The Role of Apoptosis in Preeclampsia

Roni Levy MD

Department of Obstetrics and Gynecology, Kaplan Medical Center, Rehovot, Israel
Affiliated to Hebrew University-Hadassah Medical School, Jerusalem, Israel

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Preeclampsia occurs in 5–7% of pregnancies and is a major cause of perinatal and maternal morbidity and mortality worldwide. This condition is specific to pregnancy and presents with a clinical picture of hypertension, proteinuria and different systemic manifestations. Despite intensive research the pathophysiology leading to the consequences of preeclampsia remains elusive. The most accepted concept is that immune maladaptation to the fetal allograft causes defective placentation. During preeclamptic pregnancy, cytotrophoblast invasion of the myometrium is abnormally shallow and remodeling of the uterine spiral artery incomplete. Many vessels are occluded by atherosis, with an accumulation of lipid-laden macrophages and a perivascular mononuclear cell infiltrate. The net result is uteroplacental ischemia. A yet undefined toxic circulating factor released by the ischemic placenta enters the maternal circulation and damages vascular endothelium, and is responsible for the clinical disease characterized by impaired endothelial function [1]. This circulating factor may be specific up-regulated protein, cellular debris or free fetal DNA.

Numerous articles have been published addressing the association between altered apoptosis and preeclampsia [2–7]. Apoptosis has been linked to preeclampsia at different steps in the proposed pathway leading to the clinical manifestation of preeclampsia. At each of these steps, apoptosis may play a central role. The term apoptosis (Greek for "falling off" – like autumn leaves), coined by Kerr et al. in 1972 [8], is descriptive of a unique morphology of cell death. It is distinct from cell death by necrosis, where damage to groups of cells, cytoplasmic swelling and inflammatory processes are characteristic. Unlike necrosis, apoptosis includes chromatin condensation with nuclear fragmentation and cytoplasmic condensation with cell shrinkage. The morphologic changes may be part of normal physiology or may be secondary to pathologic insult. Apoptosis occurs during normal embryonic development and in the turnover of mature tissue. However, apoptosis is also found in tissue exposed to exogenous stimuli, such as hypoxia or cytotoxic agents, and it can be induced by ligand-receptor interactions, such as with Fas and tumor necrosis factor-alpha [9].

Apoptosis is described in many physiologic as well as pathologic processes in the human body, including preeclampsia with or without fetal growth restriction. It may be argued that enhanced apoptosis may be a normal response to stimuli such as hypoxia. However, several *in vitro* and *in vivo* studies have shown that specific and unspecific scavengers of apoptosis, such as caspase inhibitor and epidermal growth factor, decreased the level of apoptosis and improved tissue function [10–12]. These studies point to an important role of apoptosis in pathologic processes. In this article, I review the evidence linking apoptosis to each step in the mechanism of preeclampsia [Figure 1].

Altered apoptosis induces maternal immune intolerance toward the fetus

The fetus and placenta are considered a semi-allograft to the mother's immune system and consequently generate an immune rejection response. From early pregnancy there is a large influx of

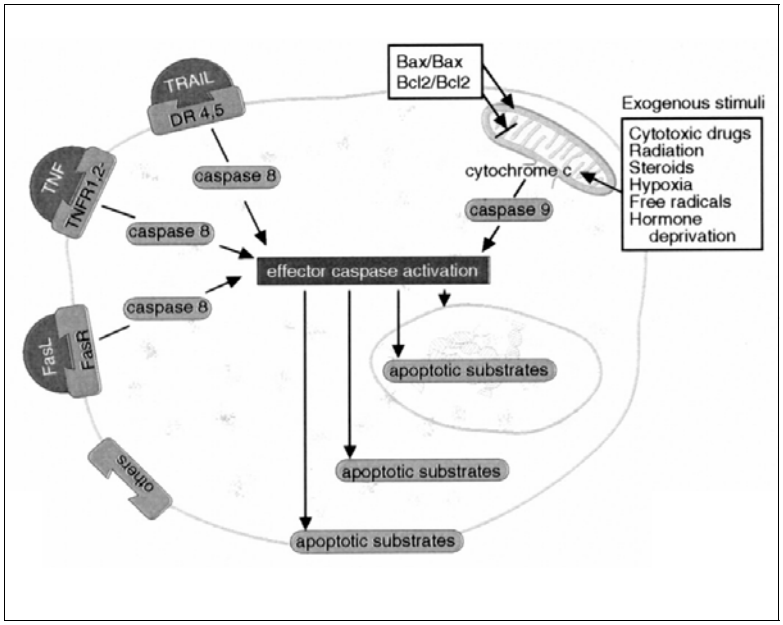

Figure 1. Apoptotic pathways. There are two main apoptotic pathways – one induced by internal or external stimuli mediated by proteins from the Bcl-2 family, via the mitochondria, and the other through ligand to receptor stimuli such as Fas and TNFα.

macrophages and lymphocytes into the decidua at the maternal-fetal interface, such that white blood cells comprise up to 40% of the cells found in the decidua during pregnancy [13]. How is the developing fetus protected from these cells? One of the most accepted theories is that human leukocyte antigen-G has an immunologically permissive role in the antigenic mismatch between mother and fetus [14]. HLA-G expression was found to be defective in extravillous cytotrophoblast of preeclamptic placentas. Further, the serum levels of a soluble HLA-G1 isoform, which down-regulates T cell and natural killer activity by inducing apoptosis, were found to be decreased in preeclamptic patients [14]. The exact mechanism by which HLA-G causes cell apoptosis is unknown.

Another theory relates to other systems of the body that are "immunologically priveleged sites." The anterior chamber of the eye and the testes are protected from antigens that penetrate these sites and fail to elict an immune response. It turns out that cells in these sites differ from the other cells of the body in that they express high levels of FasL at all times. Thus, antigen-reactive T cells, which express Fas, would be killed when they enter these sites [15]. Fas, a 45 kDa surface protein that is a member of the TNF superfamily, induces cell apoptosis upon binding to the ligand FasL [16]. Several pieces of evidence point to the key role played by the Fas-FasL system in protecting the fetus from the maternal immune system [17]. The trophoblast of first-trimester placentas expresses FasL, while Fas antigen is localized mainly on decidual cells, in particular the maternal leukocytes [18,19]. When trophoblasts expressing FasL are exposed to activated lymphocytes *in vitro*, they induce lymphocyte apoptosis [20]. In mice, FasL is positioned to prevent the exchange of activated immune cells between mother and fetus, and deters trafficking of activated Fas-expressing immune cells at the maternal-fetal interface [21]. Mice lacking FasL (gld) exhibit leukocyte infiltration of the decidual-placental interface and increased fetal loss. In preeclampsia, decidual cells exhibit lower expression of Fas antigen [6], and lower expression of FasL is seen on trophoblast cells [4]. In addition, the level of serum-soluble Fas antigen is elevated in the maternal circulation [6]. sFas, a spliced product of the Fas antigen, does not stimulate apoptosis; rather, it protects cells from apoptosis by competitive binding to the Fas antigen. Thus, elevated levels of sFas in the maternal circulation, concomitant with lower expression of Fas and FasL, result in diminished apoptotic deletion of lymphocytes in the maternal decidual system. As a consequence, there is increased apoptosis and trophoblast damage in the fetal placental compartment. Indeed, samples taken from the uterine wall of preeclamptic women demonstrated excess of macrophages. When exposed to TNFα, a known stimulator of placental apoptosis [22], these macrophages may limit trophoblast invasion of spiral arterial segments by TNFα-mediated apoptosis [23]. Indeed, TNFα is found in high concentrations in plasma, amniotic fluid and placental tissue of patients with preeclampsia [24,25]. The enhanced apoptosis of the invading trophoblasts results in limited invasion of the spiral arteries due to reduced activity of the cytotrophoblasts, so that myometrial segments of the spiral arteries remain intact, resulting in the formation of an arteriolar system with high resistance. The failure of trophoblast invasion leads to a reduction in uteroplacental perfusion, with the placenta becoming increasingly ischemic as gestation progresses. Placentas from women with preeclampsia display an increased frequency of placental infarcts and altered morphology, evidenced by abnormal cytotrophoblast proliferation and increased formation of syncytial knots [26]. In a study comparing tissue samples of the villus-uterus attachment taken from preeclamptic and control patients, samples from the former showed widespread apoptosis while those from normal pregnancies had a low rate of apoptotic cells [27]. Furthermore, the samples taken from preeclamptic patients demonstrated lower expression of one of the pro-apoptotic proteins, Bcl-2. Increased apoptosis along with decreased expression of Bcl-2 has also been reported in myocardial cells [12]. Thus, excess apoptotic activity in the placental bed of preeclamptic women inhibits trophoblast invasion into the spiral artery by increasing trophoblast apoptosis.

Several mechanisms, in addition to apoptosis, have been reported to limit extravillous invasion of the placental bed. These include the reduced expression of various proteins, among them integrin α1/β1, matrix metalloproteinase, vascular cell adhesion molecules, vascular endothelial growth factor, and heparin-binding epidermal growth factor [28]. A reduction in the expression of these proteins necessary for extravillous trophoblast differentiation along the invasive pathway is compatible with the view that cells entering the apoptotic cascade down-regulate their levels of protein transcription.

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Hypoxia induces placental apoptosis

Placental ischemia secondary to defective placentation may be a prerequisite for the development of preeclampsia. Human term trophoblasts exposed to hypoxia *in vitro* exhibited enhanced apoptosis [11]. The process was associated with increased expression of the pro-apoptotic proteins p53 and Bax and reduced expression of the anti-apoptotic Bcl-2. Enhanced apoptosis and up-regulation of p53 were also found in placental samples taken from pregnancies complicated by preeclampsia [29]. Hypoxia triggers apoptosis via a mechanism that involves predominantly mitochondrial pathways, as opposed to ligand-receptor pathways which are mediated by cytokines such as TNFα or Fas ligand [Figure 2]. In this pathway, death signals are stimulated via modulation of the expression of specific apoptosis-related genes such as p53 and

HLA = human leukocyte antigen
TNF = tumor necrosis factor
sFas = serum soluble Fas

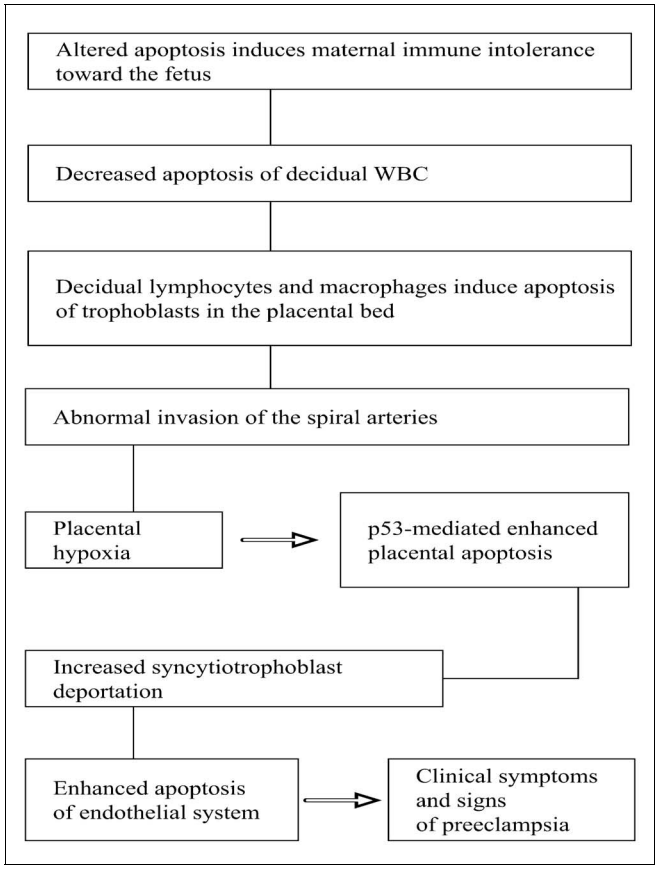

Figure 2. Apoptosis and sequences of preeclampsia

proteins of the Bcl-2 family. p53 plays a pivotal role in the cellular response to DNA damage, halting the cell cycle to allow repair of DNA. If repair is not possible, p53 promotes apoptosis. p53 is an unstable protein with a short half-life; exogenous stimuli such as hypoxia and oxidative stress stabilize it. p53 plays an important role in hypoxia-induced cell death in diverse cell types, including cardiocytes, hepatocytes and neuronal cells [30]. p53 expression is enhanced in cultured trophoblasts exposed to hypoxia [11] as well as in placental biopsies taken from pregnancies complicated by preeclampsia and fetal growth restriction.

Hypoxia may not be the sole inducer of placental apoptosis. Oxidative free radicals are frequently, although not universally, increased in preeclampsia [1]. Retention of vasoreactivity of the spiral arteries caused by defective placentation may result in intermittent perfusion of the intervillous space, fluctuating oxygen tension, and ischemia-reperfusion insult of the villus. This oxidative stress may be associated with enhanced placental apoptosis and increased turnover of the syncytiotrophoblast [31].

Thromboxane A2 levels are elevated in the circulation of pregnant preeclamptic women and in the placental villi [32]. Elevated levels of thromboxane A2 are associated with thrombocyte aggregation, which may create a predisposition to enhanced thrombosis and infarct injury of the placenta. Thromboxane A2 was recently found to enhance apoptosis in primary-term human trophoblasts [33].

Placental apoptosis increases syncytiotrophoblast deportation

One of the unresolved questions in the pathogenesis of preeclampsia is the link between placental ischemia and endothelial cell dysfunction. Systemic endothelial damage appears to be a common factor in the signs and symptoms of preeclampsia. One of the theories, presented by Redman and Sargent [34], is that systemic endothelial damage is caused by microdeposition of syncytiotrophoblast microvillous membrane particles. These particles can be detected in the plasma of normal pregnancies but increase in women with preeclampsia. The increased syncytiotrophoblast deportation in preeclampsia may be caused by enhanced apoptosis at the syncytium, affecting the integrity of the tissue. It has been proposed that apoptosis plays an important role in the renewal of the syncytium [35,36]. Apoptotic nuclei are found in syncytial knots and probably contribute to the shedding of syncytial fragments into the maternal circulation [37]. This process is enhanced in the syncytium of placentas from pregnancies complicated by preeclampsia [6,29]. When the maternal circulation cannot compensate for this enhancement, systemic endothelial damage occurs.

Altered apoptosis in preeclampsia is found not only in the placenta but also in endothelial cells [38]. Endothelial tissue apoptosis may result from the toxic effect of excessive syncytial fragments, but it may also be the result of the local hypoxic environment caused by the constriction of efferent blood vessels or from the effect mediated by free radicals. This effect may occur via

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p53-mediated endothelial apoptosis, as found in other systems [11,12]. An additional hypothesis is that increased secretion of TNFα induces activation and apoptosis of endothelial tissue [39]. Another hint for the apoptosis-induced trophoblast microfragments deportation is the increased level of free DNA in the circulation of patients with preeclampsia [40].

Summary

Altered apoptosis is involved in each step of the pathogenesis of preeclampsia. While deficient apoptosis may induce a maternal immune response against the fetus, enhanced apoptosis may interfere with the process of placentation, placental ischemia and subsequently, systemic endothelial damage. Thus, treatment modalities to inhibit or acclerate apoptosis cannot be employed in early pregnancy for prevention. Later in pregnancy, it would make sense to test treatment modalities that inhibit hypoxia-mediated apoptosis in patients in whom early testing, such as abnormal Doppler of the uterine artery, indicates the initiation of preeclampsia. This, however, has first to be tested in animal models. Since

multiple different mechanisms, rather than a single factor, could contribute to the development of apoptosis, further studies to clarify the signaling mechanisms of apoptosis in preeclampsia should be conducted before any investigational treatment modalities are employed.

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Correspondence: Dr. R. Levy, Dept. of Obstetrics and Gynecology, Kaplan Medical Center, Rehovot 73100, Israel.
Phone: (972-8) 944-1558
Fax: (972-8) 946-7045
email: Roni_L@clalit.org.il