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Activation of NF-κB in Placentas of Women with Preeclampsia

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Abstract

Objective—Placentas are oxidatively stressed during preeclampsia and produce more TNFα and more thromboxane (TX) than normal. Oxidative stress may cause these abnormalities by activating NF-κB. We measured levels of activated NF-κB in normal and preeclamptic placentas and determined whether oxidative stress activates NF-κB in a trophoblast-like cell line.

Methods—We used immunohistochemistry to determine the percentage of the total tissue area that stained for the p65 subunit of NF-κB in placentas obtained from normal and preeclamptic pregnancies. In a second set of experiments, we used a reporter plasmid bearing the NF-κB binding site and transfected it into trophoblast-like cells. The cells were incubated with medium control, linoleic acid (LA), an oxidizing solution (Ox), or Ox enriched with linoleic acid (OxLA), TNFα, or OxLA plus TNFα for 20 hours. Cell lysates were analyzed using a dual luciferase assay kit.

Results—Placentas obtained from women with preeclampsia showed nearly a 10-fold increase in the extent of area stained for activated NF-κB as compared to normal placentas. In cell culture experiments, Ox and OxLA induced a 3-fold increase in NF-κB activation as compared to medium control or LA. TNFα induced a 3-fold increase in NF-κB activation. The combination of TNFα with OxLA caused a 10-fold increase in NF-κB activation.

Conclusions—Placental NF-κB is activated nearly 10-fold in preeclampsia. Oxidative stress causes NF-κB activation in a trophoblast-like cell line, which is enhanced by TNFα. These data suggest that oxidative stress is likely an important *in vivo* activator of placental NF-κB in preeclampsia.

Keywords

Nuclear factor-κB (NF-κB); trophoblast; placenta; oxidative stress; tumor necrosis factor α (TNFα)

INTRODUCTION

Nuclear factor-κB (NF-κB), a transcription factor involved in inflammatory responses, is bound to a family of inhibitory IkappaB (IκB) proteins in the cytosol [1, 2]. Many stimuli activate NF-κB, including cytokines and oxidants [3]. Upon activation, NF-κB subunits (e.g., p65, p50) translocate to the nucleus and regulate the expression of a variety of inflammatory genes, including those that encode cytokines, such as TNFα, cytokine receptors, cyclooxygenase-2 (COX-2), growth factors, and cell adhesion molecules [1–3].

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NF-κB is activated in a wide range of human diseases that are associated with increased oxidative stress and inflammation. These include AIDS, atherosclerosis, rheumatoid arthritis, osteoporosis, Alzheimer's disease and ischemia-reperfusion injuries [3]. Reactive oxygen species (ROS) induce NF-κB activation [4–6]. In contrast, antioxidants inhibit NF-κB activation [7].

In preeclampsia, placentas are under oxidative stress and produce more lipid peroxides, more TNFα, and more of the COX-2 metabolite, thromboxane, than normal [8–11]. Considering that NF-κB regulates the expression of genes for TNFα and COX-2, and that preeclampsia is characterized by oxidative stress, we hypothesized that 1) NF-κB activation is abnormally elevated in preeclamptic placentas compared to normal placentas, and 2) trophoblast cells show increased NF-κB activation under conditions of oxidative stress.

To test our first hypothesis we used a polyclonal antibody specific for the activated form of NF-κB to immunostain normal and preeclamptic placental tissue. To evaluate our second hypothesis, we transfected a continuous line of trophoblast-like cells, ED$_{27}$, with a reporter plasmid bearing the NF-κB binding site linked to the firefly luciferase gene. The cells were then incubated in the presence or absence of oxidative stress or TNFα and assayed for activity of luciferase. We report herein that 1) the degree of NF-κB activation in preeclamptic placentas is significantly greater than in normal placentas, and 2) oxidative stress and TNFα dramatically increase NF-κB activation in trophoblast-like cells, but when oxidative stress and TNFα are combined, there is an enhanced effect.

METHODS

Study Subjects

Placentas were collected at MCV Hospitals, Virginia Commonwealth University Medical Center immediately after delivery from 4 patients with normal pregnancy and 5 patients with preeclampsia (4 with severe and 1 with mild preeclampsia). Informed consent was obtained prior to delivery. The Office of Research Subjects Protection of Virginia Commonwealth University approved this study. Exclusion criteria were infection, active STDs, chorioamnionitis, diabetes, and smoking. Preeclampsia was defined as blood pressures of 140/90 mmHg on two separate readings six hours apart, proteinuria (0.3 gm/24 hours or 2+ urine dipstick). Normal pregnancy was defined as blood pressures <140/90 mmHg and no proteinuria.

Immunohistochemistry

Pieces of villous tissue were cut from placentas immediately after delivery and were stained for the p65 subunit of NF-κB as previously described [12]. Slides were processed by hand and tissue was lightly counterstained with Hematoxylin. Diaminobenzidine reagent resulted in brown staining of antigens. An Olympus BH2 microscope with a Color 5 digital camera linked to IP Lab software (Scanalytics, Fairfax, VA) was used to digitize five different field of view regions at 400X magnification for each slide. Stained area and total tissue area for each image were quantified using IP Lab and results are reported as % area stained of total tissue area in field of view.

Cell Culture

ED$_{27}$ cells are an immortalized line of trophoblast-like cells. The cells were a gift from Douglas A. Kniss, Ph.D. of the Department of Obstetrics and Gynecology, Ohio State University, OH. The ED$_{27}$ cell line manifests many of the phenotypic properties that have served as the benchmark for trophoblast cells isolated from villous tissue obtained at term using conventional methods. For example, they express markers of both extracellular and

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villous cytotrophoblast cells, they synthesize estradiol and progesterone, and they stain positive for the α and β subunits of human chorionic gonadotropin (hCG) and placental alkaline phosphatase. When treated with dexamethasone, the cells secrete hCG which is a hormone produced by syncytiotrophoblast cells during pregnancy. The cells stain positive for cytokeratin peptide 8, but not for vimentin or von Willebrand factor (Factor VIII). The cells were originally described as a spontaneously derived continuous line of cytotrophoblast cells, but Kniss et al. [13] subsequently reported that at some point, the date of which is unknown, the ED_{27} cells acquired HeLa cell genetic markers, most likely by contamination with amnion-derived WISH cells which contain HeLa chromosome markers. Despite this contamination, the morphology and phenotypic properties of the ED_{27} cell line diverge in many key elements from HeLa and WISH cells. The ED_{27} cell line, even though containing HeLa cell markers, continues to bear the phenotype of authentic trophoblasts. The trophoblast-like phenotype has remained stable since their origin making them a useful model for the study of trophoblast function.

Luciferase Reporter Plasmids

To examine the activation of NF-κB in ED_{27} cells, transient transfection studies were done with a pGL3 luciferase reporter plasmid containing the NF-κB binding region of the human IL-8 promoter upstream from the firefly luciferase gene, as previously described [14]. The identity of the NF-κB binding site on BF^2 was confirmed using a BF^2 mutant that was created by site directed mutagenesis. For validation, negative controls received plasmid bearing the luciferase gene, but lacking an upstream promoter (pGL3-basic), while positive controls received a plasmid (pGL3-SV40) bearing the strong viral promoter SV40. pRL-TK which contains a weak herpes simplex virus thymidine kinase promoter region upstream from Renilla luciferase gene was co-transfected as a control for the transfection procedure. pRL-TK produced a low level of luminescence.

Transient Transfection Protocol

ED_{27} cells were plated in 24-well cell culture plates at a density of 40,000 cells/well and grown for 24 hours in Dulbecco's Modified Eagles Medium/Ham's F-12 (DMEM/F-12, Gibco, Grand Island, NY) supplemented with 15% fetal bovine serum (FBS, BioWhitaker, Walkersville, MD) to 50% confluence. Cells were transfected with control and reporter plasmids using SuperFect (Qiagen, Valencia, CA) for 2 hours. Cells were washed with phosphate buffered saline and then incubated for 20 hours with DMEM-F12/15% FBS alone, or with DMEM-F12/15% FBS containing either linoleic acid alone (LA, 45 μM), an oxidizing solution composed of hypoxanthine (3.6 mM) plus xanthine oxidase (0.005 U/ml) plus ferrous sulfate, 50μM, (Ox), Ox enriched with linoleic acid, 45μM, (OxLA), TNFα (2 ng/mL), or OxLA plus TNFα. Each treatment was done in triplicate. At the end of the 20 hour incubation the medium was removed from each well and passive lysis buffer (Promega, Madison, WI) was added.

Dual Luciferase Reporter (DLR) Assay

The luciferase content in the freshly lysed ED_{27} cells was measured using a commercially available DLR assay (Promega, Madison, WI). Firefly and Renilla luminescence were measured sequentially for each sample in a luminometer for 20 seconds (Berthold, Lumat LB9501, Germany). Results are expressed as fold change from cells receiving treatments (i.e. LA, Ox, OxLA, TNFα, or OxLA plus TNFα) relative to media control cells.

Statistical Analysis

Experimental and patient data were analyzed using a statistical computer software program (Graph Pad Prism 4.0 for Macintosh, Graph Pad Software, Inc., San Diego, CA,

www.graphpad.com). Unpaired t-test or Mann Whitney test was used to assess a statistical difference between two means. One-way ANOVA with Newman-Keul's post-hoc test was used to determine the statistical difference for more than 2 means. A probability level of $p < 0.05$ was considered to be statistically significant. Data are presented as mean ± SE.

RESULTS

Study Subjects

Three of the preeclamptic patients and none of the normal patients were primiparous. All patients were delivered by caesarean section except one in the preeclamptic group. The average systolic blood pressure for preeclamptic patients was 162 ± 8 mmHg and for normal patients was 125 ± 7 mm Hg ($p < 0.01$). The average diastolic blood pressure for preeclamptic patients was 94 ± 4 mmHg and for normal patients was 66 ± 1 mmHg ($p < 0.01$). Preeclamptic mothers averaged 24.5 ± 2.6 years of age and normal mothers 31.3 ± 4.8 years. Preeclamptic patients delivered at 31.0 ± 1.7 weeks of gestation and the normal patients at 39.3 ± 0.3 weeks ($p < 0.01$). Newborn weight was 1919 ± 430 g for preeclamptic patients and 3495 ± 279 g for normal patients ($p < 0.05$).

Immunohistochemistry

Both normal (n = 4) and preeclamptic (n = 5) placental tissues showed visible staining for NF-κB (Figure 1B–D). Whereas normal placental tissue displayed very low levels of staining for activated NF-κB, tissue from preeclamptic placentas stained intensely. The syncytiotrophoblast cells of preeclamptic placentas stained most intensely for NF-κB. Cytotrophoblast cells also stained. There was light to moderate staining of cells in the villous core (possibly tissue macrophages) and sometimes staining of leukocytes in placental vessels of preeclamptic placentas. Staining was evident in the nuclei, demonstrating activation of NF-κB (Panel D). When the area of NF-κB staining was quantified, preeclamptic placentas had nearly ten times the area stained of normal placentas (Figure 2: 17.6 ± 5.2 vs 1.9 ± 0.8 % area stained of total tissue area, $p < 0.05$). The placenta from the mild preeclamptic patient had less staining compared to those from patients with severe preeclampsia.

DLR Assay

Non-transfected cells and cells transfected with either pGL3 basic or the BF^2 mutant, did not exhibit any appreciable firefly luciferase activity in the presence or absence of OxLA (Figure 3). Cells that received the positive control, pGL3-SV40, showed strong luminescence (data not shown). The independent effects of LA, Ox, OxLA, and TNFα on NF-κB-induced firefly luciferase production relative to DMEM/F-12 medium control are shown in Figure 3 (n = 5). LA had no effect on NF-κB activation. In contrast, OxLA strongly activated NF-κB (3.5 ± 0.3 fold change $p < 0.01$). Ox was as effective as OxLA at activating NF-κB (5.1 ± 0.5 fold change, $p < 0.001$). TNFα also significantly increased NF-κB activation to the same extent as OxLA (3.7 ± 0.6 fold change, $p < 0.01$). The combination of OxLA plus TNFα further increased NF-κB activation both compared to medium control (10.4 ± 1.0 fold change, $p < 0.001$) and to the individual effects of OxLA and TNFα ($p < 0.001$).

DISCUSSION

Inflammation and oxidative stress play important roles in preeclampsia, so we hypothesized that the transcription factor NF-κB would be activated in placentas obtained from women with preeclampsia. We demonstrate herein that NF-κB activation is significantly increased in preeclamptic placentas compared to normal placentas. This finding corroborates prior

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work using immunohistochemistry and western blot analysis that showed increased activation of NF-κB in placentas from preeclamptic patients[15]. Other works have also implicated NF-κB in preeclampsia in leukocytes and vascular tissue [12, 16–18]. The increase in placental NF-κB activation is important because NF-κB alters the expression of many of the genes whose products are elevated in preeclampsia and that contribute to the pathogenesis of the disorder. It is unlikely that increased NF-κB activation in preeclamptic placentas was due to the lower average gestational age because in normal pregnancy NF-κB activity is not significantly increased until the onset of labor[19]. Placental NF-κB activation in preeclampsia could explain elevated placental levels of TNFα [11], COX-2 [20] and thromboxane [8].

In the present study, we examined the effects of oxidative stress on NF-κB activation in an immortalized line of trophoblast-like cells. Our results support the hypothesis that placental oxidative stress in preeclampsia could result in the activation of NF-κB by demonstrating that Ox and OxLA strongly activate NF-κB in ED_{27} cells. We previously reported that oxidative stress induces other changes in ED_{27} cells that mimic abnormalities of preeclamptic placentas. For example, OxLA treatment increased lipid peroxidation in ED_{27} cells while decreasing the activities of superoxide dismutase and glutathione peroxidase [21]. Increased lipid peroxidation and decreased antioxidant protection are prominent features of preeclamptic placentas [22, 23].

TNFα also activated NF-κB and had an additive effect to activate NF-κB when combined with OxLA. This may be significant because TNFα is an inflammatory gene whose expression is increased by NF-κB [2]. Therefore, placental oxidative stress in preeclampsia may set in motion a positive feedback loop between NF-κB and TNFα that progressively worsens inflammation in the placenta.

Inflammation in preeclampsia is not limited to the placenta. We recently demonstrated significant neutrophil infiltration into the maternal systemic vasculature in preeclampsia [24]. This infiltration was associated with inflammation of the endothelium and vascular smooth muscle as indicated by increased expression of IL-8 and ICAM-1 in the endothelium and vascular smooth muscle, as well as in the neutrophils themselves. We subsequently reported that neutrophil infiltration into the maternal vasculature is associated with NF-κB activation [12].

We chose linoleic acid as a fatty acid of concern for our study because: i) linoleic acid is significantly higher than normal early in pregnancy in women who later develop preeclampsia [25, 26]; ii) it is significantly higher than normal in women with preeclampsia [25, 27]; and iii) it is higher in the diet of women who develop preeclampsia [28]. The concentrations of linoleic acid that we used are similar to those present in preeclamptic women [27], and we previously demonstrated that OxLA induces lipid peroxidation in ED_{27} cells as evidenced by elevated thiobarbituric acid reactive substances concentrations [21]. The observation that NF-κB activation was just as great with Ox as it was for OxLA suggests ROS as the mediator of NF-κB activation rather than lipid peroxidation.

The additive effect of OxLA and TNFα to activate NF-κB is important in the context of preeclampsia because both circulating and placental levels of oxidized lipids [23] and TNFα [11, 29] are elevated. Marked NF-κB activation is consistent with many of the features of preeclampsia related to inflammation, such as elevated levels of TNFα [11, 29], IL-6 [30, 31], IL-8 [24, 32], COX-2 [12, 20], cytosolic phospholipase A_2 [33, 34], intercellular adhesion molecule-1 [24, 35, 36], and vascular cell adhesion molecule-1 [37].

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In conclusion, placental levels of activated NF-κB are significantly higher in preeclampsia than normal pregnancy. Increased placental oxidative stress and TNFα production in preeclampsia may be responsible for the increased activation of NF-κB.

Acknowledgments

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References

1. Baldwin AS. The NF-kB and IkB proteins: New discoveries and insights. Annu Rev Immunol. 1996; 14:649–681. [PubMed: 8717528]
2. Barnes PJ, Karin M. Nuclear Factor-kB: A pivotal transcription factor in chronic inflammatory diseases. New Engl J Med. 1997; 336:1066–1071. [PubMed: 9091804]
3. Winyard PG, Blake DR. Antioxidants, redox-regulated transcription factors, and inflammation. Adv Pharmacol. 1997; 38:403–421. [PubMed: 8895818]
4. Schreck R, Rieber P, Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. Embo J. 1991; 10:2247–2258. [PubMed: 2065663]
5. Los M, Droge W, Stricker K, Baeuerle PA, Schulze-Osthoff K. Hydrogen peroxide as a potent activator of T lymphocyte functions. Eur J Immunol. 1995; 25:159–165. [PubMed: 7843227]
6. Parhami F, Fang ZT, Fogelman AM, Andalibi A, Territo MC, Berliner JA. Minimally modified low density lipoprotein-induced inflammatory responses in endothelial cells are mediated by cyclic adenosine monophosphate. J Clin Invest. 1993; 92:471–478. [PubMed: 8392092]
7. Schreck R, Albermann K, Baeuerle PA. Nuclear factor kappa B: an oxidative stress-responsive transcription factor of eukaryotic cells (a review). Free Radic Res Commun. 1992; 17:221–237. [PubMed: 1473734]
8. Walsh SW. Preeclampsia: An imbalance in placental prostacyclin and thromboxane production. Am J Obstet Gynecol. 1985; 152:335–340. [PubMed: 3923838]
9. Walsh SW, Wang Y. Trophoblast and placental villous core production of lipid peroxides, thromboxane, and prostacyclin in preeclampsia. J Clin Endocrinol Metab. 1995; 80:1888–1893. [PubMed: 7775637]
10. Walsh SW, Wang Y, Jesse R. Placental production of lipid peroxides, thromboxane, and prostacyclin in preeclampsia. Hypertens Pregnancy. 1996; 15:101–111.
11. Wang Y, Walsh SW. TNFa concentrations and mRNA expression are increased in preeclamptic placentas. J Reprod Immunol. 1996; 32:157–169. [PubMed: 9023819]
12. Shah T, Walsh SW. Activation of NF-kappaB and expression of COX-2 in association with neutrophil infiltration in systemic vascular tissue of women with preeclampsia. Am J Obstet Gynecol. 2007; 196:48.e41–48.e48. [PubMed: 17240230]
13. Kniss D, Xie Y, Li Y, Kumar S, Linton E, Cohen P, Fan-Havard P, Redman C, Sargent I. ED27 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. [PubMed: 11869090]
14. Fowler AA 3rd, Fisher BJ, Sweeney LB, Wallace TJ, Natarajan R, Ghosh SS, Ghosh S. Nitric oxide regulates interleukin-8 gene expression in activated endothelium by inhibiting NF-B binding to DNA: effects on endothelial function. Biochem Cell Biol. 1999; 77:201–208. [PubMed: 10505790]
15. Dechend R, Viedt C, Muller DN, Ugele B, Brandes RP, Wallukat G, Park JK, Janke J, Barta P, Theuer J, Fiebeler A, Homuth V, Dietz R, Haller H, Kreuzer J, Luft FC. AT1 receptor agonistic antibodies from preeclamptic patients stimulate NADPH oxidase. Circulation. 2003; 107:1632–1639. [PubMed: 12668498]
16. Cooke CL, Brockelsby JC, Baker PN, Davidge ST. The receptor for advanced glycation end products (RAGE) is elevated in women with preeclampsia. Hypertens Pregnancy. 2003; 22:173–184. [PubMed: 12909002]

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17. Luppi P, Tse H, Lain KY, Markovic N, Piganelli JD, Deloia JA. Preeclampsia activates circulating immune cells with engagement of the NF-kappaB pathway. Am J Reprod Immunol. 2006; 56:135–144. [PubMed: 16836616]
18. Takacs P, Kauma SW, Sholley MM, Walsh SW, Dinsmoor MJ, Green K. Increased circulating lipid peroxides in severe preeclampsia activate NF-kappaB and upregulate ICAM-1 in vascular endothelial cells. FASEB J. 2001; 15:279–281. [PubMed: 11156936]
19. Lindstrom TM, Bennett PR. The role of nuclear factor kappa B in human labour. Reproduction. 2005; 130:569–581. [PubMed: 16264088]
20. Johnson RD, Sadovsky Y, Graham C, Anteby EY, Polakoski KL, Huang X, Nelson DM. The expression and activity of prostaglandin H synthase-2 is enhanced in trophoblast from women with preeclampsia. J Clin Endocrinol Metab. 1997; 82:3059–3062. [PubMed: 9284743]
21. Vaughan JE, Walsh SW. Oxidative stress reproduces placental abnormalities of preeclampsia. Hypertens Pregnancy. 2002; 21:205–223. [PubMed: 12517328]
22. Wang Y, Walsh SW. Antioxidant activities and mRNA expression of superoxide dismutase, catalase, and glutathione peroxidase in normal and preeclamptic placentas. J Soc Gynecol Investig. 1996; 3:179–184.
23. Walsh SW. Lipid peroxidation in pregnancy. Hypertens Pregnancy. 1994; 13:1–32.
24. Leik C, Walsh SW. Neutrophils infiltrate resistance-sized vessels of subcutaneous fat in women with preeclampsia. Hypertension. 2004; 44:72–77. [PubMed: 15148293]
25. Gratacos E, Casals E, Sanllehy C, Cararach V, Alonso PL, Fortuny A. Variation in lipid levels during pregnancy in women with different types of hypertension. Acta Obstet Gynecol Scand. 1996; 75:896–901. [PubMed: 9003089]
26. Lorentzen B, Endresen MJ, Clausen T, Henriksen T. Fasting serum free fatty acids and triglycerides are increased before 20 weeks of gestation in women who later develop preeclampsia. Hypertens Pregnancy. 1994; 13:103–109.
27. Lorentzen B, Drevon CA, Endresen MJ, Henriksen T. Fatty acid pattern of esterified and free fatty acids in sera of women with normal and pre-eclamptic pregnancy. Brit J Obstet Gynaec. 1995; 102:530–537.
28. Chung R, Davis H, Ma Y, Naivikul O, Williams C, Wilson K. Diet-related toxemia in pregnancy. I. Fat, fatty acids, and cholesterol. Am J Clin Nutr. 1979; 32:1902–1911. [PubMed: 474482]
29. Kupferminc MJ, Peaceman AM, Wigton TR, Rehnberg KA, Socol ML. Tumor necrosis factor-a is elevated in plasma and amniotic fluid of patients with severe preeclampsia. Am J Obstet Gynecol. 1994; 170:1752–1759. [PubMed: 8203436]
30. Conrad KP, Miles TM, Benyo DF. Circulating levels of immunoreactive cytokines in women with preeclampsia. Am J Reprod Immunol. 1998; 40:102–111. [PubMed: 9764352]
31. Kupferminc MJ, Peaceman AM, Aderka D, Wallach D, Socol ML. Soluble tumor necrosis factor receptors and interleukin-6 levels in patients with severe preeclampsia. Obstet Gynecol. 1996; 88:420–427. [PubMed: 8752252]
32. Laskowska M, Laskowska K, Leszczynska-Gorzelak B, Oleszczuk J. Comparative analysis of the maternal and umbilical interleukin-8 levels in normal pregnancies and in pregnancies complicated by preeclampsia with intrauterine normal growth and intrauterine growth retardation. J Matern Fetal Neonatal Med. 2007; 20:527–532. [PubMed: 17674266]
33. Jendryczko A, Drozdz M. Increased placental phospholipase A_2 activities in pre- eclampsia. Zbl Gynakol. 1990; 112:889–891.
34. Lim K-H, Rice GE, de Groot CJM, Taylor RN. Plasma type II phospholipase A_2 levels are elevated in severe preeclampsia. Am J Obstet Gynecol. 1995; 172:998–1002. [PubMed: 7892896]
35. Krauss T, Kuhn W, Lakoma C, Augustin H. Circulating endothelial cell adhesion molecules as diagnostic markers for the early identification of pregnant women at risk for development of preeclampsia. Am J Obstet Gynecol. 1997; 177:443–449. [PubMed: 9290466]
36. Clausen T, Djurovic S, Brosstad F, Berg K, Henricksen T. Altered circulating levels of adhesion molecules at 18 weeks' gestation among women with eventual preeclampsia: indicators of disturbed placentation in absence of evidence of endothelial dysfunction? Am J Obstet Gynecol. 2000; 182:321–325. [PubMed: 10694331]

37. Phocas I, Rizos D, Papoulias J, Xyni K, Sarandakou A, Salamalekis E. A comparative study of serum soluble vascular cell adhesion molecule-1 and soluble intercellular adhesion molecule-1 in preeclampsia. J Perinatol. 2000; 20:114–119. [PubMed: 10785888]

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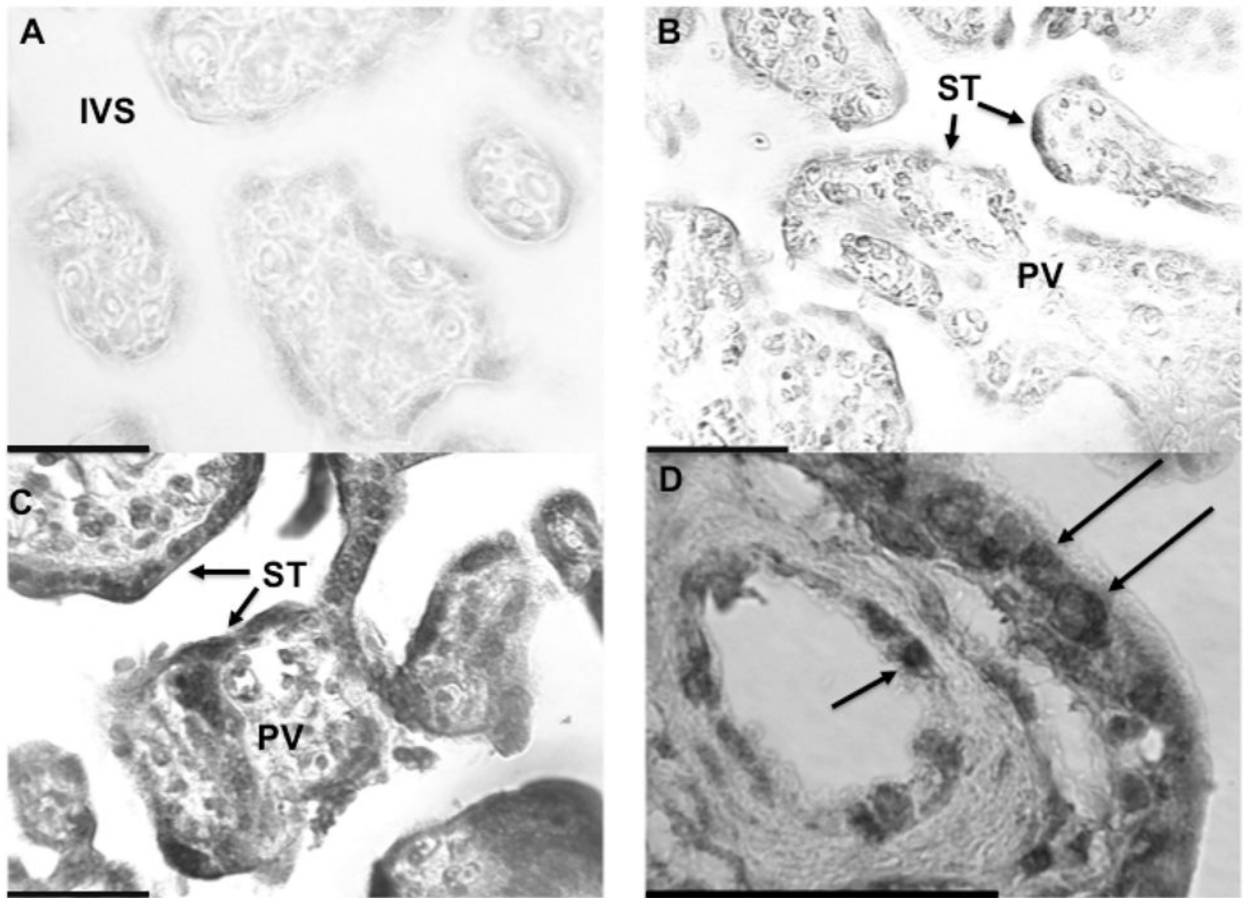

Figure 1.
Panel A: Immunohistochemical staining of placental tissue using a mouse IgG monoclonal isotype control, which served as a negative control. IVS indicates the intervillous space. Panels B&C: Immunohistochemical staining of normal and preeclamptic placental tissue for the p65 subunit of NF-κB. Normal placental tissue (panel B) showed sparse staining for NF-κB. In contrast, preeclamptic placental tissue (panel C) stained intensely for the transcription factor, particularly the syncytiotrophoblast cells (ST). Staining was also observed in cytotrophoblast cells, scattered cells in the villous core and in leukocytes contained within placental vessels (PV) in preeclamptic tissue. Images are 400x. Panel D: Magnified (1000x) view of preeclamptic placental tissue. Arrows indicate NF-κB staining in the nuclei of syncytiotrophoblast cells and endothelial cells. Scale bars indicate 50μm.

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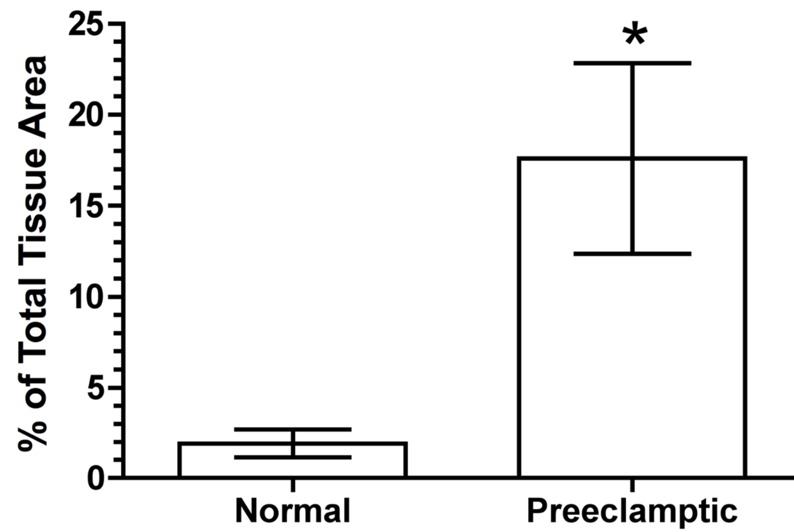

Figure 2.
Activated NF-κB in normal versus preeclamptic placentas. The area of stained regions relative to total tissue area was calculated using IP Lab software. Preeclamptic placental tissue showed almost a 10-fold increase in NF-κB staining compared to normal placental tissue. *$p < 0.05$

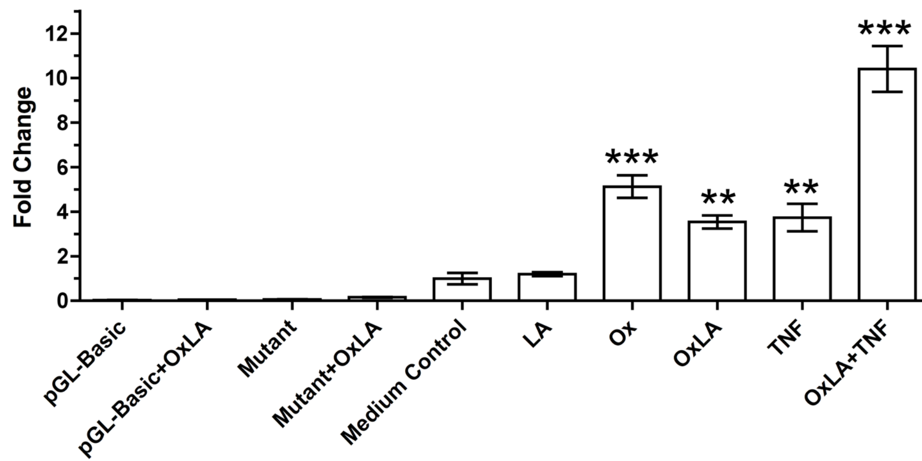

Figure 3.

NF-κB activation in ED_{27} cells, a trophoblast-like cell line, in response to oxidative stress and TNFα. A pGL3 reporter plasmid bearing the NF-κB binding site linked to the firefly luciferase gene was transfected into the cells. The cells were then incubated with either linoleic acid (LA, 45 μM), an oxidizing solution composed of hypoxanthine (3.6 mM) plus xanthine oxidase (0.005 U/ml) plus ferrous sulfate, 50μM, (Ox), Ox enriched with linoleic acid (OxLA, 45 μM), TNFα (2 ng/mL), or OxLA plus TNFα for 20 hours (n = 5). OxLA induced a 3- fold increase in activation of NF-κB as compared to medium control or LA alone. TNFα and Ox each independently increased NF-κB activation to the same extent as OxLA. The combination of TNFα with OxLA increased NF-κB activation 10-fold. These results were specific for NF-κB because OxLA was not able to induce an increase in luminescence when a pGL3 basic plasmid or a mutant reporter plasmid was substituted for the NF-κB reporter plasmid. ** $p < 0.01$, *** $p < 0.001$ Data represent mean ± SE.

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