OBSTETRICS

Fetal-placental inflammation, but not adrenal activation, is associated with extreme preterm delivery

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OBJECTIVE: Spontaneous labor at term involves the activation of placental corticotropin-releasing hormone and the fetal adrenal axis, but the basis for extreme preterm labor is unknown. Our objective was to determine whether placental corticotropin-releasing hormone is activated in extreme preterm labor.

STUDY DESIGN: One thousand five hundred six mothers delivering at less than 28 weeks' gestation were enrolled. Each mother/infant pair was assigned to the category that described the primary reason for hospitalization. Observers who had no knowledge of patient categorization assessed placenta microbiology, histology, and corticotropin-releasing hormone expression. These were correlated with the primary reason for hospitalization.

RESULTS: Among infants delivered at less than 28 weeks' gestation, spontaneous (vs induced) delivery was associated with less placental corticotropin-releasing hormone expression and more frequent signs of placental inflammation and infection.

CONCLUSION: Inflammation and infection, rather than premature activation of the fetal adrenal axis, should be the major focus of research to prevent extremely preterm human birth.

Key words: corticotropin-releasing hormone, infection, inflammation, preterm delivery

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In normal parturition, prostaglandins initiate uterine contraction, aided by a fall in progesterone's effect to maintain uterine quiescence.[1,2] As in other mammals, activation of the human fetal adrenal axis plays an important role in normal term parturition, with anthropoid primates uniquely using placental corticotropin-releasing hormone (CRH) to drive this process.[3-7] However, the mechanisms that lead to normal and very preterm delivery likely differ in ways that should guide the development of better diagnostic and therapeutic interventions.[8]

Materials and Methods

Study population

The Extremely Low Gestational Age Newborns (ELGAN) study was designed to identify pregnancy and neonatal characteristics and exposures that increase the risk of neurologic disorders in ELGANs.[9] During the years 2002-2004, women delivering between a gestation of 22 weeks 0 days and 27 weeks 6 days at 1 of 14 participating institutions in 11 cities in 5 states were asked to enroll in the study. The individual institutional review boards approved the enrollment and consent processes. Mothers were approached for consent either upon antenatal admission or shortly after delivery, depending on clinical circumstance and institutional preference.

Of 1506 infants enrolled in the ELGAN study, placentas were collected from 1411, ribonucleic acid (RNA) was prepared from 1370, and 1219 contained nondegraded RNA. These 1219 placentas constitute the sample for all the tables. The 3 samples (1506, 1411, and 1219) are comparable in their proportion of children delivered for maternal and fetal reasons and the distribution of gestational ages and birthweights.

Pregnancy variables

The clinical circumstances that led to preterm delivery were operationally defined using both data from the maternal interview and data abstracted from the

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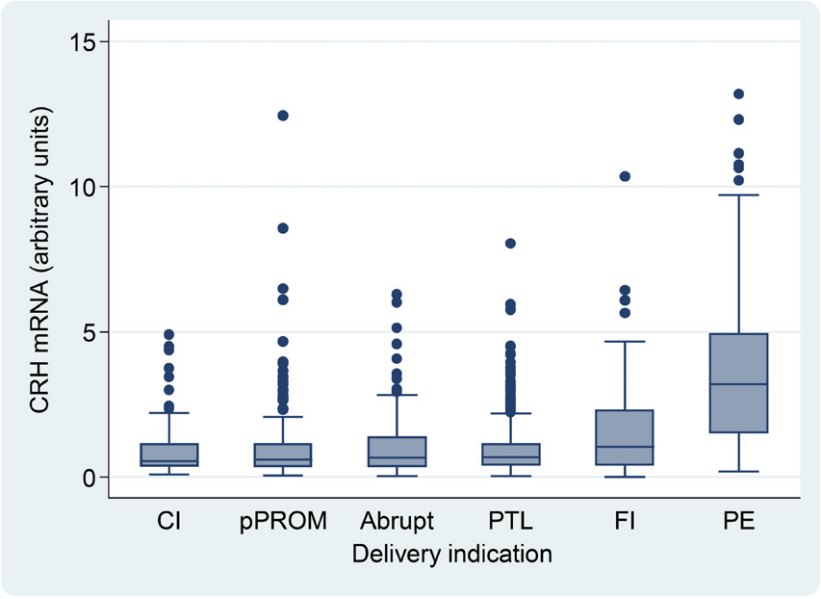
FIGURE 1
Placental CRH mRNA (qRT-PCR), gestational ages 23-27 weeks



Abrupt, placental abruption (n = 125); *CI*, cervical incompetence (n = 74); *CRH*, corticotropin-releasing hormone; *FI*, fetal indication (n = 62); *PE*, preeclampsia (n = 163); *pPROM*, prelabor premature rupture of membranes (n = 253); *PTL*, preterm labor (n = 542); *qRT-PCR*, quantitative reverse transcription-polymerase chain reaction.

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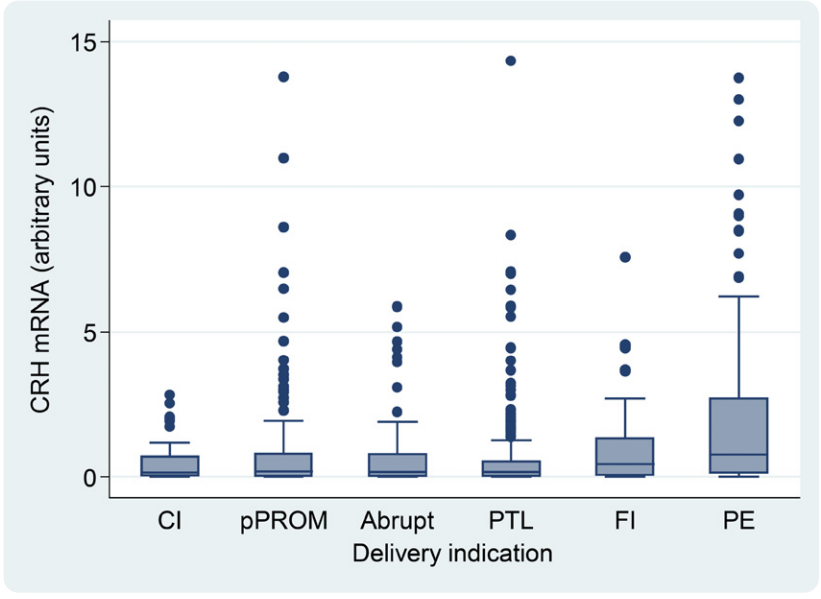
medical record.[10] Each mother/infant pair was assigned to the category that described the primary reason for hospitalization: preterm labor was defined as progressive cervical dilation with regular contractions and intact membranes. The diagnosis of preterm premature rupture of fetal membranes (pPROM) was defined as the presence of vaginal pooling with either documented nitrazine positive testing or ferning prior to regular uterine activity. Preeclampsia was defined as new-onset hypertension and proteinuria of sufficient severity to warrant delivery for either a maternal or fetal indication.

For a diagnosis of cervical insufficiency, a woman had to present with cervical dilation of greater than 2 cm, in the absence of membrane rupture and detected or perceived uterine activity. Placental abruption was defined as presentation with significant amount of vaginal bleeding (either documented in the medical record or a postpartum hematocrit <24%) and a clinical diagnosis of placental abruption in the absence of cervical change. Although painful uterine contractions were not required, most women given this diagnosis tended to present with vaginal hemorrhage often accompanied or very soon followed by labor.

In addition, placenta abruption, as defined, tended to be much more like the other spontaneous disorders of labor, prelabor rupture of membranes, and cervical insufficiency.[10,11] Presentations under the category of fetal indication/intrauterine growth restriction included severe intrauterine growth restriction based on antepartum ultrasound examination, nonreassuring fetal testing, oligohydramnious, and Doppler abnormalities of umbilical cord blood flow.

Preterm labor, prelabor premature rupture of fetal membranes, cervical insufficiency, and placental abruption were then grouped into spontaneous deliveries because they were initiated without medical assistance. "Induced for maternal or fetal reasons" is applied to deliveries that were not initiated by the mother or fetus but by the physician to preserve the health of mother or fetus.

FIGURE 2
Placental CRH mRNA (RNA blot), gestational ages 23-27 weeks



Abrupt, placental abruption (n = 96); *CI*, cervical incompetence (n = 59); *CRH*, corticotropin-releasing hormone; *FI*, fetal indication (n = 50); *PE*, preeclampsia (n = 129); *pPROM*, prelabor premature rupture of membranes (n = 206); *PTL*, preterm labor (n = 415).

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Placenta characteristics

Delivered placentas were placed in a sterile examination basin and transported to a sampling room. Eighty-two percent of the samples were obtained within 1 hour of delivery.

CRH messenger RNA content: RNA isolation. In placentas from which RNA was prepared, CRH and ACTB mRNA (actin B, a control messenger RNA [mRNA] ubiquitously expressed in all cells) expression was analyzed by quantitative reverse transcription-polymerase chain reaction (PCR) and RNA blot. Biopsies of the fetal side of the placenta to a depth of approximately 1 cm (~500 mg) were obtained shortly after birth, frozen in liquid nitrogen, and stored at −80°C. Samples (~100 mg) were crushed to a fine powder in dry ice, and total RNA was extracted using TRI reagent (Sigma, St Louis, MO) according to the manufacturer's instructions.

Control RNA was isolated from 7 normal term placentas delivered by cesarean section and pooled. This RNA was used to create CRH and ACTB complementary deoxyribonucleic acid (cDNA) to make standard curves for quantitative polymerase chain reaction (qPCR) analysis (see the following text). Serial dilutions of this RNA were also directly analyzed for CRH mRNA expression to establish a linear range for RNA blot quantification (see the following text).

CRH mRNA quantitation by quantitative reverse transcription-polymerase chain reaction (RT-PCR). Complementary DNA was prepared in 10 μL reactions using iScript cDNA synthesis (Bio-Rad Laboratories, Hercules, CA) and 0.5 μg of placental RNA. Subsequently, 2 μL of this reaction was amplified by qPCR using IQ SYBR Green Supermix (Bio-Rad Laboratories), a Bio-Rad iQ5 qPCR apparatus, and the following primers: CRH forward primer, AGA GAA AGC CCC CGG AGA, CRH reverse primer, ATG TTA GGG GCA CTC GCT TC, ACTB forward primer, CGC GAG AAG ATG ACC CAG AT, ACTB reverse primer, GTA CAT GGC TGG GGT GTT G.

The qPCR conditions consisted of denaturation (95.0°C for 3 minutes) and amplification (95.0°C for 10 seconds, 60.0°C for 40 seconds, repeated for 45 cycles). The standards were prepared by PCR amplification using these same primers and control placenta RNA (see the preceding text). ACTB results were used to confirm the quality of the mRNA and correct for total RNA amount. Of the 1370 samples, 151 contained degraded RNA (as judged by lack of ACTB and CRH signals by qRT-PCR), so that final analyses were performed on 1219 samples.

To normalize the results among the different experimental runs, all CRH standards across all 17 qPCR runs were averaged, and each of the 1219 experimental samples was evaluated against that average CRH standard curve. Similarly, all ACTB standards across all 17 qPCR runs were averaged and each of the 1219 experimental ACTB values was evaluated against that average ACTB standard curve. Each CRH value was then divided by its corresponding ACTB value to obtain the relative CRH mRNA concentrations, which are displayed in Figure 1 and Tables 1-7.

CRH mRNA quantification by blot hybridization. We initially analyzed the RNA by blot hybridization[12] to examine CRH mRNA expression in different placental regions and to assess CRH mRNA integrity and stability during the collection

TABLE 1
Percent of infants by delivery indication (column) and characteristic (row)

Characteristic	Delivery indication						Row number
	PTL	pPROM	CI	Abrupt	FI	PE	
Antenatal steroid course							
Complete	55	73	78	62	66	67	771
Partial	32	21	19	16	18	30	320
None	13	6	3	22	16	3	126
Cesarean delivery							
Yes	52	60	62	68	95	98	782
Sex							
Male	57	53	59	52	48	44	652
Type of gestation							
Singleton	59	70	58	74	44	94	812
Gestational age, wks							
23-24	32	26	39	33	16	14	340
25-26	40	43	51	42	48	45	521
27	28	32	9	25	35	40	358
Birthweight, g							
≤750	38	39	42	38	68	67	537
751-1000	42	39	42	47	19	26	469
>1000	20	22	16	14	13	7	213
Birthweight							
<−2	1	5	0	4	32	31	92
Z-score[a]							
≥−2, <−1	10	9	3	6	23	39	166
≥−1	89	86	97	90	45	30	961
Column number	542	253	74	125	62	163	1219

Columns contain column percents.
Abrupt, placental abruption; *CI,* cervical insufficiency; *FI,* fetal indication; *PE,* preeclampsia; *pPROM,* prelabor premature rupture of membranes; *PTL,* preterm labor.
[a] Yudkin standard.[27]
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TABLE 2
Percent[a] of placentas with CRH expression in highest quartile for gestational age and delivery indication

	Delivery indication						
Gestational age, wks	PTL	pPROM	CI	Abrupt	FI	PE	Row percentage
23-24	6	14	17	17	30	70	15
25-26	15	9	13	23	37	72	24
27	22	24	29	26	55	79	35
Column percentage	14	15	16	22	42	74	25

The columns are ordered by increasing total percentage of CRH in the highest quartile.
Abrupt, placental abruption; *CI,* cervical incompetence; *CRH,* corticotropin-releasing hormone; *FI,* fetal indication; *PE,* preeclampsia; *pPROM,* prelabor premature rupture of membranes; *PTL,* preterm labor.

[a] Cell-specific.

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process. We subsequently found that samples from all 4 placental quadrants had equivalent expression of CRH mRNA and that it remained intact for up to 10 hours at room temperature prior to sample retrieval and storage at −80°C. The control RNA was used to normalize the relative amount of CRH mRNA in the experimental samples among the 68 blots, which differed slightly because of variations in probe labeling, hybridization, washing, and exposure.

To correct for the amount of total RNA loaded within each gel, each filter was stripped and rehybridized to a ^{32}P-uridine 5-triphosphate-labeled antisense human beta-actin ACTB (Ambion, Austin, TX) riboprobe. Of the 1370 samples, 415 contained undetectable ACTB mRNA and were considered to contain degraded RNA, so that final analyses were performed on 955 samples. Each CRH mRNA value was then divided by its corresponding ACTB mRNA value to obtain relative CRH mRNA concentrations, which are displayed in Figure 2. The RNA blot and qRT-PCR methods revealed similar relationships between CRH mRNA expression and placental and pregnancy characteristics, except that CRH mRNA measurement by RNA blot was less sensitive and more variable (Figures 1 and 2 and data not shown).

Microbiology. The microbiologic procedures are described in detail elsewhere.[13]

TABLE 3
Odds ratios (95% confidence intervals) for CRH expression in highest quartile associated with each pregnancy disorder

	Adjustment		
Pregnancy disorder	None	GA[a] only	GA[a] plus ACS[b]
Preterm labor	1.0	1.0	1.0
Prelabor premature rupture of membranes	1.1 (0.7–1.6)	1.0 (0.7–1.6)	1.0 (0.7–1.6)
Preeclampsia	17 (11–27)[c]	16 (11–25)[c]	17 (11–16)[c]
Abruption	1.7 (1.02–2.7)[c]	1.7 (1.05–2.8)[c]	1.7 (1.05–2.8)[c]
Cervical insufficiency	1.2 (0.6–2.3)	1.4 (0.7–2.7)	1.4 (0.7–2.7)
Fetal indication	4.4 (2.5–7.6)[c]	4.1 (2.3–7.3)[c]	4.1 (2.3–7.3)[c]

The adjustments are listed at the top of each *column.* Preterm labor, the pregnancy disorder that had the lowest rate of physician-ordered ACS exposure, was set as the referent group.
ACS, antenatal corticosteroid; *CRH,* corticotropin-releasing hormone; *GA,* gestational age.

[a] GA; 23–24, 25–26, 27 weeks; [b] ACS; complete, partial, none; [c] Statistically significant elevated values.

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Briefly, the frozen samples were allowed to thaw at room temperature, a portion approximately 1 cm^3 was removed and weighed, and then diluted 1:10 with sterile phosphate-buffered saline, homogenized, and plated on selective and nonselective media, including prereduced brucella-base agar with 5% sheep blood enriched with hemin and vitamin K_1, tryptic soy agar with 5% sheep blood, chocolate agar, and A-7 agar.

Histology. The ELGAN pathologists participated in the creation of the manual of procedures as well as in defining the histologic characteristics and designing the data collection form.[14] In addition, they participated in exercises to reduce observer variability.

In keeping with the guidelines of the 1991 College of American Pathologists Conference,[15] representative sections were taken from all abnormal areas as well as 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.

Study pathologists examined the slides for histologic characteristics listed on a standardized data form. For multiple births, separate forms were filled for each newborn. Twins with fused placentas also had multiple forms filled.

The presence or absence of infarcts and intervillous fibrin, fetal stem vessel thrombosis, and decidual hemorrhage and fibrin deposition consistent with abruption were coded as present or absent. Chorionic villi were scored for syncytial knots (0, no; 1, occasional; 2, increased).

Inflammation of the membranes was described in detail. Level 3 severity of chorionic plate inflammation required more than 20 neutrophils/20x. Grade 3 inflammation of the amnion required numerous large or confluent foci, whereas grade 4 required necrosis. Inflammation in the chorion/decidua was similarly, but separately, graded.

Inflammation in the umbilical cord was graded from 0 to 5. Grade 3 required neutrophils in perivascular Wharton's jelly, grade 4 required panvasculitis and umbilical cord vasculitis extending deep into Wharton's jelly, and grade 5 required a halo lesion (ring of precipitate

in Wharton's jelly encircling each vessel). Neutrophilic infiltration into fetal stem vessels in the chorionic plate required that neutrophils appeared to have migrated toward the amnionic cavity.

Newborn blood spot collection and measurement of proteins from blood spots

The collection of newborn whole blood, preparation of dry blood spots, and their processing for protein analysis is described elsewhere.[16] Briefly, after the newborn's blood was drawn on postnatal day 1 for clinical indications, the tip of the syringe containing the remainder was blotted with filter paper and stored at −80°C. Protein elution from 12 mm punched biopsies of the frozen blood spots was performed as described.[16,17] The following 25 proteins were measured by the Meso Scale Discovery multiplex platform (MSD, Gaithersburg, MD) as described[16]: C-reactive protein (CRP), serum amyloid A (SAA), myeloperoxidase (MPO), interleukin (IL)-1β, IL-6, IL-6R, tumor necrosis factor (TNF)-α, tumor necrosis factor receptor (TNF-R)-1, TNF-R2, IL-8, monocyte chemotactic protein (MCP)-1, MCP-4, macrophage inflammatory protein (MIP)-1β, regulated upon activation, normal T cell expressed, and secreted (RANTES), interferon-inducible T cell alpha-chemoattractant (I-TAC), intercellular adhesion molecule (ICAM)-1, ICAM-3, vascular cell adhesion molecule (VCAM)-1, E-selectin (E-SEL), matrix metalloproteinase (MMP)-1, MMP-9, vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor (VEGF-R)-1, VEGF-R2, and insulin-like growth factor binding protein (IGFBP)-1.

In light of the observations that protein concentrations varied with gestational age at delivery,[18] the concentrations of most proteins did not follow a normal distribution, and because we were interested in the contribution of high concentrations, we dichotomized the distribution of each protein's concentration at the 75th centile among children in each gestational age category (ie, 23-24, 25-26, 27 weeks).

TABLE 4
Placenta histologic characteristics associated with placental CRH expression

	CRH quartile	
Placenta histologic characteristic	Lowest	Highest
None of the 8 characteristics	1.0	1.0
Infarct	1.7 (1.1–2.7)[a]	3.3 (2.2–5.0)[a]
Increased syncytial knots	1.1 (0.7–1.8)	3.2 (2.2–4.6)[a]
Inflammation chorion/decidua[b]	1.8 (1.3–2.4)[a]	0.5 (0.3–0.7)[c]
Inflammation chorionic plate[d]	1.6 (1.1–2.3)[a]	0.3 (0.2–0.6)[c]
Neutrophils in fetal stem vessels	1.6 (1.1–2.3)[a]	0.5 (0.3–0.8)[c]
Umbilical cord vasculitis[e]	1.7 (1.1–2.5)[a]	0.4 (0.2–0.7)[c]
Thrombosis fetal stem vessels	2.0 (1.1–3.7)[a]	1.4 (0.7–2.7)
Neutrophils in fetal stem vessels	1.9 (1.2–2.8)[a]	1.3 (0.8–2.1)

Odds ratios (and 95% confidence intervals) for being in the lowest or highest CRH quartile associated with each histologic characteristic listed on the *left*. The referent group for all comparisons consists of infants whose placentas had none of the histologic features. The models are adjusted for gestational age.
CRH, corticotropin-releasing hormone.

[a] Statistically significant elevated values; [b] Grades 3 and 4; [c] Statistically significant reduced values; [d] Stage 3 and severity 3; [e] Grades 3, 4, and 5.

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Data analysis

We evaluated the generalized null hypotheses that CRH mRNA expression in the placenta is not associated with the following: (1) the pregnancy disorder that led to preterm birth, (2) the recovery of an organism from the placenta parenchyma, (3) any histologic lesion of the placenta, (4) a concentration in the highest quartile for gestational age category on the first postnatal day of any inflammation-related protein; and (5) antenatal corticosteroid treatment.

CRH mRNA quantitation was performed without the knowledge of any other data associated with the study subjects. In the entire ELGAN study sample, the pregnancy disorder that preceded preterm delivery,[10] organism recovery from the placenta,[13] histologic characteristics of the placenta,[14,19] concentration of inflammation-related proteins in the newborn's circulation,[17,18] and CRH content of the placenta (Tables 1 and 2) varied with gestational age at delivery. We adjusted for gestational age in groups of weeks (23-24, 25-26, 27).

We summarize some of our data with box and whiskers displays of the central tendency and dispersion of CRH mRNA content in infants grouped by the pregnancy disorder that led to their delivery so early before term (Figures 1 and 2). The central tendency is indicated by the line close to the middle of the box, which is the median, and by the top and bottom of each box, which indicate the 25th and 75th centiles. The dispersion of CRH mRNA content is indicated by the length of the vertical lines that emanate from the box as well as by the block dots, which identify outliers. We did not see a route of delivery effect (vaginal vs cesarean) on CRH mRNA content in any of our analyses. Thus, we did not adjust for route of delivery in this study.

We created multinomial (also called polytomous or polychotomous) logistic regression models to evaluate the risk of a CRH mRNA concentration in the lowest and highest quartiles with the middle half as the referent. The contributions of antecedents/correlates are presented as risk ratios with 95% confidence intervals.

Because gestational age, pregnancy disorder, placenta histology, placenta microbiology, and CRH mRNA content were highly interrelated, we conducted additional analyses in 2 subgroups: those with spontaneous deliveries and those delivered for maternal or fetal reasons. Odds ratios for lowest and highest CRH

TABLE 5
Odds ratios (95% confidence intervals) for markers of inflammation in day-1 blood

	CRH quartile	
Protein	**Lowest**	**Highest**
CRP	1.0 (0.6–1.5)	0.7 (0.4–1.01)
SAA	0.9 (0.6–1.4)	0.6 (0.4–0.98)[a]
MPO	1.1 (0.7–1.6)	0.6 (0.4–0.9)[a]
IL-1β	1.5 (1.01–2.1)[b]	0.8 (0.5–1.1)
IL-6	1.1 (0.7–1.6)	0.6 (0.4–0.9)[a]
IL-6R	1.5 (1.02–2.2)[b]	0.6 (0.4–0.97)[a]
TNF-α	1.4 (0.96–2.0)	0.5 (0.4–0.8)[a]
TNF-R1	1.1 (0.8–1.7)	0.6 (0.4–0.95)[a]
TNF-R2	1.7 (1.1–2.5)[b]	0.6 (0.4–0.9)[a]
IL-8 (CXCL8)	1.6 (1.1–2.4)[b]	0.8 (0.5–1.2)
MCP-1 (CCL2)	0.9 (0.6–1.4)	1.0 (0.7–1.5)
MCP-4 (CCL13)	1.1 (0.7–1.6)	1.1 (0.7–1.6)
MIP-1B (CCL4)	1.5 (1.02–2.2)[b]	0.7 (0.4–1.00)
RANTES (CCL5)	1.4 (0.9–2.0)	0.7 (0.5–1.1)
I-TAC (CXCL11)	1.1 (0.8–1.7)	0.7 (0.5–1.1)
ICAM-1 (CD54)	1.3 (0.9–2.0)	0.6 (0.4–0.9)[a]
ICAM-3 (CD50)	1.2 (0.8–1.7)	0.5 (0.3–0.7)[a]
VCAM-1 (CD106)	1.2 (0.8–1.8)	0.5 (0.3–0.8)[a]
E-SEL (CD62E)	1.4 (0.97–2.1)	0.3 (0.2–0.5)[a]
MMP-1	0.8 (0.6–1.3)	0.6 (0.4–0.9)[a]
MMP-9	0.9 (0.6–1.4)	0.5 (0.3–0.7)[a]
VEGF	1.5 (1.00–2.1)	0.5 (0.3–0.8)[a]
VEGF-R1	0.9 (0.6–1.4)	1.5 (0.99–2.1)
VEGF-R2	1.5 (1.01–2.1)[b]	0.5 (0.4–0.8)[a]
IGFBP-1	0.8 (0.5–1.2)	1.9 (1.3–2.8)

Odds ratios (and 95% confidence intervals) of a day 1 concentration in the highest quartile for gestational age of the protein listed on the *left* associated with CRH expression in the lowest and highest quartiles relative to that of children whose CRH mRNA was in the middle 2 quartiles.

CCL, chemokine ligand; *CD,* cluster of differentiation; *CRH,* corticotropin-releasing hormone; *CRP,* C-reactive protein; *CXCL,* C-X-C motif ligand; *E-SEL,* E-selectin; *ICAM,* intercellular adhesion molecule; *IGFBP,* insulin-like growth factor binding protein; *IL,* interleukin; *I-TAC,* interferon-inducible T cell alpha-chemoattractant; *MCP,* monocyte chemotactic protein; *MIP,* macrophage inflammatory protein; *MMP,* matrix metalloproteinase; *MPO,* myeloperoxidase; *RANTES,* regulated upon activation, normal T cell expressed, and secreted; *SAA,* serum amyloid A; *TNF,* tumor necrosis factor; *VCAM,* vascular cell adhesion molecule; *VEGF,* vascular endothelial growth factor.

[a] Statistically significant reduced values; [b] Statistically significant elevated values.

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quartile associated with inflammation-related proteins and placental microrganisms were calculated using the middle 2 quartiles as the referent group.

To assess the contribution of a partial or complete course of antenatal corticosteroid to the CRH mRNA content, we created multivariable models of the risk of a CRH mRNA concentration in the highest quartile that included each pregnancy disorder, gestational age groups, and the antenatal corticosteroid course. Multiple separate courses were not evaluated.

RESULTS

We measured placental CRH expression and other characteristics in the 1219 pregnancies of extremely low gestational age (<28 weeks) newborns[10] and related these to the pregnancy disorders that lead to very preterm delivery (Table 1). Surprisingly, we found that spontaneous preterm deliveries were associated with lower CRH expression and higher frequency of markers of placental inflammation and infection than deliveries induced for maternal or fetal reasons.

In our cohort, the placentas of women who were induced to deliver because of preeclampsia tended to have the highest CRH expression, those who were induced to deliver for a fetal indication had intermediate CRH expression, and those from women who delivered spontaneously had the lowest CRH expression (Figure 1 and Tables 2 and 3). As gestational age at birth increased, so did the percent of placentas that had CRH expression in the highest quartile (Table 2). However, these differences in CRH among the various pregnancy disorders are not explained by disparities in gestational age or antenatal corticosteroid use (Table 3). The association of induced delivery with the highest quartile of the CRH RNA quartile was thus not confounded by corticosteroid receipt.

Placentas that had an infarct or an increased number of syncytial knots were significantly more likely than those without these characteristics to have a CRH concentration in the highest quartile, even after adjusting for gestational age (Table 4). In contrast, placentas that had inflammation were significantly less likely than placentas without inflammation to have a CRH concentration in the highest quartile and at increased risk of having a CRH concentration in the lowest quartile.

The histologic characteristics less clearly associated with inflammation (thrombosis of the fetal stem vessels, decidual hemorrhage, and fibrin deposition) were also associated with an increased risk of a CRH concentration in the lowest quartile. Placenta infarcts were also associated with increased odds of being in the lowest CRH quartile (Table 4).

As further evidence for an association between inflammation and low placental CRH expression, those newborns whose placental CRH concentration was in the

lowest quartile were more likely to have an elevated concentration in day 1 blood of IL-1β, IL-6R, TNF-R2, IL-8, MIP-1β, and VEGF-R2 (Table 5). In contrast, those with CRH in the highest quartile were significantly less likely to have an elevated concentration in day 1 blood of SAA, MPO, IL-6R, TNFα, TNF-R1, TNF-R2, ICAM-1, ICAM-3, VCAM-1, E-SEL, MMP-1, MMP-9, VEGF, and VEGF-R2 and more likely to have an elevated concentration of IGFBP-1.

One potential cause of inflammation during pregnancy is infection. Compared with placentas that did not yield any organism, those that harbored any of the group of organisms evaluated (ie, pure cultures, mixed cultures, aerobe, anaerobes, Mycoplasma species, normal skin flora, or normal vaginal flora) were at significantly reduced risk of having a CRH concentration in the highest quartile (Table 6).

All of the preceding data document that preeclampsia and inflammatory pregnancy disorders have opposite relationships with CRH expression. To evaluate whether inflammation had an independent effect (separate from the one reflecting merely the absence of preeclampsia), we limited 1 set of analyses to placentas of infants delivered spontaneously and excluded placentas of infants induced to deliver for maternal or fetal indications, including preeclampsia and fetal growth restriction (Table 7). In a model with gestational age variables only (left data column), low gestational age was associated with the lowest CRH mRNA concentrations. Histologic inflammation variables by themselves were also strongly associated with low CRH expression (middle data column). When variables for both gestational age and histologic inflammation were evaluated in the same model, the strong relationship between inflammation and low CRH expression persists, as does the strong relationship between low gestational age and low CRH expression (right data column).

TABLE 6
Odds ratios (95% confidence intervals) for CRH expression quartile associated with placental organisms

	CRH quartile	
Microorganism	**Lowest**	**Highest**
No organism	1.0	1.0
Number of species isolated		
1	1.3 (0.9–1.8)	0.5 (0.3–0.7)[a]
≥2	1.2 (0.8–1.6)	0.4 (0.3–0.6)[a]
Any organism	1.2 (0.9–1.6)	0.5 (0.3–0.6)[a]
Any aerobe	1.3 (0.9–1.7)	0.4 (0.3–0.6)[a]
Any anaerobe	1.1 (0.8–1.6)	0.6 (0.4–0.8)[a]
Any mycoplasma	1.5 (0.97–2.3)	0.2 (0.1–0.4)[a]
Skin organisms[b]	1.0 (0.7–1.4)	0.5 (0.4–0.7)[a]
Vaginal organisms[c]	1.1 (0.7–1.6)	0.4 (0.2–0.6)[a]

The referent group for all comparisons consists of infants whose CRH expression was in the middle 2 quartiles. Data are adjusted for gestational age.
CRH, corticotropin-releasing hormone.

[a] Statistically significant reduced values; [b] *Corynebacterium sp, Propionebacterium sp, Staphylococcus sp*; [c] *Prevotella bivia, Lactobacillus sp, Peptostrep magnus, Gardnerella vaginalis.*

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COMMENT

In this large cohort of prospectively enrolled infants delivered prior to 28 weeks, the expression of CRH was lowest in the placentas of infants who delivered spontaneously and highest in those of infants delivered for maternal or fetal reasons. The one claim that CRH mRNA expression is higher in preterm placentas that are inflamed, which is contrary to what we found, was based on only 6 inflamed placentas (vs 481 in our cohort) and did not compare these placentas with any delivered for fetal or maternal reasons.[20]

Supporting our results, Struwe et al[21] reported elevated CRH expression in placentas of infant with intrauterine growth restriction. They also found increased expression of placental IGFBP-1,[21] consistent with our findings in newborn blood. We found that spontaneous deliveries were most likely associated with infection and inflammation of the placenta and with systemic inflammation. These findings were not due to differences in gestational length or antenatal steroid use or whether preeclampsia was present.

Our study has several strengths. First, we included a large number of infants, making it unlikely that we have missed important associations because of the lack of sta-

TABLE 7
Odds ratios (95% confidence intervals) of lowest CRH mRNA expression quartile associated with gestational age, inflammation, and both gestational age and inflammation, among pregnancies with spontaneous reasons for preterm delivery

Variable	GA alone	Inflammation alone	GA and inflammation
Inflammation[a]	—	1.9 (1.4–2.5)[b]	1.8 (1.3–2.4)[b]
GA 23-24 wk	2.3 (1.6–3.4)[b]	—	2.2 (1.5–3.1)[b]
GA 25-26 wk	1.3 (0.9–1.9)	—	1.3 (0.9–1.8)

Odds ratio (95% confidence interval) for being in the lowest CRH quartile associated with inflammation and gestational age among infants delivered for preterm labor, prelabor premature ruptured membranes, abruption, or cervical insufficiency. Infants delivered for maternal or fetal reasons have been excluded.
CRH, corticotropin-releasing hormone; *GA,* gestational age.

[a] Inflammation of the chorion/decidua or chorionic plate, neutrophilic infiltration of the fetal stem vessels, and umbilical cord vasculitis; [b] Statistically significant elevated values.

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tistical power. Second, we selected infants based on gestational age, not birthweight, to minimize confounding because of factors related to fetal growth restriction. Third, we collected all of our data prospectively. Fourth, the examiners were not aware of the medical histories of the placentas they examined, thereby minimizing bias. Fifth, we measured CRH mRNA expression using 2 completely different methods that yielded similar results. Sixth, our protein data are of high quality and have high content validity.[11,16] The weaknesses of our study are those of all observational studies. We are unable to distinguish between causation and association as explanations for what we found.

Although normal delivery near term depends on linking the timing of fetal maturation to parturition via the fetal adrenal-placental unit driven by placental CRH,[2,4,5,7] extreme preterm delivery is likely driven by different mechanisms, conceptualized as the preterm parturition syndrome.[22] For example, a single measurement of maternal blood CRH at 16-20 weeks' gestation predicted the timing of spontaneous labor in an unselected population of pregnant women[7] but not in women at high risk for preterm delivery.[23]

Progesterone supplementation is only half as effective preventing extreme preterm delivery as compared with later recurrent preterm delivery.[24] In addition, early preterm labor is more likely than term labor to be associated with intrauterine infection and cytokine production.[25] Cytokines, via the prostaglandins that they stimulate,[26] increase uterine contraction[27] and might directly initiate labor, independent of fetal signals and relatively unresponsive to progesterone's quiescent effects.

In summary, among infants born before the 28th week of gestation, spontaneous deliveries were not associated with premature activation of the fetal adrenal axis, but were associated with inflammation. This suggests that the latter should be the major focus of research to prevent extremely preterm human birth. ■

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