Pediatric and Developmental Pathology 11, 15–22, 2008
DOI: 10.2350/07-06-0285.1

Characterization of Chorioamnionitis in 2nd-Trimester C-Section Placentas and Correlation with Microorganism Recovery from Subamniotic Tissues

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Received June 1, 2007; accepted September 13, 2007; published online September 17, 2007.

ABSTRACT

Prolonged exposure to infection appears to influence fetal/neonatal development. We characterize the relationship between histologic patterns of inflammation and microorganism recovery from the placentas of live born infants delivered before the 28th postmenstrual week. The subamniotic parenchyma of 835 placentas delivered by cesarean section were cultured and evaluated for specific histologic patterns of inflammation in a blinded fashion. Cases with prolonged membrane rupture were excluded. Microorganisms were recovered from 41% of placentas. Microorganisms found more frequently in placentas with high-grade chorionic plate inflammation include *Actinomyces, Prevotella bivia, Corynebacterium* sp., *Escherichia coli*, *Peptostreptococcus magnus*, multiple species of *Streptococci*, and *Mycoplasma* sp., including *Ureaplasma urealyticum*. These microorganisms were also associated with fetal vasculitis (neutrophilic infiltration of chorionic plate stem vessels or umbilical cord). Recovery of microorganisms from placental parenchyma is associated with histologic inflammation. The same microorganisms responsible for inciting high-grade chorionic plate inflammation are also most likely to promote fetal inflammation.

Key words: chorioamnionitis, culture, microorganisms, placenta

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INTRODUCTION

Chorioamnionitis has been associated with morbidity and mortality in children born prematurely [1,2]. Although most infections eliciting placental inflammation are due to recent ascending infection, cultures of routine amniocentesis fluid from asymptomatic women indicate that bacterial colonization or low-level infection may be present long before delivery [3]. Less likely, endometrial cultures taken from asymptomatic women prior to pregnancy and postpartum also indicate that the endometrial cavity may not be sterile and could provide a source of infection [4–6]. The duration of infection is relevant to the risk of fetal injury, since such morbidity depends on the presence of a fetal inflammatory response and fetal response typically occurs later than the maternal response [7,8]. Our culture technique (parenchyma rather than membrane) and selected population (cesarean-section [C-section] delivery and C-section within 1 hour of membrane rupture) were chosen to emphasize that the infection was of sufficient duration to have elicited a fetal response.

Studies based on cultures of placental membranes and amniotic fluid show a close association between histologic chorioamnionitis and infection [9–12] but have not comprehensively described the organisms associated with specific fetal and maternal patterns of inflammation in early gestation. Our primary purpose was to examine the relationship between microorganism recovery and specific patterns and severity of chorioamnionitis in a large cohort at early gestation.

Table 1. The percent of delivered placentas for the indication listed on the left that were enrolled, had cultures taken, were delivered by cesarean section (C-section), and were delivered within 1 hour of membrane rupture (columns indicate percent)

	Enrolled ($n = 1506$)	Cultured and evaluated histologically ($n = 1292$)	C-section ($n = 835$)	C-section and ROM <1 hour ($n = 548$)
PTL	44	44	37	41
pPROM	22	21	20	2
PEC	13	13	20	29
Abruption	10	10	11	12
Cervical insufficiency	6	6	6	5
Fetal indications	7	5	7	11

ROM indicates rupture of membranes; PTL, preterm labor; pPROM, prelabor premature rupture of membranes; PEC, preeclampsia.

Organisms recovered from noninflamed placentas may represent early infection in babies delivered for other indications or true low-level infections that would not have led to delivery; we wish to at least quantify the phenomenon.

The methods used were chosen to minimize contamination and to maximize microorganism isolation and identification, and issues of culture contamination are discussed.

METHODS

Population

As we are interested in the detrimental effects of inflammation on neurologic development, we focus on premature infants, since they have a higher prevalence of inflammation and infection [13,14]. The Extremely Low Gestational Age Newborn (ELGAN) Study enrolled infants born before the beginning of the 28th postmenstrual week at 14 sites in 5 states between the years 2002 and 2004; the study was conducted with the permission of the individual institutional review boards. While 1506 infants were enrolled, only 1292 placentas were sampled, cultured, and submitted for histologic evaluation.

Because contamination might occur during passage through the vagina, we restricted the sample for this analysis to the 835 placentas delivered by C-section. Restricting the analysis to C-section cases preferentially excludes women presenting in preterm labor and preterm premature rupture of membranes (pPROM), a population known to be at risk for acute ascending infection near the time of delivery. This limit is compatible with our goals, since we are most interested in infections that preceded delivery and were of a duration sufficient to elicit a fetal response. On the other hand, this limitation severely restricts our ability to draw inferences about acute infections in pregnancies with preterm labor or prelabor preterm rupture of membranes (Table 1). For some analyses, we further limited the sample to placentas delivered within 1 hour of membrane rupture.

Microbiologic assessment

Placentas were placed in a sterile exam basin and transported to a sampling room. Eighty-two percent of the samples were obtained within 1 hour of delivery. Longer delay in the remaining cases is unlikely to have had a major impact on organism recovery, since most anaerobes that are part of the vaginal microflora are considered 'moderately' oxygen sensitive and can survive for up to 4 hours with exposure to room air [15]. Since the placenta no longer has vascular flow, low oxygen concentrations may also provide some protection for the organisms. In addition, we found no difference in organism recovery rates based on processing time (81% cultured within 1 hour of delivery and 90% within 3 hours of delivery).

The area to be sampled was at the midpoint of the longest distance between the cord insertion and the edge of the placental disk. Once the area was identified, the uppermost layer of the membranes (amnion) was lifted with a set of sterile forceps and cut with sterile scissors. Using forceps, the amnion was gently pulled away from the underlying chorion. The amnion was then snipped open with the scissors and peeled away from the initial site of entry, thus exposing the chorion. With a 2nd set of sterile forceps and scissors, traction was put on the chorion and underlying trophoblast tissue by gently pulling on it. A piece of tissue was removed by cutting at the base of the section with the sterile scissors and placed into a sterile 2-ml cryovial. The sample was immediately flash frozen in liquid nitrogen and then stored in a −80°C freezer. Snap freezing obligate anaerobes does not alter their viability or quantitative recovery [16,17]. Periodically, the samples were shipped on dry ice from the 14 sites to the central microbiology laboratory located in Boston, MA, USA.

Samples were kept frozen at −80°C until processing. The sample was then removed from the freezer and allowed to thaw at room temperature. Subsequently, it was placed into a sterile Petri dish. With sterile forceps and a disposable sterile scalpel, a portion of approximately 1 cm^2 was removed and weighed. Sterile phosphate-buffered saline (PBS) was added to the vial containing the sample to achieve a 10-fold weight to volume dilution of the sample. The sample was then homogenized using a handheld Pro-200 homogenizer (PRO Scientific, Inc, Oxford, CT, USA) until the placental tissue was completely dispersed in the PBS. Serial 10-fold dilutions of the homogenized sample were made in PBS and aliquots of the sample and dilutions plated onto selective and nonselective media.

The culture media for recovering anaerobes was prereduced Brucella-base agar with 5% sheep blood enriched with hemin and vitamin K_1; for facultative

anaerobes tryptic soy agar with 5% sheep blood was used (PML, Microbiologicals, Mississauga, ON, Canada). Chocolate agar was used for the recovery of fastidious microorganisms, and A-7 agar (Northeast Laboratory, Waterville, ME, USA) was used for the detection of *Mycoplasma* and *Ureaplasma*. Anaerobic culture plates and A-7 plates were incubated in an anaerobic chamber with an atmosphere of 10% carbon dioxide, 10% hydrogen, and 80% nitrogen for a minimum of 120 hours at 35°C before enumeration. Tryptic soy agar plates were incubated in air and chocolate plates in 5% carbon dioxide for 48 hours. After incubation, the various colony types were enumerated, isolated, and identified by established criteria.

Gram-positive, catalase-negative microaerophilic or anaerobic bacilli that produced a large amount of lactic acid, as determined by gas-liquid chromatography, were classified as *Lactobacillus* sp. Obligate anaerobes were classified by Gram stain and by gas-liquid chromatographic analysis of glucose fermentation products, and the final identification was determined using the Microbial Identification System (MIDI, Inc, Newark, NJ, USA) or the Rapid ANA II system (Remel Inc, Lenexa, KS, USA). *Mycoplasma* and *Ureaplasma* were identified based on their differentiating morphological characteristics of growth on A-7 agar [18]. Cultures were quantified, and all counts were reported as log10 colony-forming units (CFUs) per gram of tissue.

Histologic assessment

Histologic examination of the placenta was performed following College of American Pathologists guidelines [19]. Representative sections were taken from all abnormal areas; we also sampled routine sections of the umbilical cord and a membrane roll and full-thickness sections from the center and a paracentral zone of the placental disc. After the creation of a manual with definitions and illustrations, and after completion of procedures to minimize observer variability, a pathologist at each site examined the slides for the histologic characteristics listed on the data form. For multiple births, separate forms were filled out for each newborn. Multiple forms were also completed for twins with fused placentas.

Inflammation of the membranes was described in detail using a scoring system similar to that proposed by Redline and colleagues [20]. At the chorionic plate of the disc, acute inflammation was assigned a stage from 0 to 3 (0 indicated none, 1 indicated neutrophils collecting in the subchorionic space, 2 indicated neutrophils into the chorionic plate, and 3 indicated neutrophils up to the amnionic epithelium) and a grade (1 was 1 to 9 neutrophils/×20 field, 2 was 10 to 19 neutrophils/×20 field, 3 was >20 neutrophils/×20 field). In the external membranes, inflammation of the chorion/decidua was graded from 0 to 4 (0 indicated none, 1 indicated a single focus of 5 to 10 neutrophils, 2 indicated several small foci or a single focus of >10 neutrophils, 3 indicated numerous large or confluent foci, and 4 indicated necrotizing).

Chorionic plate vasculitis was defined as neutrophilic infiltration of the fetal stem vessels in the chorionic plate. Umbilical cord vasculitis was defined by the presence of neutrophils in a cord vessel. Fetal vasculitis was defined as chorionic plate vasculitis and/or umbilical cord vasculitis.

Data analysis

We evaluated the following 6 hypotheses:

1. Inflamed placentas are more likely to harbor a single microorganism than are noninflamed placentas. Is inflammation a good predictor of organism recovery?
2. Inflamed placentas are more likely to harbor 2 or more microorganisms than are noninflamed placentas. Are infections less likely to be polymicrobial when they are severe enough to elicit inflammation?
3. Among placentas that harbor a microorganism, those that are inflamed tend to have higher median CFUs than noninflamed placentas. Might organism recovery in noninflamed placentas reflect contamination or early/low-level infection?
4. Microorganisms differ in their propensity for association with high-grade chorionic plate inflammation. Which organisms are more likely to elicit a maternal response?
5. Microorganisms differ in their propensity for association with fetal vasculitis. Which organisms are more likely to elicit a fetal response?
6. Microbial species that predict high-grade chorionic plate inflammation differ from the species that predict fetal vasculitis.

We compared microorganism prevalence among placental subgroups (Table 2) by constructing a series of separate 2×3 tables, with the number of microorganisms grouped as zero, single, and multiple and with calculated Fisher exact P values. We used the Wilcoxon rank sum test to calculate P values for comparisons of median CFUs between subgroups (adjacent rows in Table 2).

Corrections for multiple comparisons are not presented. Corrections are appropriate when comparisons are independent. We do not view our multiple comparisons as independent. Instead of making any corrections, we offer caution here and in the Discussion section, advising that inferences be drawn in light of these multiple comparisons.

Inflammation is modulated by many factors, including the timing of delivery after membrane rupture (neutral to increased with interval to delivery) [21,22], the route of delivery (higher in vaginal than in C-section deliveries), treatment with antenatal corticosteroids (decreased if membranes are intact) [23], and gestational age (decreased with age) [24]. Our restriction of the sample to C-section deliveries eliminated the need to adjust for route of delivery. The multivariate analyses presented in Table 3 are adjusted for antenatal corticosteroid receipt and week of gestation at the time of birth. Instead of adjusting for duration of membrane rupture in the multivariate analyses, we further restricted the sample to the 548 placentas delivered within 1 hour of membrane rupture.

PRINCIPAL RESULTS

Microorganisms were recovered from 41% of cultured placentas. The more intense the histologic chorioamnionitis

Table 2. Histologic evaluation of placental inflammation in relation to microorganism recovery in culture

Histologic characteristic		No. of isolates: Single (row%)	No. of isolates: 2+ (row%)	*P* value	CFU[a]: Single (median)	*P* value	CFU[a]: 2+ (median)	*P* value
Stage of inflammation chorionic plate	0–1	22	10	≤0.001	2.3	≤0.001	3.2	≤0.001
	2–3	37	29		3.7		4.1	
Grade of inflammation chorionic plate	1	31	16	0.005	3.0	0.05	3.5	0.02
	2–3	36	30		3.8		4.1	
Chorionic plate inflammation, stage 2 to 3 and grade 2 to 3	No	21	10	≤0.001	2.3	≤0.001	3.1	≤0.001
	Yes	36	32		3.8		4.1	
Chorionic plate vasculitis	Absent	23	11	≤0.001	2.3	≤0.001	3.5	0.003
	Present	38	31		4.1		4.1	
Umbilical cord vasculitis	Absent	23	11	≤0.001	2.3	≤0.001	3.3	≤0.001
	Present	37	29		3.9		4.5	
Grade of membrane inflammation (chorion/decidua)	0–1	21	9	≤0.001	2.3	≤0.001	3.2	≤0.001
	2–4	35	25		3.7		4.0	
Grade of membrane inflammation (amnion)	0–1	23	11	≤0.001	2.3	≤0.001	3.4	≤0.001
	2–4	36	29		3.8		4.4	

[a]Log_{10} colony-forming units (CFUs)/g tissue.

Table 3. Point estimate and 95% confidence interval of the risk of a histologic characteristic among placentas harboring a microorganism in the parenchyma relative to the risk among placentas without a microorganism. The sample is restricted to all placentas delivered by cesarean, and adjustment is made for antenatal corticosteroid >24 hours before delivery and gestational age

Histologic characteristic	Odds ratio (95% confidence interval): Cesarean delivery	Cesarean delivery & ROM <1 hour
Acute inflammation chorionic plate, stage 2 to 3 and grade 2 to 3	4.6 (3.2, 6.7)	6.1 (3.2, 12)
Chorionic vasculitis	3.9 (2.6, 5.8)	4.0 (2.0, 8.0)
Umbilical cord vasculitis	3.4 (2.2, 5.4)	4.7 (1.6, 14)
Membrane inflammation, chorion/decidua, grade 2 to 4	3.3 (2.4, 4.5)	2.7 (1.9, 4.2)
Membrane inflammation, amnion, grade 2 to 4	3.5 (2.5, 5.0)	3.7 (2.1, 6.6)

ROM indicates rupture of membranes.

score, the more likely it was that a microorganism was recovered from the placental parenchyma (Table 2). This was true for multiple measures of inflammation, including stage and grade of neutrophilic accumulation in the chorionic plate, and vasculitis in chorionic plate vessels and umbilical cord. The relationship between histologic indicators of inflammation and recovery of bacteria applied to single microorganism cultures, as well as to polymicrobial cultures. Higher median numbers of CFUs were noted with more intense inflammatory responses.

Placentas with high grade/stage acute inflammation of the chorionic plate, chorionic vasculitis (neutrophils in fetal stem vessels), or umbilical cord vasculitis were 3.5 to 4 times more likely to harbor a microorganism than were placentas without these features (Table 3). Restricting the C-section-delivered sample to those delivered within an hour of membrane rupture increased the likelihood that a microorganism had been recovered. Placentas with histologic features characteristic of preeclampsia (for example, infarct or increased syncytial knots) were at reduced risk of harboring a microorganism (data not shown).

The following species were found twice as often in placentas with stage 2 to 3 and grade 2 to 3 inflammation in the chorionic plate as in placentas without this characteristic (Table 4): *Actinomyces* sp.; *Prevotella bivia*; *Corynebacterium* sp.; *Escherichia coli*; *Peptostreptococcus magnus*; coagulase-negative *Staphylococcus* sp.; Group B, Group D, alpha hemolytic, and anaerobic *Streptococci*; *Gardnerella vaginalis*; *Mycoplasma* sp. (other than *Ureaplasma urealyticum*); and *U. urealyticum*. These same microorganisms, with the exception of coagulase-negative *Staphylococcus* sp. and *G. vaginalis*, were also associated with fetal vasculitis (neutrophilic infiltration of the fetal stem vessels of the chorionic plate or the umbilical cord vessels) (Table 5).

Table 4. The percent of all cesarean-delivered placentas delivered with, or without, inflammation in the chorionic plate (stage 2 to 3 and grade 2 to 3) that also had each microorganism alone or with another microorganism (columns indicate percent)

	Inflammation in the chorionic plate, stage 2 to 3 and grade 2 to 3					
	Yes ($n = 184$)			No ($n = 566$)		
Number of isolates	**1**	**2+**	**Any**	**1**	**2+**	**Any**
Microorganisms						
Actinomyces sp.	1	6	6	1	1	2
Prevotella bivia	1	6	7	0	1	1
Corynebacterium sp.	1	3	4	0	1	1
Escherichia coli	6	6	12	1	1	2
Lactobacillus sp.	0	2	2	2	3	5
Peptostreptococcus magnus	1	3	3	0	0	0
Propionibacterium sp.	2	3	6	7	3	10
Staphylococcus sp.[a]	2	4	6	3	4	3
Group B *Streptococcus*	3	5	8	1	0	1
Group D *Streptococcus*	1	5	6	0	1	1
Alpha hemolytic *Streptococcus*	3	6	8	1	1	2
Anaerobic *Streptococcus*	3	6	9	0	1	1
Gardnerella vaginalis	2	2	4	1	1	2
Mycoplasma sp.[b]	6	6	11	1	1	2
Ureaplasma urealyticum	3	4	7	0	1	2

[a]Other than *S. aureus.*
[b]Other than *Ureaplasma urealyticum.*

Table 5. The percent of all cesarean-delivered placentas delivered with, or without, fetal vasculitis (neutrophils in fetal stem vessels or in umbilical cord vessels) that also had each microorganism alone or with another microorganism (columns indicate percent)

	Fetal vasculitis					
	Yes ($n = 203$)			No ($n = 582$)		
Number of isolates	**1**	**2+**	**Any**	**1**	**2+**	**Any**
Microorganisms						
Actinomyces sp.	0	4	5	1	1	2
Prevotella bivia	1	5	6	1	1	2
Corynebacterium sp.	0	3	4	0	1	1
Escherichia coli	7	6	13	2	0	2
Lactobacillus sp.	0	1	1	2	3	5
Peptostreptococcus magnus	0	2	3	0	0	1
Propionibacterium sp.	2	3	5	6	3	9
Staphylococcus sp.[a]	1	3	5	3	4	7
Group B *Streptococcus*	4	5	9	1	0	1
Group D *Streptococcus*	0	5	6	0	1	2
Alpha hemolytic *Streptococcus*	3	6	9	1	1	2
Anaerobic *Streptococcus*	2	4	7	1	2	2
Gardnerella vaginalis	1	1	2	1	1	3
Mycoplasma sp.[b]	4	5	10	1	1	2
Ureaplasma urealyticum	5	3	8	1	2	2

[a]Other than *S. aureus.*
[b]Other than *Ureaplasma urealyticum.*

Many of the microorganisms associated with high-grade inflammation in the chorionic plate or with fetal vasculitis were identified in polymicrobial cultures rather than as pure cultures (Tables 4,5). This is apparent for low-pathogenicity microorganisms routinely found in the vagina (for example, *Prevotella bivia* and *P. magnus*) or skin (for example, *Corynebacterium* sp.) as well as for *Actinomyces* and various *Streptococci*.

Microorganisms associated more often with fetal vasculitis than with high-grade chorionic plate inflammation are *Actinomyces* sp., Group B, Group D, and alpha hemolytic *Streptococci.*

Microorganisms that were more prevalent in the noninflamed placentas included *Lactobacillus*, *Propionibacterium*, coagulase-negative *Staphylococcus,* and *G. vaginalis*.

CONCLUSIONS

This is the largest study to date describing the microbiology associated with chorioamnionitis in live born gestations delivered before 28 weeks. The sampling technique and population selection as well as our focused analysis on fetal inflammatory response are intended to identify protracted infections in a population at risk for neurologic developmental abnormalities.

Chorioamnionitis was a frequent finding in our population. Inflammation in either the chorionic plate or the cord was found in 26% of specimens, and microorganisms were recovered from more than 67% of those cases. In addition, intense inflammation indicated a high likelihood of microorganism recovery and a higher median number of CFUs. These rates of inflammation and microorganism recovery are comparable to, or slightly lower than, those in other reports about pregnancies that ended prematurely [25,26], but they are much higher than those found in reports of term gestations [9].

The histologic characteristics most strongly correlated with microorganism recovery were high-grade inflammation at the chorionic plate, vasculitis of the fetal stem vessels, as well as vasculitis of umbilical cord vessels. Inflammation of umbilical cord vessels is the histologic hallmark of a fetal inflammatory response [27,28]. The contribution of the fetus to placenta inflammation, sometimes measured biochemically as an elevation in the fetal plasma concentration of interleukin-6, has been associated with preterm delivery and adverse perinatal outcome [27,29–31]. Fetal vasculitis at the chorionic plate is a feature not generally noted in pathology reports, but it represents a true cytokine-driven process within the fetal circulation [23] and is clinically comparable to cord inflammation. In contrast, chorionic plate infiltration is viewed as histologic evidence of a maternal response that is not as closely linked to fetal morbidity unless it is severe (grade 3, stage 3) [32,33] or accompanied by cord inflammation [23,28].

The intensity of placental inflammation is thought to evolve in a sequence developing from chorionic plate inflammation to fetal vasculitis at the chorionic plate to cord inflammation; each stage may be substaged and graded as indicated in Methods [32,34]. The intensity may reflect the duration and extent of infection [35]. This sequence implies that maternal response precedes fetal response. Consistent with this model, we observe that most cord inflammation occurs in combination with high grade/stage plate inflammation. In addition, a similar group of microorganisms was associated with both high-grade chorionic plate inflammation and fetal vasculitis. We did not find any microorganism associated with high-grade chorionic plate inflammation that was not also associated with fetal vasculitis.

Despite the model, an accelerated fetal response may sometimes occur. A subset of cases showed isolated umbilical cord inflammation. We identified cord inflammation in 3% of placentas that had no chorionic inflammation and in 8% that did not have chorionic plate vasculitis. Similar findings have been observed in 5% to 8% of preterm and 17% of term placentas [9]. These cases may represent undersampling when histologic chorioamnionitis does not involve the entire chorioamniotic membranes. However, we also found that some microorganisms are more likely to promote fetal vasculitis than high-grade chorionic plate inflammation.

We have shown that certain microorganisms are more likely to be recovered from placentas in mixed cultures than as single microorganism cultures. Whether this represents co-colonization or contamination is unclear. The possibility exists that growth of such low-virulence microorganisms facilitates the growth of more virulent pathogens [36,37]. Contamination is less likely since (1) polymicrobial cultures show statistically significant correlation with chorioamnionitis; (2) specific organisms that were components of polymicrobial cultures distinguished between placentas with and without fetal vasculitis, including *Actinomyces* sp., *P. bivia, Corynebacterium* sp., *P. magnus*, and anaerobic *Streptococcus* sp.; (3) such positive cultures decrease with gestational age in the expected manner; and (4) microbiologists rely on CFUs to help distinguish contaminants from biologically relevant microorganisms. The working rule is that contaminants tend to have lower numbers of CFUs than pathogens. In our sample, inflamed placentas were not only more likely to harbor a microorganism or groups of microorganisms, but they also tended to have higher CFUs.

We recovered microorganisms from one third of placentas that were not inflamed. Similar findings have been reported in 3rd-trimester placentas with microorganisms recovered from 51% to 71% of placentas with histologic chorioamnionitis and from 23% to 45% in those without inflammation [11,26]. Colonization without inflammation may be related to virulence factors, since many of the microorganisms recovered were low-virulence, apparent bystanders, such as *Lactobacillus*, *Propionebacteria*, and coagulase-negative *Staphylococcus,* that may not be capable of inducing a generalized infection and inflammatory response. In contrast, more invasive microorganisms, such as *E. coli* or group B *Streptococcus,* are fully capable of escaping from colonized tissue and causing a more generalized infection and inflammatory process [26,38].

Alternatively, the discrepancy between microorganism recovery and inflammation may represent very recent arrival of a pathogen with insufficient time to develop an inflammatory response or microorganisms capable of inhibiting local inflammation. A portion of these cases

may also represent a low level of inflammation that was missed by our limited histologic sampling.

Culture contamination is less likely since our cultures were taken from the presumably protected tissue below the amnion, and we limited our analyses to placentas delivered by C-section within 1 hour of membrane rupture. In addition, contamination is expected to show polymicrobial cultures. Tables 4 and 5 demonstrate that many of the noninflamed placentas harbor pure cultures. The rate of organism recovery varies with gestational age in the noninflamed placentas, a feature not expected for contamination. Contamination of specimens usually occurs with recovery of very low levels of microorganisms. This is due to the source of the microorganisms (skin with less than 10^5 CFU/cm^2). When a microorganism is present at levels at least 2 logs greater than the average for the sample group, colonization rather than contamination appears the more likely interpretation. The uniformly high median counts (Table 2) indicate that most of the microorganisms were not contaminants.

We observed negative cultures in inflamed placentas. In our sample, a microorganism could not be recovered from 40% of placentas with grades 2 through 4 free membrane inflammation. The percent was only slightly lower when the placenta had neutrophilic infiltration of the chorionic plate fetal stem vessels (31%) or of the umbilical cord vessels (34%). Others have also failed to recover a microorganism when the placenta is inflamed [39]. One possible explanation is that infections start in the amniotic fluid and must invade the placental parenchyma to be detected in our study. Fetal and maternal cytokine response, however, may be stimulated by the diffusion of nonviable bacterial components from the fluid and do not require bacterial invasion [38]. While the inability to culture a microorganism from these specimens may represent a failure of culture techniques, this is not likely based on our inability to detect unculturable microorganisms using polymerase chain reaction and universal primers. The sensitivity of the polymerase chain reaction assay used for these studies was approximately 1000 copies/g tissue, based on standardization of the assay and primers with a variety of bacterial DNA [40]. Alternatively, the findings may represent nonbacterial stimuli or effects of antibiotics given shortly before delivery, since 31% of women received an antibiotic sometime during pregnancy and 67% received antibiotic during their delivery admission.

A surprising finding was the high rate of organism recovery in placentas delivered by C-section in the absence of labor or rupture of membranes. While 45% of placentas delivered after labor and 53% delivered after membrane rupture harbored a microorganism, 25% of placentas delivered as a result of preeclampsia were also infected. As argued above, these results cannot be entirely explained by contamination. Recent literature indicates that these infections may represent prepregnancy endometrial colonization. When appropriate culture techniques are used, approximately 80% of women in their childbearing years are documented to harbor microorganisms in their endometrium before [6] or after [5] a pregnancy. These findings have challenged the view that the uterus is normally sterile [4].

This is the largest study to date examining placentas delivered before the 28th postmenstrual week, and one of the few to culture placenta parenchyma. The culture techniques were designed to minimize contamination while maximizing detection of fastidious microorganisms, and the histology was assessed in a uniform manner.

In our restricted sample of 835 preterm placentas delivered by C-section, we found support for the following hypotheses: (1) Microorganisms can be found in noninflamed placentas, (2) Inflamed placentas sometimes do not harbor a recoverable microorganism, (3) The more severe the inflammation, the higher the rate of microorganism recovery and the higher the median number of CFUs, (4) The same microorganisms that promote high-grade maternal inflammation (chorionic plate) also appear to promote fetal inflammation, (5) Both pure and polymicrobial cultures predict maternal and fetal inflammation.

ACKNOWLEDGMENTS

Authors are from the Department of Pathology at their respective institutions unless otherwise noted. We wish to thank several members of the ELGAN placental pathology committee for their contribution to the data collection for this study: Barbara J. Doss, MD (Spectrum Health, Grand Rapids, MI, USA), Drucilla Roberts, MD (Massachusetts General Hospital, Boston, MA, USA), John Christie, MD, PhD (Eastern Carolina Medical Center, Greenville, NC, USA), Bo Xu, MD (formerly at University of Massachusetts Memorial Medical Center, Worcester, MA, USA), Chung-Ho Chang, MD (William Beaumont Hospital, Royal Oak, MI, USA), Dennis W. Ross, MD, PhD (Wake Forest University School of Medicine, Winston-Salem, NC, USA), Patricia Senagore, MD (Michigan State University, East Lansing, MI, USA), and Aliya Husain, MD (University of Chicago, Chicago, IL, USA). Gillian Levy (pathology resident, Yale University School of Medicine) also assisted in data collection.

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