BASIC SCIENCE: OBSTETRICS

Natural antimicrobial production by the amnion

Sarah J. Stock, MBChB; Rodney W. Kelly, PhD; Simon C. Riley, PhD; Andrew A. Calder, MD

OBJECTIVE: The purpose of this study was to determine the expression of natural antimicrobials in primary cultured amnion epithelial cells and to examine their regulation by interleukin-1 beta (IL-1β).

STUDY DESIGN: Primary amnion epithelial cells were cultured from samples that were obtained at prelabor cesarean section (n = 12) and stimulated with IL-1β. Natural antimicrobial messenger RNA expression was determined by real-time quantitative polymerase chain reaction, and protein was measured by enzyme-linked immunosorbent assay. Data was analyzed by analysis of variance.

RESULTS: Primary amnion epithelial cells express messenger RNA for human beta defensin (HBD) 1 to 3, secretory leukocyte protease inhibitor and elafin, but not HBD4. IL-1β 10 ng/mL stimulates HBD2 messenger RNA in a biphasic pattern, with a 51-fold increase at 6 hours and a 67-fold at 12 hours ($P < .001$). HBD2 protein production is significantly increased by 24 hours ($P < .05$).

CONCLUSION: The amnion produces potent natural antimicrobials that may help protect the pregnancy from infection. HBD2 production is dramatically upregulated by the labor-associated inflammatory cytokine IL-1β.

Key words: amnion, defensin, infection, natural antimicrobial, pregnancy

Cite this article as: Stock SJ, Kelly RW, Riley SC, Calder AA. Natural antimicrobial production by the amnion. Am J Obstet Gynecol 2007;196:255.e1-255.e6.

Labor is an inflammatory process that is characterized by leukocyte invasion of the uterine tissue, and increased cytokine and prostaglandin production.[1] Intrauterine infection in pregnancy can stimulate this process prematurely and is responsible for approximately one third of all cases of preterm labor.[2] Prevention of ascending infections is paramount in maintaining a healthy pregnancy.

Natural antimicrobials are peptides that are essential components of the innate immune system that provide broad-spectrum protection against bacteria, yeasts, and some viruses.[3] The human beta defensins (HBDs) are a major family of vertebrate natural antimicrobials; HBD1-4 are expressed widely at mucosal surfaces.[4] In addition to their antimicrobial activity, HBDs have chemoattractant properties that suggest that they interact between the innate and adaptive immune systems.[5,6] A related family of molecules with important antimicrobial activities is the antileukoproteinases.[7] This group includes elafin (skin-derived antiproteinase) and secretory leukocyte protease inhibitor (SLPI).

Natural antimicrobial proteins are expressed throughout the nonpregnant female reproductive tract.[8,9] In pregnancy, natural antimicrobials are found in the amniotic fluid[10-13] and have been localized in the placenta, decidua, and fetal membranes.[14-18]

The amnion is positioned critically between the normally sterile amniotic cavity and the contaminated extrauterine environment, but amniotic production of natural antimicrobials has not been elucidated. The bacterial product lipopolysaccharide has been shown to stimulate HBD3 messenger RNA (mRNA) in so-called amnion-derived FL cells.[17] However, this cell line is derived from cervical He-La cell contaminants[19] (www.atcc.org), thus the validity of this finding is unclear. The current study examined natural antimicrobial expression and regulation in primary cultured amnion cells. We also compared the expression with that in the contaminated FL cell lines and the similarly contaminated WISH cell line[20] (www.atcc.org) to determine whether they are a suitable model for the study of amniotic innate immune responses.

METHODS

Samples

The Lothian Local Research Ethics Committee approved this study, and written informed consent was obtained from all participants. Fetal membranes were collected from 12 women who underwent elective cesarean section at 39 weeks of gestation. All of the women had uncomplicated singleton pregnancies, with no signs of labor or infection. Amnion for culture was stripped from underlying chorion, washed, and transported to the

From the Department of Obstetrics and Gynaecology, University of Edinburgh (Drs Stock and Riley and Professor Calder), and the Medical Research Council Human Reproductive Sciences Unit (Professor Kelly), Centre for Reproductive Biology, Queen's Medical Research Institute, Edinburgh, UK.

Presented at the Eleventh Annual Conference of the British Maternal and Fetal Medicine Society, Cardiff, UK, April 7, 2006.

Received Jun. 27, 2006; accepted Oct. 27, 2006.

Reprint requests: Sarah Stock, MBChB, Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh, EH16 4TJ UK; sarah.stock@ed.ac.uk.

Supported by The Jennifer Brown Research Fund, administered by UK Charity PiggyBankKids, Registration Number 1092312.

0002-9378/$32.00

doi: 10.1016/j.ajog.2006.10.908

TABLE 1
Sequences of primers and probes for Taqman polymerase chain reaction

Variable	Forward primer	Reverse primer	Probe
HBD1	*TCAGCAGTGGAGGGCAATG*	*CCTCTGTAACAGGTGCCTTGAAT*	*TCTATTCTGCCTGCCCGATCTTTACCAA*
HBD2	*CTGATGCCTCTTCCAGGTGTTT*	*CTGGATGACATATGGCTCCACTCT*	*AAGGCAGGTAACAGGATCGCCTATACCACCA*
HBD3	*CAGAGGCGGCCGGTGT*	*CGAGCACTTGCCGATCTGTT*	*CTGTGCTCAGCTGCCTTCCAAAGGA*
HBD4	*GGCAGTCCCATAACCACATATTC*	*TGCTGCTATTAGCCGTTTCTCTT*	*TGTCCAATTCAAATTCGCTTCTCACTGGA*
SLPI	*GCATCAAATGCCTGGATCCT*	*GCATCAAACATTGGCCATAAGTC*	*TGACACCCCAAACCCAACAAGGAGG*
Elafin	*TGGCTCCTGCCCCATTATC*	*CAGTATCTTTCAAGCAGCGGTTAG*	*ATCCGGTGCGCCATGTTGAATCC*

laboratory in sterile phosphate-buffered saline solution (Sigma, Poole, Dorset, UK).

Three endometrial samples were used as positive controls for natural antimicrobials. Because individual natural antimicrobials are expressed differentially throughout the menstrual cycle,[21] an endometrial sample from the period of maximal expression for each natural antimicrobial was used (menstrual sample for elafin and HBD2; proliferative sample for HBD4; mid-secretory sample for HBD1, HBD3, and SLPI). They were collected from women who underwent gynecologic procedures for benign conditions. All of the women had regular menstrual cycles (28-35 days) and had not received any hormonal treatments for 3 months before biopsy collection. Menstrual cycle stage was determined from the date of the patient's last menstrual period, histologic dating, and circulating serum estradiol and progesterone concentrations. Endometrial biopsy specimens were immersed in Tri reagent (Sigma) for RNA extraction. A portion was also fixed in 10% neutral buffered formalin overnight at 4°C, stored in ethanol, and then wax embedded for subsequent histologic examination, which was normal in all cases.

Cell culture

Amnion epithelial cells were isolated with a method adapted from Bennett et al.[22] Amniotic membrane was washed and steeped in EDTA (0.5 mmol/L; Sigma) for 15 minutes. Cells were dissociated by incubation in a solution of Dispase (Gibco, Paisley, UK) for 45 minutes at 37°C, and released by agitation in Roswell Park Memorial Institute 1640 medium (Sigma).

Primary amnion cells were plated in 6-well plates (Nunc; Gibco) at a density of 1.5 to 2×10^6cells/mL. FL, WISH, and He-La cells (ATCC, Manassas, VA) were plated at a density of 0.5×10^6cells/mL. Cells were cultured in Roswell Park Memorial Institute 1640 medium that was supplemented with 10% fetal calf serum (Mycoplex; PAA Laboratories, Teddington, UK), penicillin (50 μg/mL; Sigma), streptomycin (50 μg/mL; Sigma,) and L-glutamine (2 mmol/L; Sigma) and was maintained at 37°C in 5% Co_2 and 95% air.

After 24 hours of culture, cells were washed, and fresh medium was added. After 72 hours, when confluent, medium was changed to serum-depleted (2% fetal calf serum) for 20 hours before treatments were added; 2% fetal calf serum was used because preliminary experiments showed decreased cell viability after 48 hours of culture in the absence of serum. The epithelial origin of primary cultured cells was confirmed by immunocytochemistry, with >95% of cells positive for the epithelial cell marker pan cytokeratin (Dako, Ely, Cambs, UK).

Treatments

Cells were incubated with recombinant human interleukin (IL)-1β (Peprotech, London, UK) to stimulate natural antimicrobial production. Initial experiments used a dose of 10 ng/mL and treatment times of 6 and 12 hours, because these were the optimal conditions in a previous study.[23] Subsequent experiments investigated the effects of dose or treatment time by using doses of 0.1-100 ng/mL or treatment times of 1, 2, 3, 6, 12, 16, 24, or 48 hours, after which time the effects diminished. All experiments were performed in quadruplicate, with 1 set of duplicates being used to determine mRNA expression by real-time TaqMan quantitative polymerase chain reaction (PCR) and 1 set being used for protein analysis by enzyme-linked immunosorbent assay. Media from the second set of duplicates were stored at –20°C. Treatments had no significant effect on cell numbers or viability at 48 hours, as determined by trypan blue exclusion.

RNA extraction and quantitative PCR

RNA was extracted from amnion with RNeasy minispin columns (Qiagen, Crawley, West Sussex, UK), and from endometrium with the use of Tri-reagent (Sigma), according to manufacturer's protocols. RNA quantity and quality were assessed by the Agilent 2100 bioanalyzer system in combination with RNA_{6000}nano chips (Agilent Technologies, Cheshire, UK). Only RNA that displayed intact 18S and 28S peaks was reverse transcribed to complementary DNA for real-time PCR.

RNA samples were reverse transcribed with the use of random primers (Taqman RT-PCR kit; Applied Biosystems, Foster City, CA) and amplified by ABI Prism TaqMan 7900 (Applied Biosystems), according to standard protocols. Target mRNA was quantified in relation to 18S ribosomal RNA abundance in each sample. Negative controls were: i) a negative reverse transcribed-sample (RNA template with no reverse transcriptase enzyme); ii) reverse tran-

scribed-H_2O (water in place of RNA template); and iii) a TaqMan-reaction negative control (complementary DNA replaced with H_2O). Positive controls were provided by mRNA that were extracted from endometrium.

Primer/probe combinations were designed with Primer Express software (Applied Biosystems) (Table 1). Sequences were validated by basic local alignment search tool (BLAST) searches to show that the amplified sequence was unique; the linearity of response was checked by the amplification of serially diluted complementary DNA samples and by plotting the corrected values against dilution.

Enzyme-linked immunosorbent assay

HBD2 and SLPI concentrations in cell culture medium were determined with the use of a commercially available HBD2 assay kit (PeproTech) and SLPI assay kit (HyCult, Uden, the Netherlands), according to manufacturer's protocol in 96-well assay plates (Nunc Maxi-sorp; Gibco). An assay for total cellular protein (Bio-Rad Laboratories, Hercules, CA) was performed on the remaining cells, according to manufacturer's protocols. The amount of total cellular protein was used as a denominator for HBD2 and SLPI levels to avoid errors that could result from variation in cell numbers.

Statistical analysis

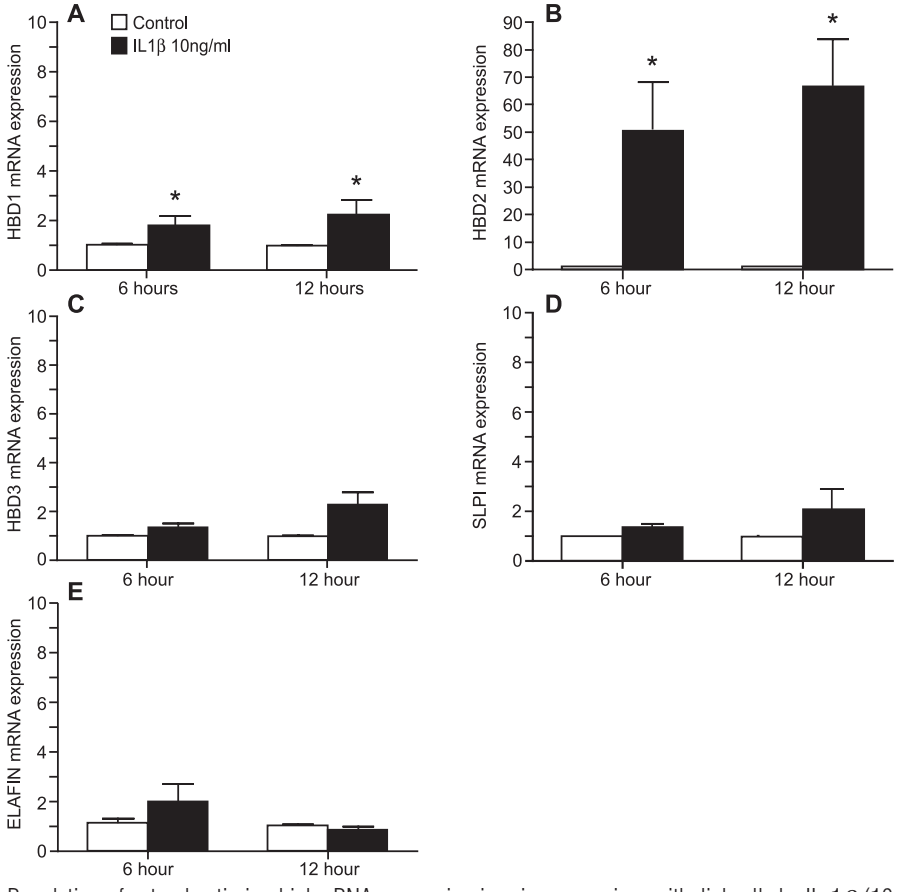
Analysis was carried out with the GraphPad Prism software package (GraphPad Software, Inc, San Diego, CA). Statistical significance was determined by 1-way analysis of variance with Tukey's test to assign individual differences. Nonparametric data were analyzed with the use of the Kruskal-Wallace test. A probability value of $<.05$ was regarded as significant.

RESULTS

Natural antimicrobial mRNA expression by primary cultured amnion epithelial cells

The expression of HBD1, HBD2, HBD3, SLPI, and elafin mRNA was detectable by reverse transcribed quantitative PCR in primary cultured amnion epithelial cells, whereas HBD4 was undetectable (n = 5). HBD1 mRNA was slightly upregulated by IL-1β 10 ng/mL treatment, which exhibited a 1.81-fold increase that was seen at 6 hours and a 2.24-fold increase that was seen at 12 hours ($P < .05$; Figure 1**A**). HBD2 mRNA was increased dramatically by IL-1β 10 ng/mL treatment, which exhibited a 51-fold increase at 6 hours and a 67-fold increase at 12 hours ($P < .001$; Figure 1**B**). Levels of HBD3, SLPI, and elafin mRNA were not affected significantly by IL-1β treatment (Figure 1**C-E**).

FIGURE 1
Natural antimicrobial expression in amniotic epithelial cells



Regulation of natural antimicrobial mRNA expression in primary amnion epithelial cells by IL-1β (10 ng/mL; n = 5). Data are presented as mean ± SEM, relative to untreated controls at 6 and 12 hours. **A**, HBD1 mRNA expression. The *asterisk* denotes a probability value of $<.05$ that was determined by analysis of variance. **B**, HBD2 mRNA expression. The *asterisk* denotes a probability value of $<.001$ that was determined by analysis of variance. **C**, HBD3 mRNA expression. **D**, SLPI mRNA expression. **E**, Elafin mRNA expression.

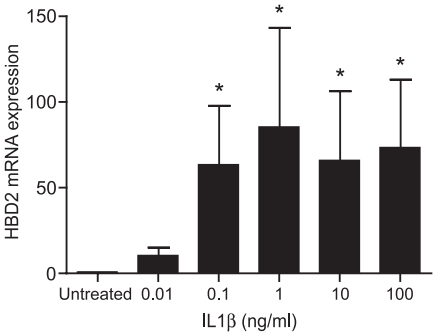
Dose responsive effect of IL-1β on HBD2 production

IL-1β exhibited a dose-responsive effect on HBD2 mRNA that was maximal at 1 ng/mL, which then plateaued ($P < .05$; Figure 2). Subsequent time course experiments used a dose of 10 ng/mL to ensure that sufficient protein was available throughout the whole experiment, despite any possible metabolism.

Pattern of HBD2 mRNA expression in primary cultured amnion epithelial cells

The pattern of HBD2 mRNA expression in response to IL-1β was examined over a 48-hour period in 5 different biologic samples. Treatment significantly upregulated HBD2 mRNA production compared with unstimulated control at all time points that were examined ($P < .05$; Figure 3**A**). There was no significant change in HBD2 mRNA

FIGURE 2
IL-1β dose response of HBD2



HBD2 mRNA expression IL-1β dose response in primary amnion epithelial cells. Data are presented as mean ± SEM, relative to untreated controls at 6 hours. Cells were treated with IL-1β (0.01 ng/mL, 0.1 ng/mL, 1 ng/mL, 10 ng/mL, or 100 ng/mL; n = 3). The *asterisk* denotes a probability value of <.05 determined by analysis of variance.

expression in unstimulated controls over the 48-hour period (Figure 3**B**). In all 5 samples, HBD2 mRNA was produced in a biphasic pattern (Figure 3C) with 2 peaks in expression. A typical response from 1 sample is shown, because the periodicity of the response varied between samples; thus the pattern was not as evident when data were averaged. The early response of HBD2 to IL-1β treatment was investigated in 4 samples. This showed that HBD2 mRNA expression became significantly upregulated after 3 hours of treatment with IL-1β 10 ng/mL ($P < .05$; Figure 3**D**).

Natural antimicrobial protein expression by primary cultured amnion epithelial cells

HBD2 and SLPI protein levels were measured in cell culture medium by sandwich enzyme-linked immunosorbent assays. Significantly higher levels of HBD2 protein were produced by cells that were treated with IL-1β 10 ng/mL for 24 and 48 hours ($P < .05$; Figure 4). We found no increase in SLPI levels (data not shown).

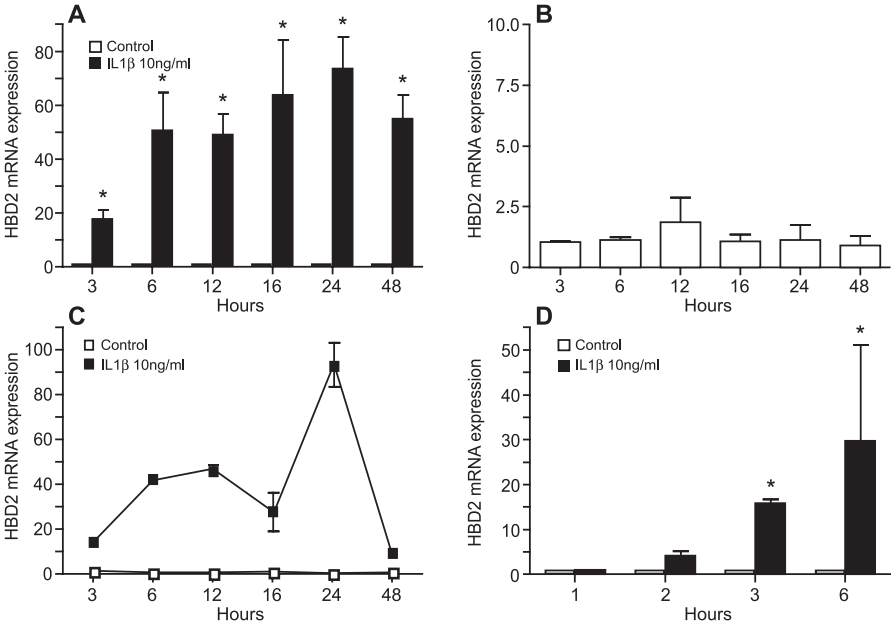
Natural antimicrobial mRNA expression by cell lines

Natural antimicrobial mRNA expression in primary amnion cells was significantly different from that in FL and WISH cell lines (Table 2). HBD2 mRNA was highly expressed in primary amnion cells but undetectable in FL and WISH cells. HBD1 and HBD3 were expressed at significantly higher levels in primary amnion cells than in FL and WISH cells ($P < .05$). Conversely, SLPI and elafin mRNA were significantly lower in primary amnion cells than in either FL or WISH cells ($P < .05$). Natural antimicrobial mRNA expression in FL and WISH cells was similar to that seen in the He-La cervical cell line that contaminates them, except for the observation that SLPI and elafin mRNA expression was slightly lower in FL cells than in He-La cells ($P < .05$).

COMMENT

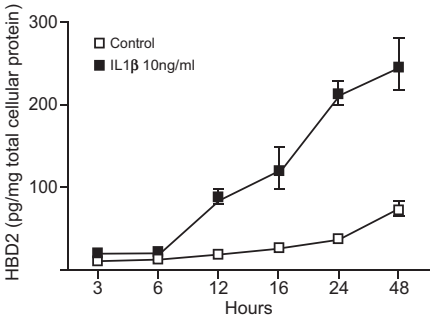
To our knowledge, this is the first study of natural antimicrobial production in primary cultured amnion epithelial cells and demonstrates the expression of HBD1, 2, and 3, SLPI, and elafin. This is consistent with findings in other studies that show HBD1-3, elafin, and SLPI im-

FIGURE 3
Pattern of HBD2 mRNA expression in response to IL-1β



HBD2 mRNA expression in response to IL-1β (10 ng/mL) in primary amnion epithelial cells over time. Data are presented as mean ± SEM. **A** HBD2 mRNA expression in primary amnion epithelial cells at 3 hours, 6 hours, 12 hours, 16 hours, 24 hours, and 48 hours (n = 5), relative to untreated control at the same time point. **B** HBD2 mRNA expression in unstimulated primary amnion epithelial cells at 3 hours, 6 hours, 12 hours, 16 hours, 24 hours, and 48 hours (n = 5), relative to 3-hour control. **C** Typical pattern of HBD2 mRNA expression in primary amnion epithelial cells (n = 1). **D** HBD2 mRNA expression in primary cultured amnion epithelial cells treated with IL-1β (10 ng/mL) at 1 hour, 2 hours, 3 hours, and 6 hours (n = 4), relative to untreated control at the same time point.

FIGURE 4
HBD2 protein production in response to IL-1β



HBD2 protein expression in response to IL-1β in primary amnion epithelial cells over 48 hours. Data represent mean ± SEM in picograms of HBD2 per milligram total cellular protein (n = 5).

TABLE 2
Comparison of natural antimicrobial mRNA expression in primary amnion cells (n = 5), FL cells (n = 3), WISH cells (n = 3), and He-La cells (n = 3)

	Cells			
Variable	Primary amnion	FL	WISH	He-La
HBD1	5.3 ± 2.0*	0.04 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
HBD2	4.0 ± 1.4*	Undetectable	Undetectable	Undetectable
HBD3	9.2 ± 2.9*	1.9 ± 0.6	1.1 ± 0.07	1.8 ± 0.6
SLPI	0.10 ± 0.01*	0.11 ± 0.01†	0.34 ± 0.06	0.20 ± 0.03
Elafin	0.05 ± 0.01*	0.21 ± 0.06‡	0.21 ± 0.06	0.40 ± 0.03

Data are presented as mean ± SEM, relative to the amount in an endometrial positive control.

* $P < .05$ between primary amnion cells and FL, WISH and He-La cells.

† $P < .05$ between FL cells and He-La and WISH cells.

‡ $P < .05$ between FL cells and He-La cells.

munopositivity in the amniotic epithelial layer[10,16,24] and SLPI and elafin mRNA expression in amnion tissue.[10,18]

IL-1β dramatically upregulates the production of HBD2 in primary amnion epithelial cells in a dose and time-dependent manner, which is evidenced at both mRNA and protein levels. Labor is associated with increased numbers of inflammatory cells and production of cytokines such as IL-1β.[1] This suggests that HBD2 production could be increased during parturition, which is a time when the fetus is particularly vulnerable to ascending infection. Because HBD2 is chemotactic for neutrophils[5] and immature dendritic cells and T cells,[6] it could also participate in the recruitment of inflammatory cells that are seen at this time.

Intrauterine infection is associated with an exaggerated inflammatory response and is a major cause of preterm labor.[2] IL-1β is implicated in this process, and women with preterm labor and amniotic infection have elevated amniotic fluid IL-1β concentrations and activity.[25] In this situation, HBD2 production may be upregulated and could act to limit the spread of infection. Because HBD2 mRNA is also stimulated by IL-1β in primary cultured chorion cells,[16] these adjacent tissues may act in tandem.

HBD2 mRNA production in response to IL-1β that showed a biphasic pattern was intriguing. This suggests that there are 2 distinct components to this response. The first peak in production occurs within a few hours and is typical of a rapid innate immune response. The second peak is slower and may represent upregulation that is controlled by a secondary gene product. This requires elucidation, and the production of HBD2 by the fetal membranes in normal and preterm labor settings is under further investigation.

HBD1 mRNA expression in primary amnion epithelial cells was also slightly upregulated by IL1β treatment, although this was only marginal and may not be biologically relevant. Until recently, it was accepted widely that HBD1 was produced constitutively by epithelia, whereas HBD2 was highly inducible by various cytokines, microbes, and bacterial products.[26] New studies, however, have demonstrated that HBD1-4 may all be produced in a constitutive or inducible manner, depending on the site of production and stimulus that is applied.[27] Defensin production in the amnion may also be influenced by different cytokines and inflammatory stimuli.

In this study, IL-1β did not influence amnion epithelial SLPI mRNA. SLPI protein expression was similarly unaffected by IL-1β treatment, which suggests that there is neither de novo production of SLPI nor secretion of preformed protein in response to IL-1β. This differs from findings by Zhang et al[10] that demonstrated an increase in SLPI protein production by primary amnion cells after 48 hours of stimulation with IL-1β. This may reflect differences in culture technique, because we allowed our cells to become confluent before treatment as opposed to stimulating them immediately after dissociation.

We found that IL-1β treatment had no significant effect on the expression of elafin mRNA in amnion epithelial cells, which is in contrast to the stimulatory effect of IL-1β on elafin mRNA production in chorion trophoblast cells.[16] In whole fetal membranes, elafin mRNA expression is decreased in cases of preterm membrane rupture but is increased in cases of chorioamnionitis.[18] Together this evidence suggests that the chorion provides the greater contribution to production of elafin, which may have an important role in the regulation of protease activity within the fetal membranes.

The pattern of natural antimicrobial expression in both FL and WISH cells markedly differed from that in primary cultured amnion epithelial cells. These findings show that cell lines are not representative of amnion and illustrate the care that must be taken when the results are extrapolated from commercial cell lines.

In summary, HBD2 is a potent natural antibiotic that is produced by amnion epithelial cells in response to IL-1β, which also interacts with the adaptive immune system. We believe that it may have an important role in protecting the fetus and could also be an important chemokine that is involved in the onset of parturition. Further study of this response may allow development of new strategies and treatments to help decrease the incidence of premature birth. ■

ACKNOWLEDGMENTS

We thank Professor Hilary Critchley for providing the endometrial messenger RNA for use as positive controls and Professor Phillip Bennett for helping with the technique of primary amnion culture.

REFERENCES

1. Keelan JA, Blumenstein M, Helliwell RJ, Sato TA, Marvin KW, Mitchell MD. Cytokines, prostaglandins and parturition: a review. Placenta 2003;24(suppl A):S33-46.

2. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med 2000;342:1500-7.
3. Bals R. Epithelial antimicrobial peptides in host defense against infection. Respir Res 2000;1:141-50.
4. Ganz T. Defensins: antimicrobial peptides of innate immunity. Nat Rev Immunol 2003;3: 710-20.
5. Niyonsaba F, Ogawa H, Nagaoka I. Human beta-defensin-2 functions as a chemotactic agent for tumor necrosis factor-alpha-treated human neutrophils. Immunology 2004;111:273-81.
6. Yang D, Chertov O, Bykovskaia SN, et al. Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science 1999;286:525-8.
7. Sallenave JM. Antimicrobial activity of antiproteinases. Biochem Soc Trans 2002;30: 111-5.
8. King AE, Fleming DC, Critchley HO, Kelly RW. Regulation of natural antibiotic expression by inflammatory mediators and mimics of infection in human endometrial epithelial cells. Mol Hum Reprod 2002;8:341-9.
9. Quayle AJ. The innate and early immune response to pathogen challenge in the female genital tract and the pivotal role of epithelial cells. J Reprod Immunol 2002;57:61-79.
10. Zhang Q, Shimoya K, Moriyama A, et al. Production of secretory leukocyte protease inhibitor by human amniotic membranes and regulation of its concentration in amniotic fluid. Mol Hum Reprod 2001;7:573-9.
11. Denison FC, Kelly RW, Calder AA, Riley SC. Secretory leukocyte protease inhibitor concentration increases in amniotic fluid with the onset of labor in women: characterization of sites of release within the uterus. J Endocrinol 1999; 161:299-306.
12. Akinbi HT, Narendran V, Pass AK, Markart P, Hoath SB. Host defense proteins in vernix caseosa and amniotic fluid. Am J Obstet Gynecol 2004;191:2090-6.
13. Espinoza J, Chaiworapongsa T, Romero R, et al. Antimicrobial peptides in amniotic fluid: defensins, calprotectin and bacterial/permeability-increasing protein in patients with microbial invasion of the amniotic cavity, intra-amniotic inflammation, preterm labor and premature rupture of membranes. J Matern Fetal Neonatal Med 2003;13:2-21.
14. Feng Y, Pan X, Huang N, Wu Q, Wang B. [The human beta-defensins expression in female genital tract and pregnancy-related tissues]. Sichuan Da Xue Xue Bao Yi Xue Ban 2003;34:217-9.
15. Zhao C, Wang I, Lehrer RI. Widespread expression of beta-defensin hBD-1 in human secretory glands and epithelial cells. FEBS Lett 1996;396:319-22.
16. King AE, Paltoo A, Kelly RW, Sallenave JM, Bocking AD, Challis JR. Expression of natural antimicrobials by human placenta and fetal membranes. Placenta 2007;28:161-9.
17. Buhimschi IA, Jabr M, Buhimschi CS, Petkova AP, Weiner CP, Saed GM. The novel antimicrobial peptide beta3-defensin is produced by the amnion: a possible role of the fetal membranes in innate immunity of the amniotic cavity. Am J Obstet Gynecol 2004;191:1678-87.
18. Tromp G, Kuivaniemi H, Romero R, et al. Genome-wide expression profiling of fetal membranes reveals a deficient expression of proteinase inhibitor 3 in premature rupture of membranes. Am J Obstet Gynecol 2004; 191:1331-8.
19. Nelson-Rees WA, Flandermeyer RR. HeLa cultures defined. Science 1976;191:96-8.
20. Kniss DA, Xie Y, Li Y, et al. ED(27) trophoblast-like cells isolated from first-trimester chorionic villi are genetically identical to HeLa cells yet exhibit a distinct phenotype. Placenta 2002;23:32-43.
21. King AE, Critchley HO, Kelly RW. Innate immune defenses in the human endometrium. Reprod Biol Endocrinol 2003;1:116-24.
22. Bennett PR, Rose MP, Myatt L, Elder MG. Preterm labor: stimulation of arachidonic acid metabolism in human amnion cells by bacterial products. Am J Obstet Gynecol 1987;156: 649-55.
23. McDermott AM, Redfern RL, Zhang B, Pei Y, Huang L, Proske RJ. Defensin expression by the cornea: multiple signaling pathways mediate IL-1beta stimulation of hBD-2 expression by human corneal epithelial cells. Invest Ophthalmol Vis Sci 2003;44:1859-65.
24. Buhimschi IA, Jabr M, Buhimschi CS, Petkova AP, Weiner CP, Saed GM. The novel antimicrobial peptide beta3-defensin is produced by the amnion: a possible role of the fetal membranes in innate immunity of the amniotic cavity. Am J Obstet Gynecol 2004;191:1678-87.
25. Romero R, Mazor M, Brandt F, et al. Interleukin-1 alpha and interleukin-1 beta in preterm and term human parturition. Am J Reprod Immunol 1992;27:117-23.
26. Krisanaprakornkit S, Weinberg A, Perez CN, Dale BA. Expression of the peptide antibiotic human beta-defensin 1 in cultured gingival epithelial cells and gingival tissue. Infect Immunol 1998;66:4222-8.
27. Joly S, Organ CC, Johnson GK, McCray PB Jr, Guthmiller JM. Correlation between beta-defensin expression and induction profiles in gingival keratinocytes. Mol Immunol 2005; 42:1073-84.