity ($P = 0.262$) or placental subamnionic chorion positivity ($P = 0.521$). Endometrial epithelial positivity, placental subamnionic chorion positivity, chorionic villus positivity, and total HSV positivity were all associated with progressively older gestational ages (8.1 [$P = 0.006$], 9.8 [$P = 0.006$], 11.0 [$P = 0.028$], and 11.4 [$P = 0.001$] weeks). Therapeutic abortions, as compared with spontaneous abortions, occurred in younger women (27.2 versus 29.7 years, $P = 0.05$). The gestational age at the time of abortion was not associated with inflammation ($P = 0.966$) or blighted ova ($P = 0.282$).

Clinical/Pathologic Endometrial Curettage Correlations

Table 3 lists the statistical correlations between the endometrial phases, abnormalities, and inflammation for endometrial epithelial negativity and endometrial epithelial positivity for 180 cases. There was no significant difference between the ages of the two groups (HSV-negative, 41.5 years; HSV-positive, 39.8 years; $P = 0.765$) or for the volume of endometrial tissue removed, excluding blood clots and mucus (<3 mm^3 versus 3 to 5 mm^3 versus >5 mm^3, $P = 0.797$).

The detection of endometrial epithelial positivity was significantly different in the different phases of the cycle. Normal proliferative (10 per cent), hyperplastic (14 per cent, $P = 0.697$), and atrophic (6.7 per cent, $P > 0.990$) endometria were not statistically different. Normal secretory (40 per cent) and abnormal secretory (67 per cent) endometria were significantly different from normal proliferative ($P = 0.017$ and <0.001), hyperplastic ($P = 0.001$ and <0.001), and atrophic ($P = 0.014$ and <0.001) endometria, as well as from each other ($P = 0.040$). Furthermore, the cycle date in the cases of normal secretory endometrium was significantly earlier for the HSV-negative cases (day 20.6) than for the HSV-positive cases (day 22.6, $P = 0.004$), suggesting activation of HSV protein synthesis in the latter portion of the normal secretory phase. The percentage of HSV positivity in normal secretory phase endometrium (40 per cent) was similar to that found in material from both therapeutic (41 per cent) and spontaneous (48 per cent) abortions. The presence of HSV positivity in chronic active endometritis depended on the phase and was similar to the degree of HSV positivity found in the corresponding noninflamed normal phases.

TABLE 3. Clinical/Pathologic Correlations for Abortion and Curettage Data

Parameter*	Population A No. Yr/Mo/% ± SD	Population B No. Yr/Mo/% ± SD	*P* Value
Significant correlations			
Patient age (yr)†	SAb (101) 29.7 ± 6.1	TAb (99) 27.2 ± 6.0	<0.05
Total inflammation	SAb (101) 92.1 +	TAb (99) 65.7 +	<0.001
Villous Hofbauer cells +	Sab (74) 13.5 +	TAb (85) 0.0 +	<0.001
Villous Hofbauer cells +	SAC⁻ (111) 1.8 +	SAC⁺ (48) 16.7 +	0.001
Subamnionic chorion⁺	SAb (101) 38.6 +	TAb (99) 13.1 +	<0.001
Subamnionic chorion⁺	EMET⁻ (139) 20.1 +	EMET⁺ (58) 39.7 +	0.004
Endometrial + Abortion	Normal (40) 45.0 +	Tot Inflam⁺ (157) 25.6 +	0.022
Endometrial + Abortion	Normal (40) 45.0 +	Acute CD (98) 23.5 +	0.012
Gestational age (wk)	SAb (90) 9.2 ± 2.6	TAb (97) 8.5 ± 2.5	0.038
Gestational age (wk)	Tot HSV⁻ (106) 7.0 ± 1.1	Tot HSV⁺ (81) 11.4 ± 1.6	<0.001
Gestational age (wk)	SAC⁻ (142) 8.5 ± 2.3	SAC⁺ (44) 9.8 ± 2.9	0.006
Gestational age (wk)	VH⁻ (143) 9.0 ± 2.6	VH⁺ (8) 11.0 ± 2.1	0.028
Gestational age (wk)	EMET⁻ (130) 9.0 ± 2.3	EMET⁺ (55) 8.1 ± 2.7	0.006
Blighted ovum	SAb (73) 78.1 +	TAb (85) 27.1 +	0.001
Blighted ovum	Normal (36) 27.8 +	Tot Inflam (122) 45.6 +	0.002
Blighted ovum	Normal (36) 27.8 +	Acute CD (77) 48.1 +	0.043
Blighted ovum	Normal (36) 27.8 +	Chronic CD (45) 73.3 +	0.001
Blighted ovum	Acute CD (77) 48.1 +	Chronic CD (45) 73.3 +	0.007
Blighted ovum	SAC⁻ (110) 43.7 +	SAC⁺ (48) 66.7 +	0.008
Patient Genital HSV +	Partner genital⁻ (60) 6.7 +	Partner genital⁺ (6) 50.0 +	0.001
Multiparity	Normal (41) 58.5 +	Chronic CD (56) 78.6 +	0.034
Endometrial + D/C	Secretory (57) 40.4 +	Prolif (19) 10.5 +	0.017
Endometrial + D/C	Secretory (57) 40.4 +	Abn Secretory (21) 66.7 +	0.040
Endometrial + D/C	Secretory (57) 40.4 +	Atrophic (15) 6.7 +	0.014
Endometrial + D/C	Secretory (57) 40.4 +	Hyperplastic (35) 14.3 +	0.001
Endometrial + D/C	Endometritis (15) 40.0 +	Prolif (19) 10.5 +	0.047
Endometrial + D/C	Endometritis (15) 40.0 +	Hyperplasia (35) 14.3 +	0.046
Endometrial + D/C	Atrophic (15) 6.7 +	Abn Secret (21) 66.7 +	<0.001
Endometrial + D/C	EMET Date HSV⁻ (33) 20.6d	EMET Date HSV⁺ (22) 22.6d	0.004
Trends			
Multiparity	SAC⁻ (144) 64.6 +	SAC⁺ (46) 78.3 +	0.084
Multiparity	EMET⁻ (130) 69.2 +	EMET⁺ (57) 75.4 +	0.179
Multiparity	Tot inflam⁻ (42) 59.5 +	Tot inflam⁺ (148) 70.3 +	0.189
Multiparity	Tot HSV⁻ (107) 63.6 +	Tot HSV⁺ (82) 73.5 +	0.143
Recurrent SAb	EMET⁻ (130) 18.2 +	EMET⁺ (57) 29.8 +	0.143
Recurrent SAb	VH⁻ (145) 23.4 +	VH⁺ (8) 50.0 +	0.092
Blighted Ovum	VH⁻ (148) 49.3 +	VH⁺ (10) 70.0 +	0.207
Endometrial + Abortion	Normal (40) 45.0 +	Chronic CD (58) 29.3 +	0.113
Subamnionic chorion +	Normal (41) 17.1 +	Tot Inflam⁺ (158) 28.5 +	0.139
Subamnionic chorion +	Normal (41) 17.1 +	Chronic CD (59) 30.5 +	0.129
Villous Hofbauer cells +	Partner oral⁻ (45) 4.4 +	Partner oral⁺ (17) 17.6 +	0.091
Villous Hofbauer cells +	Normal (36) 0.0 +	Tot inflam⁺ (123) 8.1 +	0.078
Villous Hofbauer cells +	Normal (36) 0.0 +	Acute CD⁺ (78) 7.7 +	0.089
Villous Hofbauer cells +	Normal (36) 0.0 +	Chronic CD⁺ (45) 8.9 +	0.068
Nonsignificant correlations			
Acute versus chronic CD	SAb (93) 64.6 +	TAb (65) 60.0 +	0.565
Total HSV⁺	SAb (101) 47.5 +	TAb (99) 41.4 +	0.386
Endometrial + Abortion	SAb (98) 25.5 +	TAb (99) 33.3 +	0.230
Endometrial + Abortion	Acute CD (98) 23.5 +	Chronic CD (58) 29.3 +	0.421
Endometrial + Abortion	Partner oral⁻ (60) 35.0 +	Partner oral⁺ (18) 22.2 +	0.311
Endometrial + Abortion	Partner genital⁻ (60) 35.0 +	Partner genital⁺ (6) 33.3	0.935
Endometrial + Abortion	Partner oral/genital⁻ (60) 35.0 +	Partner oral/genital⁺ (24) 25.0 +	0.378
Total HSV⁺	Acute CD (99) 42.4 +	Chronic CD (59) 42.4 +	0.995
Subamnionic chorion⁺	Normal (41) 17.1 +	Acute CD (99) 27.0 +	0.202
Subamnionic chorion⁺	Acute CD (99) 27.3 +	Chronic CD (59) 30.5 +	0.664
Subamnionic chorion⁺	Partner oral⁻ (60) 25.0 +	Partner oral⁺ (18) 33.3 +	0.487
Subamnionic chorion⁺	Partner genital⁻ (60) 25.0 +	Partner genital⁺ (6) 33.3 +	0.659
Subamnionic chorion⁺	Partner oral/genital⁻ (60) 25.0 +	Partner oral/genital⁺ (24) 33.3 +	0.783
Villous Hofbauer cells⁺	Acute CD (78) 7.7 +	Chronic CD (45) 8.9 +	0.816
Villous Hofbauer cells⁺	Partner genital⁻ (45) 4.4 +	Partner genital⁺ (4) 0.0 +	0.670
Villous Hofbauer cells⁺	EMET⁻ (111) 7.2 +	EMET⁺ (45) 4.4 +	0.525
Gestational age (wk)	Normal (40) 9.0 ± 2.8	Tot inflam (146) 8.8 ± 2.5	0.966
Gestational age (wk)	Normal (40) 9.0 ± 2.8	Chronic CD (54) 9.2 ± 2.7	0.766
Gestational age (wk)	Normal (40) 9.0 ± 2.8	Acute CD (92) 8.6 ± 2.4	0.685
Gestational age (wk)	Acute CD (92) 8.6 ± 2.4	Chronic CD (54) 9.2 ± 2.7	0.270
Gestational age (wk)	Blighted ovum⁻ (77) 8.8 ± 2.3	Blighted ovum⁺ (73) 9.4 ± 2.9	0.282
Blighted ovum	Tot HSV⁻ (83) 45.8 +	Tot HSV⁺ (75) 56.0 +	0.201

TABLE 3. *Continued*

Parameter*	Population A No. Yr/Mo/% ± SD	Population B No. Yr/Mo/% ± SD	*P* Value
Blighted ovum	EMET⁻ (110) 50.9 +	EMET⁺ (45) 51.1 +	0.820
Patient cold sore	Tot HSV⁻ (45) 20.0 +	Tot HSV⁺ (36) 25.0 +	0.593
Patient cold sore	SAC⁻ (62) 22.6 +	SAC⁺ (19) 21.0 +	0.889
Patient cold sore	EMET⁻ (54) 20.4 +	EMET⁺ (27) 29.2 +	0.573
Patient cold sore	VH⁻ (62) 22.6 +	VH⁺ (5) 40.0 +	0.383
Recurrent SAb	Tot HSV⁻ (107) 19.6 +	Tot HSV⁺ (83) 26.5 +	0.262
Recurrent SAb	SAC⁻ (144) 21.5 +	SAC⁺ (46) 26.1 +	0.521
Patient genital HSV	Tot HSV⁻ (107) 5.6 +	Tot HSV⁺ (83) 3.6 +	0.522
Patient genital HSV	SAC⁻ (144) 5.6 +	SAC⁺ (46) 2.2 +	0.349
Patient genital HSV	VH⁻ (145) 6.2 +	VH⁺ (8) 0.0 +	0.469
Patient genital HSV	EMET⁻ (130) 4.6 +	EMET⁺ (57) 5.3 +	0.849
Patient genital HSV	Partner oral⁻ (60) 6.7 +	Partner oral⁺ (18) 5.6 +	0.867
Patient genital HSV	Primipara (4) 6.6 +	Multipara (5) 3.9 +	0.425
Partner oral HSV	Tot HSV⁻ (59) 42.4 +	Tot HSV⁺ (16) 50.0 +	0.588
Partner genital HSV	Tot HSV⁻ (59) 42.4 +	Tot HSV⁺ (5) 40.0 +	0.918
Multiparity	VH⁻ (145) 66.9 +	VH⁺ (8) 75.0 +	0.635
Multiparity	Normal (41) 58.5 +	Acute CD (92) 65.2 +	0.462
Endometrial + D/C	Prolif (19) 10.5 +	Hyperplasia (35) 14.3 +	0.697
Endometrial + D/C	Prolif (19) 10.5 +	Atypical hyperplasia (9) 11.1 +	0.963
Endometrial + D/C	Secretory (57) 40.4 +	Menstrual (10) 20.0 +	0.223
Endometrial + D/C	Secretory (57) 40.4 +	Endometritis (15) 40.0 +	0.980
Endometrial + D/C	Menstrual (10) 20.0 +	Endometritis (15) 40.0 +	0.303
Endometrial + D/C	Abn Secret (21) 66.7 +	Endometritis (15) 40.0 +	0.118
Endometrial + D/C	Menstrual (10) 20.0 +	Atrophic (15) 6.7 +	>0.990
Endometrial + D/C	Prolif (19) 10.5 +	Atrophic (15) 6.7 +	>0.990
Endometrial + D/C	HSV⁻ endomet volume	HSV⁺ endomet volume	0.797
Endometrial + D/C	HSV⁻ Age 41.4 ± 14.1	HSV⁺ age 39.8 ± 11.7	0.765

* PARAMETERS: Total inflammation, presence of acute or chronic choriodeciduitis (CD); normal, no acute or chronic CD; total HSV⁺, staining in one or more of the following patterns—chorionic villus Hofbauer cells (VH), subamnionic chorion (SAC), or endometrial epithelium (EMET) (see Results section and figures 1 and 3); blighted ovum, destruction of the embryo as defined by lack of nucleated embryonic erythrocytes and capillaries in the chorionic villi; multiparity, more than one pregnancy; SAb and TAb, spontaneous and therapeutic abortions, respectively; recurrent SAb, more than one spontaneous abortion; gestational age, the gestational age of the embryo at the time of the abortion; patient or partner cold sore, clinical history of at least one episode of oral HSV infection in the patient or partner at the time of pregnancy, respectively; patient or partner genital HSV, clinical history of genital HSV infection in the patient or partner, respectively.

† The patient ages were normally distributed for all categories and were analyzed by the Anderson-Darling test, while all other parameters were nonparametric and were analyzed by the Wilcoxon nonpaired rank sum test.

DISCUSSION

The specificity of the screening rabbit anti-HSV antibody in detecting only HSV antigens underlies the concepts about latent intrauterine and neonatal HSV infection presented in this study. Our contention that the antigens that we detected in endometrial, placental, and cordal tissues are specific for HSV, probably of type 2 origin, is strongly supported by the control data. The control studies included numerous non-HSV virus-infected tissues, HSV-negative tissues, and bacterium-infected tissue. In addition, numerous HSV-positive and -negative antibodies derived from different animals and from different routes of infection with HSV viruses of both type 1 and 2 were used as controls. Confirmation of this HSV specificity will have to be accomplished by DNA and/or RNA extraction and/or in situ hybridization techniques, if the necessary technical sensitivity can be achieved.[11,14,15]

Mouse monoclonal antibodies have been useful in defining the HSV-specific nature of the detected antigens, especially the amnionic intranuclear staining of the immediate/early antigen, ICP4. Exact delineation of the HSV-specific proteins present in our tissues was difficult, for at least two reasons.

First, there are 15 to 35 different virion proteins in the HSV virion,[16,17] and the number of available monoclonal antibodies to these proteins is limited. Virus-specific nonvirion and cellular proteins also are present in HSV-infected cells; some of these proteins may have been detected with the Dako antibody, which recognizes both HSV-specific virion and nonvirion antigens.[18] The antibody response to HSV is polyclonal, and, in humans, at least 31 HSV 1 and 27 HSV 2 virus-specific proteins can be antigenic with great individual variation.[12]

Second, little is known about the fate or degradation sequence of the various HSV-specific virion and nonvirion proteins in phagocytic cells (primarily macrophages) that organize (i.e., clean up) the cellular and viral debris after acute HSV infection. HSV proteins are degraded differentially in different cell

lines after acute infections in cell culture.[19] Therefore, redundant antigenic sites on the virus-specific proteins[20–23] may be either exposed or lost during the degradation process in macrophages or other phagocytic cells, such as some choriodecidual cells and the Hofbauer cells of the chorionic villi. This uncertainty may make identification of specific HSV protéins by immunohistochemistry and/or isolation difficult in subacute or chronic infections in animal tissues. Antigenic variants of the viral glycoproteins may also occur.[24] The long-term (weeks to months) fate of the numerous HSV virion and nonvirion protein antigenicities in acutely infected tissues has not been studied in animal models.

Our working hypothesis is that a latent endometrial epithelial infection is established by HSV type 2 intrauterine infection through one or more of the following routes: ascending cervical, transneural, maternal primary viremia during pregnancy, postnatal primary viremia in childhood or adulthood, and activated endometrial intrauterine embryonic or fetal infection with or without germ cell involvement. Once this latent endometrial infection is established, sporadic transient focal acute infections may be activated, as in the oral and genital regions.

This endometrial activation is maximal at the end of the secretory phase, when endometrial prostaglandin synthesis is maximal[25] and when local immunosuppression is occurring to protect the implanting embryo (L. Olding, unpublished data) if fertilization has occurred. Prostaglandins produce local and systemic immunosuppression,[26] and both immunosuppression[27] and prostaglandins[8,29] activate latent HSV infection.

The level of endometrial prostaglandin synthesis is also quite high during pregnancy,[30] and genital HSV activation and asymptomatic shedding of HSV are increased during the gestational period.[31] Furthermore, the presence of ICP4, the immediate/early protein detected in our study, is necessary, but not sufficient, for HSV activation and replication. Further events are required for virus production,[32,33] possibly prostaglandin synthesis and/or local immunosuppression. The immune response to HSV infection in both the pregnant and nonpregnant states is quite complex and involves antibody and complement, antibody and natural killer cells, macrophages, and polymorphonuclear leukocytes.[1,27] Maternal antibody in the amnionic fluid may protect the embryo or fetus.[34] The cellular immune response to HSV 2 infection is decreased during pregnancy.[35]

If conception has not occurred, the acute activated infection is not detected clinically, because the endometrium has no sensory pain nerves, and there is no transcervical discharge or spotting. If conception has occurred, however, the infection has the potential to spread to the placenta and amniochorionic sac. If the infection is not inhibited by maternal antibody in the amnionic fluid,[34] it may continue into the embryonic or fetal circulation and/or tissues, as evidenced by antigen psotivity in the chorionic villi. If the embryo or fetus is infected, the probability of spontaneous abortion, stillbirth, or neonatal complications is high. If the infection is limited to the amniochorionic sac, however, the chance of embryonic or fetal damage is greatly reduced (see companion paper[4]). Spontaneous abortion and infants small for the gestational age are associated with nonspecific villitis,[36] a condition that we found to be associated with HSV antigen positivity. Specimens from maternal placental floor infarction[37,38] were HSV antigen-positive in five of the eight cases assayed. Our companion paper[4] describes the neonatal complications associated with HSV antigen positivity.

The observational and clinical data in support of this hypothesis are as follows. First, endometrial epithelial positivity is maximal in the late secretory phase (day 22.4, 40 per cent positive) as compared with the proliferative phase (10 per cent, $P = 0.017$), endometrial hyperplasia (14 per cent, $P = 0.001$), and the atrophic state (7 per cent, $P = 0.014$). Maximal positivity occurred in abnormal secretory phase endometria (68 per cent, $P = 0.040$ versus normal secretory phase), probably a time of altered prostaglandin synthesis.[39]

Second, the mean gestational age increases progressively for endometrial epithelial positivity, placental subamnionic chorion positivity, and chorionic villus positivity (8.1 weeks, $P = 0.006$, versus 9.8 weeks, $P = 0.006$, versus 11 weeks, $P = 0.028$, respectively). The increasing gestational age for antigen positivity at these sites supports the concept that the infection begins in the endometrium and progresses into the amniochorion and, finally, into the chorionic villi (i.e., the embryo or fetus).

Third, placental subamnionic chorion positivity is dependent on endometrial epithelial positivity ($P = 0.004$), and chorionic villus positivity is dependent on placental subamnionic chorion positivity ($P = 0.001$). This finding supports the concept that amniochorionic infection cannot occur without endometrial infection, and chorionic villus infection cannot occur without amniochorionic infection. The reason for which chorionic villus positivity is not statistically dependent on endometrial epithelial positivity could be that most epithelium-positive endometria may not produce placental subamnionic chorion-positive or chorionic villus-positive infections. Another reason for the discordance in individual specimens between endometrial epithelial positivity, placental subamnionic chorion positivity, and chorionic villus positivity is that very little of the endometrium in the uterus accompanies the placental tissue at the time of abortion. Furthermore, little of the removed endometrium may be submitted for examination, because of the difficulty in separating it from the placental and decidual tissue during gross examination.

Fourth, material from spontaneous abortions is more likely to be inflamed (92 per cent, $P < 0.001$) than is that from therapeutic abortions (66 per cent).

Both placental subamnionic chorion positivity and chorionic villus positivity show trends ($P = 0.139$ and 0.078, respectively) toward an association with inflammation, while endometrial epithelial positivity is significantly associated with noninflamed normal tissue ($P = 0.022$). Some therapeutic abortions are associated with inflammation and blighted ova, because they are incipient spontaneous abortions that have not been recognized clinically. Therapeutic abortions occur earlier than spontaneous abortions (at 8.5 versus 9.2 weeks of gestation, $P = 0.038$).

Fifth, placental subamnionic chorion positivity (39 versus 13 per cent, $P < 0.001$) and chorionic villus positivity (14 versus 0.0 per cent, $P < 0.001$) are more frequent in spontaneous than in therapeutic abortions, while the incidences of endometrial epithelial positivity are similar (26 versus 33 per cent, $P = 0.230$).

Sixth, blighted ova are associated more frequently with spontaneous than with therapeutic abortions (78 vs 27 per cent, $P = 0.001$), with acute (48 per cent, $P = 0.043$) and chronic (73 per cent, $P = 0.007$) inflammation more frequently than with no inflammation, with placental subamnionic chorion positivity more frequently than with subamnionic chorion negativity (67 versus 44 per cent, $P = 0.008$), and with chorionic villus positivity more frequently than with chorionic villus negativity (70 versus 49 per cent, $P = 0.207$, trend), but not with endometrial epithelial positivity or negativity (51 versus 51 per cent, $P = 0.820$).

The data presented in the fourth, fifth, and sixth points support the concept that HSV infection of the amniochorion (placental subamnionic chorion positivity) and chorionic villi (chorionic villus positivity) can destroy the embryo (blighted ovum) and produce spontaneous abortion, as suggested previously.[40-43] It is difficult to determine the percentage of spontaneous abortions caused by HSV infection. The identified causes of spontaneous abortion are chromosomal abnormalities (about 50 per cent), bacterial infection (about 10 per cent), CMV (rare), and chlamydial infection (unkown frequency); the cause is unknown in up to 40 per cent of cases).[1] Since our data show an increase of at least 50 per cent in blighted ova when spontaneous abortion material has placental subamnionic chorion positivity (67 per cent/44 per cent = 152 per cent) and since HSV infection itself may produce chromosomal damage (double-stranded DNA virus), a significant proportion of the 40 per cent of spontaneous abortions of unknown etiology may be caused by HSV infection.

Recurrent spontaneous abortions may be associated with endometrial epithelial positivity (30 versus 28 per cent, $P = 0.143$) and chorionic villus positivity (50 versus 23 per cent, $P = 0.207$), but the present data show only a trend, and the number of mothers with recurrent spontaneous abortions in this study was small (38 cases). Recurrent spontaneous abortions are not associated with placental subamnionic chorion positivity (26 vs 22 per cent, $P = 0.521$). This finding might correlate with the amount of maternal anti-HSV antibody in the amnionic fluid.[34] That is, the infection is blocked at the amniochorionic level, without transmission into the embryo or fetus (i.e., chorionic villi). Recurrent neonatal HSV infection has not been documented in successive pregnancies of the same mother.[43] Two of the HSV-positive patients in this study who had spontaneous abortions subsequently had normal full-term infants with HSV antigen-positive placentae and cords, but with negative chorionic villi. In a guinea pig model of placental CMV infection, only 27 per cent of the fetuses that had CMV-positive placentae were CMV-positive by direct culture.[44]

Multiparity is associated with chronic inflammation more than with the absence of inflammation (79 versus 58 per cent, $P = 0.034$), and shows a trend toward associations with endometrial epithelial positivity (75 versus 69 per cent, $P = 0.179$) and placental subamnionic chorion positivity (78 versus 65 per cent, $P = 0.084$). The greater the number of pregnancies, the greater is the potential for latent endometrial infection (endometrial epithelial positivity) to activate and produce acute infection with intrauterine infection (placental subamnionic chorion positivity). Multiparity was not associated with increased risk of embryonic infection (chorionic villus positivity, 75 versus 67 per cent, $P = 0.635$).

The only significant association between oral and genital HSV infections in the patient and/or the father of the aborted embryo or fetus was between genital HSV infection in both individuals (50 per cent of patients with clinical histories of genital HSV infection had partners with clinical histories of genital HSV infection, and 6.7 per cent of patients with positive clinical histories had partners with negative clinical histories, $P = 0.001$). Only 3.6 per cent of 83 HSV antigen-positive patients and 5.6 per cent of 107 HSV antigen-negative patients had clinical histories of genital herpes infection ($P = 0.522$). Asymptomatic genital HSV 2 infection, however, is common, occurring in more than 50 per cent of nonpregnant women with primary infections,[45] 25 per cent of nonpregnant women with recurrent infections,[46] and 13 to 33 per cent of women during pregnancy.[31,41,48] The optimal time to detect asymptomatic shedding from an activated endometrial infection in nonpregnant women would be the first day of menstrual flow, as maximal antigen detection occurs in the late secretory phase (day 22 to 23).

The data reported in this study suggest that latent intrauterine HSV infection is etiologically important in spontaneous abortion. The accompanying paper describes the neonatal problems produced by infections of this type.[4] Future studies should include the following investigations: determination of maternal anti-HSV antibody status; extraction of DNA from, and in situ DNA/RNA hybridization of,

aborted and curettage tissues to determine whether HSV-specific nucleic acid sequences are present; and direct culture of first-day menstrual flow material for infectious HSV.

Note added in proof. In collaboration with Dr. David Myerson, we have detected HSV-specific DNA in amnionic and chorionic nuclei in serial sections from the specimen in figures 1 and 9. In situ hybridization using a biotinylated HSV DNA probe as described in reference 11 was used. Evaluation of endometrial tissues is underway.

Acknowledgments. The authors are grateful to Karen Gray, Linda Kitabayashi, Jean Langworthy, Brenda Robb, Marla Robb, Pamela Sam-Fong, Christine Shawger, and Kimberly Stecker for their technical assistance; to Dr. Frank Howell for the use of his photomicroscope; and to the Department of Academic Affairs of the Scripps Clinic Research Foundation for photographic printing. The gifts of antisera from Drs. G. D. Hsiung and Z. M. Zheng (guinea pig[50,51]), Douglas Richman (rabbit[49]), and Martin Zweig (mouse[19]) are sincerely appreciated. Dr. Fred Rapp is thanked for his presubmission review of the manuscript.

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