Chapter 2
Chorioamnionitis and Oxidative Stress: New Ideas from Experimental Models

Boris W. Kramer, Suhas G. Kallapur, and Alan H. Jobe

Introduction

Chorioamnionitis is commonly associated with preterm labor and preterm delivery [1]. Although chorioamnionitis can be diagnosed by clinical criteria [2] or by microbiology [3], the most accepted definition is histologic inflammation of the amnion/chorion/decidua [4]. Fetal inflammation is diagnosed by the presence of funisitis or chorionic vasculitis [4]. In addition to triggering preterm labor, chorioamnionitis is also associated with an increased risk for inflammatory diseases in the preterm infant including bronchopulmonary dysplasia, necrotizing enterocolitis [5], and injury to the white matter of the brain. Infection in chorioamnionitis is largely restricted to the amniotic compartment and is not systemically disseminated since only 5 % of preterm infants with histologic chorioamnionitis have early onset sepsis by organisms recovered in routine cultures [6]. However, a higher percentage is blood culture positive for genital mycoplasma and ureaplasma when selective culture methods are used [7, 8]. Therefore, inflammatory diseases of different fetal organs are thought to be mediated by fetal inflammatory response syndrome (FIRS) induced by chorioamnionitis with colonization of the fetus by bacteria in the amniotic fluid. FIRS is the systemic response of the fetus to chorioamnionitis, generally diagnosed clinically as inflammatory cells in the umbilical cord or increased cytokines/acute phase reactants in the cord blood [9, 10].

B.W. Kramer, M.D., Ph.D. (✉)
Department of Pediatrics, School of Oncology and Developmental Biology, School of Mental Health and Neuroscience, Maastricht University Medical Center, Maastricht, The Netherlands
e-mail: b.kramer@maastrichtuniversity.nl

S.G. Kallapur • A.H. Jobe
Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, OH, USA

P.A. Dennery et al. (eds.), *Perinatal and Prenatal Disorders*,
Oxidative Stress in Applied Basic Research and Clinical Practice,
DOI 10.1007/978-1-4939-1405-0_2

Oxidative stress can both cause and be induced by inflammation. Therefore, it is logical to ask "what is the contribution of oxidative stress to chorioamnionitis-induced fetal organ injury?" However, chorioamnionitis is a unique infection and inflammatory disorder: (1) the organisms causing chorioamnionitis have low virulence, e.g., ureaplasma/mycoplasma species [11]. (2) The FIRS from chorioamnionitis is compartmentalized in experimental models with the most affected organs being those that are in contact with the amniotic fluid – the lung, amnion/chorion, gut, and the skin [12–15]. Unlike the cytokine storm associated with adult systemic inflammatory response [16], FIRS is a low-grade inflammatory disease [17]. (3) Although chorioamnionitis can occur in term infants, it is most prevalent in preterm infants and therefore largely a disease of a developmentally immature host, and (4) since the host is a fetus with low ambient intrauterine oxygen tension, the oxidant load is much lower than that in post natal life. Not much is known about oxidant stress in human fetuses, and therefore this review will focus on data from animal models of chorioamnionitis.

Animal Models of Chorioamnionitis

Most chorioamnionitis in humans is an ascending infection, where the organisms in the lower genital area ascend in to the chorio-decidual space or the chorioamnion space through the cervix [18]. Organisms are thought to spread diffusely through the chorio-decidual or the chorioamnion plane and then invade the amniotic cavity. However, a recent study using molecular microbiologic techniques in human placentae demonstrated that the initial event is a localized chorio-decidual infection, which then invades locally into the amniotic cavity and thereby infecting amniotic fluid and the fetus prior to diffuse chorio-decidual inflammation [19]. This sequence is consistent with experiments in the Rhesus macaque demonstrating that localized chorio-decidual infection with live *Group B streptococci* did not trigger preterm labor until the amniotic fluid was colonized [20]. However, a transient chorio-decidual infection induced cytokine production in the amniotic fluid, which resulted in fetal lung inflammation without overt infection of amniotic fluid or preterm labor [21]. Therefore, animal models of chorioamnionitis resulting from injection of inflammatory agents or organisms into the amniotic fluid may reproduce the pathology of most cases of chorioamnionitis associated with preterm delivery. Models of chorioamnionitis have been described with intrauterine injection of proinflammatory agonists or live bacteria in the mouse [22, 23] and the rabbit [24–26]. Chorioamnionitis can also be induced by intra-amniotic injection in the sheep using proinflammatory agonists that include IL-1ß [27], IL-1α [27], LPS (ligand for TLR4) [28], and live *Ureaplasma parvum* [29]. In the Rhesus macaque, intra-amniotic injections of *Group B streptococci* [30], *Ureaplasma parvum* [31], IL-1ß [32, 33], or TNF [32] cause chorioamnionitis. Since most of the data on oxidants in chorioamnionitis are described in the preterm sheep model, we will focus on this experimental system.

Inflammation and Oxidants

The major endogenous sources of the reactive oxygen species are the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, mitochondrial respiratory chain, enzymatic activation of cytochrome p450, and others [34]. Although the general belief is that reactive oxygen species are invariably harmful, they also mediate important biological functions that are adaptive in nature [35]. NADPH oxidases are activated by insulin, platelet-derived growth factors and other growth factors, and proinflammatory molecules such as TNF, complement 5a, leukotriene B4, and others [34]. ROS can regulate the activity of NF-kB, prolyl hydroxylases which can in turn modulate the activity of hypoxia-inducible factor (HIF)-1α. Thus, ROS can regulate cell proliferation, survival, differentiation, and redox homeostasis [36]. The classic role of ROS in innate immunity is killing of microorganisms by phagocytes [37]. Accordingly, patients with chronic granulomatous disease, a disorder characterized by increased susceptibility to infections, have mutations in NOX_2, a subunit of NADPH oxidase, the main source of ROS in neutrophils and monocytes [38]. Further, ROS can also activate the inflammasome NLRP3 leading to production of IL-1ß [39]. ROS also regulate adaptive immune responses via their action in B-cells. Ca^{2+} and reactive oxygen intermediates generated upon B cell receptor activation rapidly engage in a cooperative interaction that acts in a feedback manner to amplify the early signal generated and thereby influence downstream pathways [40]. Therefore, ROS effects range from adaptive to maladaptive with tissue injury depending on the concentration of these molecules and host defense against the ROS. The ROS effects are regulated by suppression of pathways leading to ROS production, catabolism of ROS, and repair of ROS-mediated damage [35]. To what extent these mechanisms operate in the preterm are not understood but constitute an important area of future study.

Developmental Aspects of Oxidants/Antioxidants

Endogenously produced reactive oxygen species are part of the normal development and the homeostasis. The highly toxic superoxide radical O_2^- is, for example, produced by NAD(P)H oxidases. The balance between oxidants and antioxidants is however essential for the normal development, especially after preterm birth [41].

The antioxidant system in the fetus is not upregulated until very late in gestation. During the last 15 % of gestation, the antioxidant enzyme system is increasingly expressed [42, 43]. Nonenzymatic antioxidants also cross the placenta at this late gestation. The enzyme activity of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GP) increased with the development of the lungs in rabbit fetuses during late gestation. The expression of these enzymes increased in parallel with the surfactant synthesis in late gestation [44]. SOD and catalase are secreted into pulmonary surfactant probably to mediate protection of the lipid layer in the alveolus for gas exchange.

The function of SOD is to transform the highly toxic superoxide radical O_2^- into hydrogen peroxide (H_2O_2) and water. Hydrogen peroxide is then further converted to water by catalase, GP, and glutathione reductase. The enzyme activity increases by more than 150 % in the last 15 % of the gestation [44].

Gas exchange in the placenta is also causing oxidative stress for which SOD, catalase, and thioredoxin are locally expressed in gestation-dependent pattern. In human placenta, there was a positive association between the detection of mitochondrial manganese SOD mRNA and chorioamnionitis [45]. The functional implications are unclear.

Apparently, evolution does not prepare the fetus for the abrupt transition from the low oxygen environment in utero to air breathing with higher oxygen exposure and oxidative stress until very late in gestation. The treatment with antenatal maternal glucocorticoids matures the antioxidant enzymes in the preterm fetus which may be part of the beneficial effects of antenatal glucocorticoids [46, 47].

Chorioamnionitis and Oxidative Stress

There are limited data on oxidative stress to the fetus and the counter-regulatory antioxidant enzymes after exposure to chorioamnionitis in the preterm fetus. Intra-amniotic (IA) injection of *E. coli* LPS in preterm sheep at about 80 % gestation causes chorioamnionitis (Table 2.1). The predominant inflammation is in the fetal lung, although modest inflammation, immune modulation, and injury response is also seen in the ileum [48], skin [13], brain [49, 50], thymus [51], and liver [52]. The lung inflammation is maximally induced 1–2 days after IA LPS exposure with large increases in IL-1ß, IL-8, and IL-6 expression and a robust recruitment of neutrophils and monocytes to the lung [53, 54]. Compared to controls, protein carbonyls, markers of oxidative stress, increased by threefold in the airways of fetuses 7 days after exposure to IA LPS but not at 2 days after IA LPS [55]. Similar increases were also noted in myeloperoxidase levels in the airways 7 days after IA LPS [55]. Mirroring the increased oxidants in the lungs, protein carbonyls also increased in the plasma of fetuses 7 days after IA LPS but not at 2 days post LPS [55]. However, the increase in plasma protein carbonyls was more modest compared to the lung response. Since the maximal inflammation preceded increases in oxidants, the oxidant stress response appears to be a consequence rather than a cause of inflammation in this model. It should also be noted that these oxidant stress responses are modest compared to increases after postnatal hyperoxia.

A major mechanism to reduce oxidant damage is the antioxidant enzyme system. The major enzymes include superoxide dismutase, catalase, and glutathione peroxidase. In preterm sheep delivered at 125 days gestational age (80 % gestation) after 7 days exposure to IA LPS, the antioxidant enzyme activity of glutathione peroxidase, catalase, and superoxide dismutase increased in the fetal lung in a dose-dependent fashion [56]. Pulmonary glutathione peroxidase activity increased at 2 days post IA LPS, whereas superoxide dismutase increased by 4 days, and catalase

Table 2.1 Summary of results in sheep models of chorioamnionitis

Chorioamnionitis induced by	Organ	Readout	Reference
LPS intra-amniotically	Lung	Hydrogen peroxide production in cells from fetal airways	[53]
	Lung	Glutathione peroxidase activity increased within 2 days	[56]
		Catalase activity increased within 4 days	
		Superoxide dismutase activity increased within 7 days	
		No sustained effects after 15 days	
	Airways	Protein carbonyls increased after 7 days	[55]
		Myeloperoxidase increased after 7 days	
	Lung	No difference in protein carbonyls, superoxide dismutase, and peroxiredoxin 1 after 7 days	[55]
	Plasma	Protein carbonyl was increased after 7 days	[55]
Interleukin-1 alpha intra-amniotically	Lung	Superoxide dismutase activity, catalase activity, and glutathione peroxidase activity did not increase	[57]

activity increased by 7 days after IA LPS. The induction of antioxidant enzyme systems in the lung was short-lived since the values declined to near control levels 15 days after IA LPS [56]. However, antioxidant enzyme activity did not increase in the fetal sheep lung after exposure to IA IL-1 alpha, although the lung inflammatory response is roughly equivalent to LPS responses [57]. These experiments suggest a time-dependent and ligand-dependent antioxidant enzyme induction in the preterm sheep in different chorioamnionitis models.

Interactive Phenomenon Between Antenatal Inflammation and Postnatal Oxidative Stress

Postnatal hyperoxia inhibits alveolar development in the developing neonatal lungs of mice [58]. Antenatal inflammation in the preterm sheep also inhibits alveolar development, although the effects are milder [59]. Similar changes of inhibited alveolar development were also demonstrated in the rats exposed to IA LPS [60]. Interestingly, IA-induced aberrant lung development was reversed after moderate but not severe postnatal hyperoxia [60]. These results demonstrate the complex interactions between chorioamnionitis or antenatal inflammation and injury resulting from postnatal hyperoxia. In these experiments, the oxidant stress response was not measured, and therefore it is not clear whether the effects were attributable to oxidative lung damage or other mechanisms leading to lung injury. These interactive phenomena are reminiscent of other experiments showing adaptive effects of oxygen exposure.

Adult rats exposed to 95 % oxygen for 7 days had 100 % mortality [61]. However, if the rats were exposed to 95 % oxygen for 2 days followed by 1 day of either exposure to room air or 50–75 % oxygen followed by exposure to 95 % oxygen for continued periods, the mortality was prevented [61]. This tolerance to hyperoxia after exposure to a sublethal oxygen exposure was attributed to increased pulmonary antioxidant enzyme activity [61]. These experiments demonstrate that inflammation and oxidative stress responses can either potentiate injury responses or can cause tolerance leading to reduced organ injury response. Preterm infants are exposed to multiple injurious insults both antenatally and postnatally, and the net result on organ injury can be unpredictable based on the animal studies. The magnitude of the effects of oxidants from chorioamnionitis to fetal organ injury seems to be much less than that following preterm birth and oxygen exposure.

References

1. Goldenberg RL, et al. Infection-related stillbirths. Lancet. 2010;375(9724):1482–90.
2. Tita AT, Andrews WW. Diagnosis and management of clinical chorioamnionitis. Clin Perinatol. 2010;37(2):339–54.
3. DiGiulio DB, et al. Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. PLoS One. 2008;3(8):e3056.
4. Andrews WW, et al. The Alabama Preterm Birth study: polymorphonuclear and mononuclear cell placental infiltrations, other markers of inflammation, and outcomes in 23- to 32-week preterm newborn infants. Am J Obstet Gynecol. 2006;195(3):803–8.
5. Been JV, et al. Chorioamnionitis as a risk factor for necrotizing enterocolitis: a systematic review and meta-analysis. J Pediatr. 2013;162(2):236–42 e2.
6. Strunk T, et al. Histologic chorioamnionitis is associated with reduced risk of late-onset sepsis in preterm infants. Pediatrics. 2012;129(1):e134–41.
7. Viscardi RM. Ureaplasma species: role in diseases of prematurity. Clin Perinatol. 2010; 37(2):393–409.
8. Goldenberg RL, et al. The Alabama Preterm Birth Study: umbilical cord blood Ureaplasma urealyticum and Mycoplasma hominis cultures in very preterm newborn infants. Am J Obstet Gynecol. 2008;198(1):43 e1–5.
9. Redline RW, et al. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. Pediatr Dev Pathol. 2003;6(5):435–48.
10. Gomez R, et al. The fetal inflammatory response syndrome. Am J Obstet Gynecol. 1998; 179:194–202.
11. Onderdonk AB, et al. Detection of bacteria in placental tissues obtained from extremely low gestational age neonates. Am J Obstet Gynecol. 2008;198(1):110 e1–7.
12. Kemp MW, et al. Inflammation of the fetal ovine skin following in utero exposure to Ureaplasma parvum. Reprod Sci. 2011;18(11):1128–37.
13. Kemp MW, et al. Exposure to in utero lipopolysaccharide induces inflammation in the fetal ovine skin. Reprod Sci. 2011;18(1):88–98.
14. Kramer BW, et al. Modulation of fetal inflammatory response on exposure to lipopolysaccharide by chorioamnion, lung, or gut in sheep. Am J Obstet Gynecol. 2010;202(1):77 e1–9.
15. Newnham JP, et al. The fetal maturational and inflammatory responses to different routes of endotoxin infusion in sheep. Am J Obstet Gynecol. 2002;186(5):1062–8.
16. Matsuda N, Hattori Y. Systemic inflammatory response syndrome (SIRS): molecular pathophysiology and gene therapy. J Pharmacol Sci. 2006;101(3):189–98.

17. Gotsch F, et al. The fetal inflammatory response syndrome. Clin Obstet Gynecol. 2007;50(3):652–83.
18. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med. 2000;342(20):1500–7.
19. Kim MJ, et al. Widespread microbial invasion of the chorioamniotic membranes is a consequence and not a cause of intra-amniotic infection. Lab Invest. 2009;89(8):924–36.
20. Grigsby PL, et al. Choriodecidual inflammation: a harbinger of the preterm labor syndrome. Reprod Sci. 2010;17(1):85–94.
21. Adams Waldorf KM, et al. Choriodecidual group B streptococcal inoculation induces fetal lung injury without intra-amniotic infection and preterm labor in Macaca nemestrina. PLoS One. 2011;6(12):e28972.
22. Hirsch E, Saotome I, Hirsh D. A model of intrauterine infection and preterm delivery in mice. Am J Obstet Gynecol. 1995;172(5):1598–603.
23. Elovitz MA, et al. Intrauterine inflammation, insufficient to induce parturition, still evokes fetal and neonatal brain injury. Int J Dev Neurosci. 2011;29(6):663–71.
24. Davies JK, et al. Histologic inflammation in the maternal and fetal compartments in a rabbit model of acute intra-amniotic infection. Am J Obstet Gynecol. 2000;183(5):1088–93.
25. Kannan S, et al. Microglial activation in perinatal rabbit brain induced by intrauterine inflammation: detection with 11C-(R)-PK11195 and small-animal PET. J Nucl Med. 2007;48(6): 946–54.
26. Yoon BH, et al. Experimentally induced intrauterine infection causes fetal brain white matter lesions in rabbits. Am J Obstet Gynecol. 1997;177(4):797–802.
27. Willet KE, et al. Intra-amniotic injection of IL-1 induces inflammation and maturation in fetal sheep lung. Am J Physiol Lung Cell Mol Physiol. 2002;282:L411–20.
28. Jobe AH, et al. Effects of antenatal endotoxin and glucocorticoids on the lungs of preterm lambs. Am J Obstet Gynecol. 2000;182:401–8.
29. Moss TJ, et al. Intrauterine Ureaplasma infection accelerates fetal lung maturation and causes growth restriction in sheep. Am J Obstet Gynecol. 2005;192:1179–86.
30. Gravett MG, et al. Fetal and maternal endocrine responses to experimental intrauterine infection in rhesus monkeys. Am J Obstet Gynecol. 1996;174(6):1725–31; discussion 1731–3.
31. Novy MJ, et al. Ureaplasma parvum or Mycoplasma hominis as sole pathogens cause chorioamnionitis, preterm delivery, and fetal pneumonia in rhesus macaques. Reprod Sci. 2009; 16(1):56–70.
32. Sadowsky DW, et al. Preterm labor is induced by intraamniotic infusions of interleukin-1beta and tumor necrosis factor-alpha but not by interleukin-6 or interleukin-8 in a nonhuman primate model. Am J Obstet Gynecol. 2006;195(6):1578–89.
33. Kallapur SG, et al. Intra-amniotic IL-1ß induces fetal inflammation in rhesus monkeys and alters the regulatory T cell/IL-17 balance. J Immunol. 2013;191:1102–9.
34. Nathan C, Cunningham-Bussel A. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. Nat Rev Immunol. 2013;13(5):349–61.
35. Buonocore G, Perrone S, Tataranno ML. Oxygen toxicity: chemistry and biology of reactive oxygen species. Semin Fetal Neonatal Med. 2010;15(4):186–90.
36. Bae YS, et al. Regulation of reactive oxygen species generation in cell signaling. Mol Cells. 2011;32(6):491–509.
37. Sbarra AJ, Karnovsky ML. The biochemical basis of phagocytosis. I. Metabolic changes during the ingestion of particles by polymorphonuclear leukocytes. J Biol Chem. 1959;234(6): 1355–62.
38. Roos D, et al. Hematologically important mutations: X-linked chronic granulomatous disease (third update). Blood Cells Mol Dis. 2010;45(3):246–65.
39. Zhou R, et al. A role for mitochondria in NLRP3 inflammasome activation. Nature. 2011;469(7329):221–5.
40. Singh DK, et al. The strength of receptor signaling is centrally controlled through a cooperative loop between Ca2+ and an oxidant signal. Cell. 2005;121(2):281–93.

41. Davis JM, Auten RL. Maturation of the antioxidant system and the effects on preterm birth. Semin Fetal Neonatal Med. 2010;15(4):191–5.
42. Sosenko IR, Nielsen HC, Frank L. Lack of sex differences in antioxidant enzyme development in the fetal rabbit lung. Pediatr Res. 1989;26(1):16–9.
43. Frank L, Groseclose EE. Preparation for birth into an O2-rich environment: the antioxidant enzymes in the developing rabbit lung. Pediatr Res. 1984;18(3):240–4.
44. Matalon S, et al. Characterization of antioxidant activities of pulmonary surfactant mixtures. Biochim Biophys Acta. 1990;1035(2):121–7.
45. Than NG, et al. Mitochondrial manganese superoxide dismutase mRNA expression in human chorioamniotic membranes and its association with labor, inflammation, and infection. J Matern Fetal Neonatal Med. 2009;22(11):1000–13.
46. Walther FJ, Jobe AH, Ikegami M. Repetitive prenatal glucocorticoid therapy reduces oxidative stress in the lungs of preterm lambs. J Appl Physiol. 1998;85(1):273–8.
47. Been JV, et al. Antenatal steroids and neonatal outcome after chorioamnionitis: a meta-analysis. BJOG. 2011;118(2):113–22.
48. Wolfs TG, et al. Endotoxin induced chorioamnionitis prevents intestinal development during gestation in fetal sheep. PLoS One. 2009;4(6):e5837.
49. Nitsos I, et al. Chronic exposure to intra-amniotic lipopolysaccharide affects the ovine fetal brain. J Soc Gynecol Investig. 2006;13(4):239–47.
50. Gavilanes AW, et al. Chorioamnionitis induced by intraamniotic lipopolysaccharide resulted in an interval-dependent increase in central nervous system injury in the fetal sheep. Am J Obstet Gynecol. 2009;200(4):437 e1–8.
51. Kuypers E, et al. Ovine fetal thymus response to lipopolysaccharide-induced chorioamnionitis and antenatal corticosteroids. PLoS One. 2012;7(5):e38257.
52. Wilson TC, et al. Pulmonary and systemic induction of SAA3 after ventilation and endotoxin in preterm lambs. Pediatr Res. 2005;58(6):1204–9.
53. Kramer BW, et al. Dose and time response for inflammation and lung maturation after intra-amniotic endotoxin in preterm lambs. Am J Respir Crit Care Med. 2001;164:982–8.
54. Kallapur SG, et al. Intra-amniotic endotoxin: chorioamnionitis precedes lung maturation in preterm lambs. Am J Physiol Lung Cell Mol Physiol. 2001;280(3):L527–36.
55. Cheah FC, et al. Oxidative stress in fetal lambs exposed to intra-amniotic endotoxin in a chorioamnionitis model. Pediatr Res. 2008;63(3):274–9.
56. Sosenko IR, Jobe AH. Intraamniotic endotoxin increases lung antioxidant enzyme activity in preterm lambs. Pediatr Res. 2003;53(4):679–83.
57. Sosenko IR, et al. IL-1alpha causes lung inflammation and maturation by direct effects on preterm fetal lamb lungs. Pediatr Res. 2006;60(3):294–8.
58. Warner B, et al. Functional and pathological effects of prolonged hyperoxia in neonatal mice. Am J Physiol. 1998;275:L110–7.
59. Willet KE, et al. Antenatal endotoxin and glucocorticoid effects on lung morphometry in preterm lambs. Pediatr Res. 2000;48:782–8.
60. Tang JR, et al. Moderate postnatal hyperoxia accelerates lung growth and attenuates pulmonary hypertension in infant rats after exposure to intra-amniotic endotoxin. Am J Physiol Lung Cell Mol Physiol. 2010;299(6):L735–48.
61. Frank L, et al. New “rest period” protocol for inducing tolerance to high O2 exposure in adult rats. Am J Physiol. 1989;257(4 Pt 1):L226–31.

http://www.springer.com/978-1-4939-1404-3

Perinatal and Prenatal Disorders
Dennery, P.A.; Buonocore, G.; Saugstad, O.D. (Eds.)
2014, XIV, 435 p. 67 illus., 28 illus. in color., Hardcover
ISBN: 978-1-4939-1404-3
A product of Humana Press