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Premature labor: a state of platelet activation?

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Abstract

Objective: This study was undertaken to determine whether premature labor is associated with changes in the maternal plasma concentration of soluble CD40 ligand (sCD40L), a marker of platelet activation.

Methods: A cross-sectional study included patients in the following groups: 1) non-pregnant (n=21); 2) normal pregnancy (n=71); 3) normal pregnancy at term with (n=67) and without labor (n=88); 4) preterm labor (PTL) with intact membranes (n=136) that was divided into the following sub-groups: 4a) PTL who delivered at term (n=49); 4b) PTL without intra-amniotic infection and/or inflammation (IAI) who delivered preterm (n=54); and 4c) PTL with IAI who delivered preterm (n=33). sCD40L concentrations were measured by ELISA.

Results: The median maternal plasma sCD40L concentration was higher in pregnant than non-pregnant women

(P=0.017). Patients with PTL had a higher median maternal plasma sCD40L concentration than women with normal pregnancies, regardless of the presence or absence of IAI and gestational age at delivery (P<0.001 for all comparisons). IAI was not associated with a higher median maternal plasma concentration of sCD40L.

Conclusions: Normal pregnancy is a state in which there is a physiologic increase of sCD40L. PTL was associated with an increased median maternal plasma sCD40L concentration that could not be accounted for by IAI. Thus, our findings suggest that platelet activation occurs during an episode of preterm labor.

Keywords: Coagulation; inflammation; labor; prematurity; platelet; thrombin; sCD40L.

Introduction

Platelet activation and degranulation are key steps in the formation of a primary hemostatic plug and clot generation following blood vessel laceration [12, 15, 90, 92, 98, 122]. In addition to their classic hemostatic role, activated platelets participate in the process of acute and chronic inflammation [28, 94, 101, 103, 139], in which platelet degranulation, as well as the direct contact of activated platelets with circulating monocytes, can generate an inflammatory response [28, 94, 101, 139].

CD40 ligand (CD40L), a member of the tumor necrosis factor superfamily [57], was originally identified on activated CD4⁺ T cells and has been associated with T cell regulation of B cell function [6, 45, 57, 79, 132]. In addition, CD40L is associated with platelet pro-inflammatory activity [124], activation of the vascular endothelium [19, 52, 80, 115, 130, 135], as well as cyclooxygenase-2 (COX-2) expression and prostaglandin synthesis [97, 109].

Upon activation, platelets express CD40L on their membrane (from which it is subsequently cleaved by CD40), and the soluble form of CD40L (sCD40L) can be measured in the plasma [52, 53]. Activated platelets are the source of more than 90% of sCD40L in the plasma [52], and this cytokine has been proposed as a measurable marker of platelet activation [85, 107]. Circulating sCD40L has a role in platelet activation and arterial thrombi stabilization [4, 110]. High concentrations of sCD40L have been reported in chronic inflammatory diseases associated with platelet activation, such as cystic fibrosis [37], inflammatory bowel disease [24–26, 76, 89, 106], systemic sclerosis [73], and systemic lupus erythe-

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matosis [22, 44, 70, 131]. Moreover, elevated sCD40L plasma concentrations are associated with a higher risk for cardiovascular disease in asymptomatic patients [120] and for coronary artery restenosis after balloon angioplasty [23].

Platelet count and mean platelet volume do not change significantly with pregnancy [51, 129]. However, especially during the third trimester, a significant decrease in platelet count has been proposed to result from an increased consumption of platelets in the utero-placental unit [51]. Moreover, the inverse relationship between the mean platelet volume and the platelet count observed during normal pregnancy led to the proposal that pregnancy is a state of compensated thrombocytolysis [129]. There are inconsistent reports concerning the degree of platelet activation and the release of vasoactive products during normal pregnancy [8, 51, 60, 113, 114]. Women who develop obstetric complications, such as pre-eclampsia and fetal growth restriction, have a higher degree of platelet activation than both normal pregnant and non-pregnant women [1, 9, 11, 13, 38, 48, 50, 59, 62, 64, 65, 74, 75, 82, 86, 88, 96, 99, 102, 108, 116, 128, 137]; however, little information is available regarding the association between maternal platelet activation and preterm labor (PTL) [117].

Preterm labor is associated with an increased thrombin generation that is reflected by elevated maternal plasma concentrations of thrombin-antithrombin (TAT) complexes [18, 32]. Thrombin activates platelets [61, 67], thus, the increased thrombin generation [18, 32], along with the moderate maternal systemic inflammation observed in patients with PTL [43], may lead to subsequent platelet activation and an increased secretion of CD40L, which can further enhance this process.

The aims of this study were: 1) to determine maternal platelet activation through the marker sCD40L in patients with PTL; and 2) to investigate whether platelet activation is associated with the presence of intra-amniotic infection and/or inflammation (IAI) or a history of vaginal bleeding during pregnancy.

Material and methods

Study groups and inclusion criteria

This cross-sectional study included patients in the following groups: 1) non-pregnant women ($n=21$); 2) normal pregnancy ($n=71$); 3) women with a normal pregnancy at term with ($n=67$) and without labor ($n=88$); 4) women with PTL ($n=136$) that were divided into the following sub-groups: 4a) women with PTL who delivered at term ($n=49$); 4b) patients with PTL without IAI who delivered preterm ($n=54$); and 4c) women with PTL and IAI who delivered preterm ($n=33$). Patients with multiple pregnancies or fetuses with congenital and/or chromosomal anomalies were excluded.

Samples and data were retrieved from our bank of biological samples and clinical databases. Many of these samples have

been employed to study the biology of inflammation, hemostasis, angiogenesis regulation, and growth factor concentrations in non-pregnant women, normal pregnant women, and those with pregnancy complications. All women provided a written informed consent prior to the collection of maternal blood. The Institutional Review Boards of both Wayne State University and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD/NIH/DHHS) approved the collection and utilization of samples for research purposes.

Clinical definitions

Preterm labor was diagnosed by the presence of at least two regular uterine contractions every 10 min associated with cervical changes that required admission to the hospital before 37 weeks of gestation. In women with PTL amniotic fluid collection was performed by trans-abdominal amniocentesis under ultrasonographic guidance in order to determine the microbiologic state of the amniotic cavity. Amniotic fluid was transported to the laboratory in a capped plastic sterile syringe and cultured for aerobic and anaerobic bacteria, as well as for genital mycoplasmas. White blood cell (WBC) count, glucose concentration, and Gram stain for microorganisms were performed in amniotic fluid shortly after collection. Intra-amniotic infection was defined by the presence of positive amniotic fluid cultures for microorganisms and intra-amniotic inflammation by an amniotic fluid WBC count ≥ 100 cells/mL. The results of the amniotic fluid analyses were used for clinical management. A small for gestational age (SGA) neonate was defined as birthweight below the 10th percentile [3]. Placental histologic findings were classified according to a diagnostic schema proposed by Redline et al. [112].

Blood samples collection

All blood samples were collected with a Vacutainer[®] into 0.109 M trisodium citrate anticoagulant solution (BD; San Jose, CA, USA). Samples were centrifuged at 1300 *g* for 10 min at 4°C and stored at -70°C until assay.

Human sCD40L immunoassays

Maternal plasma sCD40L concentrations were determined by sensitive and specific immunoassays obtained from R&D Systems (Minneapolis, MN, USA). The assay was conducted according to the manufacturer's recommendations. The sensitivity of the sCD40L assay was 4.2 pg/mL. The intra-assay coefficient of variation is 5%, while the inter-assay coefficient of variation is 6.2%.

Statistical analysis

The Shapiro-Wilk and the Kolmogorov-Smirnov tests were used to test if the data was normally distributed. Soluble sCD40L plasma concentrations were not normally distributed; therefore, Kruskal-Wallis and Mann-Whitney *U* tests were employed for comparisons of continuous variables, and Chi-square test was used to compare categorical variables. Spearman correlation was used to detect an association between the concentrations of sCD40L and gestational age at sample collection in women with a normal pregnancy. A *P*-value < 0.05 was considered sta-

Table 1 Demographic and clinical characteristics of the study population.

	Normal pregnancy (n = 71)	PTL without IAI (n = 54)	PTL with IAI (n = 33)	PTL delivered at term (n = 49)
Maternal age (years)	24.0 (21.0, 27.0)	22.0 (19.0, 29.0)	23.0 (19.0, 27.0)	20.0 (18.0, 24.0)*
Gravidity ^a				
1	14 (20.3)	8 (14.8)	12 (36.4)	11 (22.4)
2–5	45 (65.2)	39 (72.2)	17 (51.5)	32 (65.3)
≥6	10 (14.5)	7 (13)	4 (12.1)	6 (12.2)
Parity ^b				
1	39 (55.7)	30 (55.6)	24 (72.7)	31 (63.3)
2–5	30 (42.9)	21 (38.8)	6 (18.2)	18 (36.7)
≥6	1 (1.4)	3 (5.6)	2 (6.1)	0
Ethnic origin ^c				
African-Americans	53 (77.9)	42 (79.2)	27 (81.8)	42 (87.5)
Caucasian	11 (16.1)	8 (15.1)	5 (15.2)	3 (6.2)
Hispanic	2 (3)	3 (5.7)	0	1 (2.1)
Asian	2 (3)	0	1 (3)	2 (4.2)
Gestational age at blood collection (weeks)	31.6 (27.4, 35.0)	29.5* (25.1, 32.2)	26.1* (24.6, 31.6)	31.4 (29.3, 32.5)
Gestational age at delivery (weeks)	39.6 (38.4, 40.4)	31.6* (26.2, 34.7)	27.9* (25, 33.5)	38.2* (37.3, 38.9)
Neonatal birthweight (g)	3330 (3050, 3700)	1690* (880, 2335)	1040* (642.5, 1755)	2948* (2710, 3255)
Cesarean delivery*	22 (33.8)	20 (37.7)	6 (18.2)	0*
SGA	0	5 (9.4)*	5 (15.1)*	12 (24.5)*

Data are presented as median (25th–75th interquartile range) or numbers (%).

*P < 0.05 in comparison to normal pregnancy.

^a = Normal pregnancy (n = 69).

^b = Normal pregnancy (n = 70); PTL with IAI (n = 32).

^c = Normal pregnancy (n = 68); PTL without IAI (n = 53); PTL delivered at term (n = 48).

PTL = preterm labor, IAI = intraamniotic infection/inflammation, SGA = small for gestational age.

tistically significant. Analysis was performed with SPSS package, version 12 (SPSS Inc., Chicago, IL, USA).

Results

Demographic and clinical characteristics of women with normal pregnancies and PTL sub-groups are presented in Table 1. The rate of SGA in patients with PTL who delivered at term was significantly higher than in patients with PTL without IAI and in women with a normal pregnancy.

Changes in maternal plasma sCD40L concentrations during normal pregnancy and labor at term

Women with a normal pregnancy had a significantly higher median maternal plasma sCD40L concentration than non-pregnant women (normal pregnancy: median 369.5 pg/mL, range 63.5–1848.7 vs. non-pregnant median 270.4 pg/mL, range 94.2–568, P = 0.017) (Figure 1). There was no correlation between maternal plasma sCD40L concentrations and gestational age at sample collection ($r = 0.18$, P = 0.13) (Figure 2).

Patients with normal pregnancy in labor at term had a significantly higher median maternal plasma sCD40L concentration than those at term not in labor (term in labor: median 384 pg/mL, range 104–3784 vs. term no labor 338.5 pg/mL, range 32.9–4125, P = 0.04) (Figure 3).

Changes in the maternal plasma sCD40L concentration in patients with preterm labor

The median maternal plasma sCD40L concentrations were significantly different in patients in all PTL sub-groups (Kruskal-Wallis, P < 0.001) than in women with normal pregnancies (PTL without IAI: median 781.5

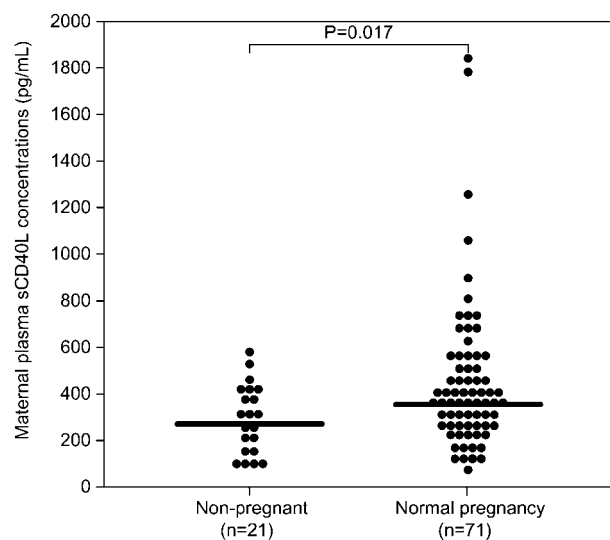


Figure 1 Comparison of maternal plasma sCD40L concentrations between non-pregnant and pregnant women.

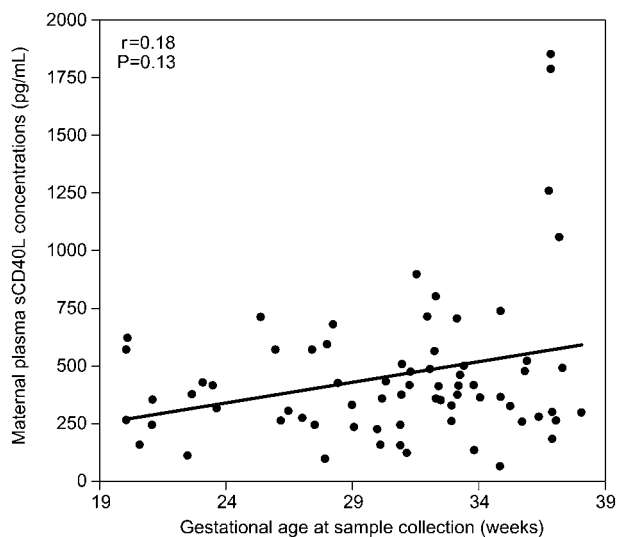


Figure 2 The correlation between gestational age at sample collection and maternal plasma sCD40L concentrations.

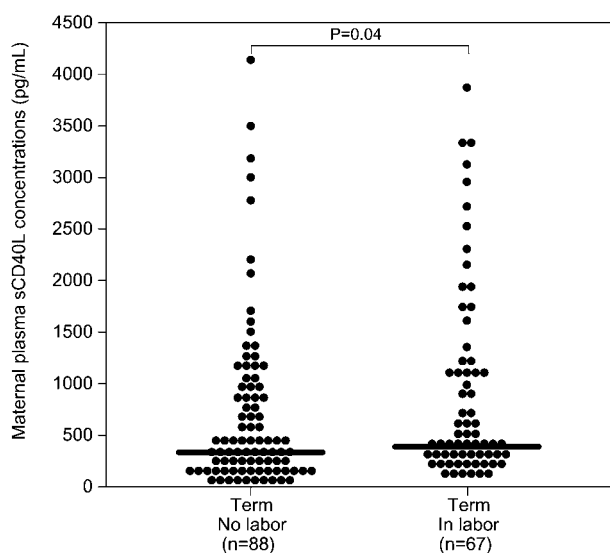


Figure 3 The comparison of the median maternal plasma sCD40L concentration between patients with and without labor at term.

pg/mL, range 107.1–3206.4 vs. normal pregnancy: median 369.5 pg/mL, range 63.5–1848.7, $P < 0.001$; PTL with IAI: median 988.2 pg/mL, range 123.2–4422 vs. normal pregnancy: median 369.5 pg/mL, range 63.5–1848.7, $P < 0.001$; and PTL who delivered at term: median 686.7 pg/mL, range 109.6–3102 vs. normal pregnancy: median 369.5 pg/mL, range 63.5–1848.7, $P < 0.001$ (Figure 4).

Amniocentesis was performed in 83.8% (114/136) of the patients presenting with PTL, of which 14% (16/114) had a positive amniotic fluid culture. All patients without

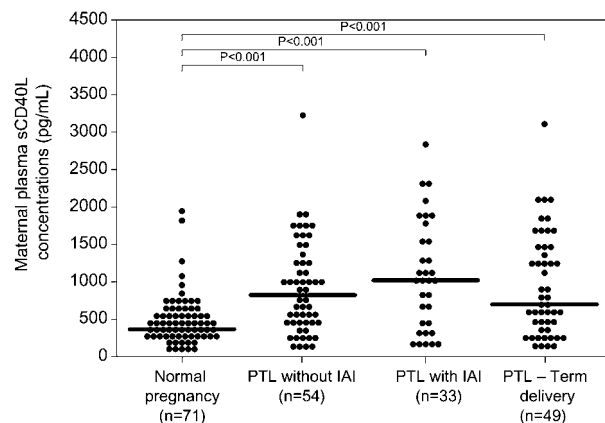


Figure 4 Comparison of median maternal plasma sCD40L concentration among patients with preterm labor (PTL) who delivered preterm (with/without intraamniotic infection/inflammation) or at term and patients with normal pregnancy who delivered at term.

amniocentesis were women with an episode of PTL who delivered at term. Among patients in the PTL sub-groups, there was no significant difference in the median maternal plasma concentration of sCD40L between those with (median 988.2 pg/mL, range 123.2–4422) and without (median 781.5 pg/mL, range 107.1–3206.4) IAI. Moreover, no significant differences in the median plasma concentration of sCD40L were detected between patients with PTL who delivered preterm (with or without IAI) and those with PTL who delivered at term (median 686.7 pg/mL, range 109.6–3102) (Kruskal-Wallis, $P = 0.3$). There was no correlation between the admission-to-delivery interval and maternal plasma sCD40L concentrations ($r = -0.04$, $P = 0.7$).

Data concerning episodes of vaginal bleeding during pregnancy were available in 88.2% (120/136) of the patients with PTL. Vaginal bleeding was not associated with significant differences in the median maternal plasma sCD40L concentration in the different PTL sub-groups (Kruskal-Wallis, $P = 0.7$).

Placental histologic findings of chorioamnionitis, funisitis, maternal underperfusion, and fetal vascular thrombo-occlusion were not associated with significant changes in the median maternal plasma concentration of sCD40L in the different PTL subgroups (data not shown).

Discussion

Principal findings of the study

- 1) The median maternal plasma sCD40L concentration is higher in pregnant than in non-pregnant women;
- 2) patients with preterm labor, regardless of the presence of IAI or gestational age at delivery, have a significantly higher median maternal plasma concentration of sCD40L

than women with normal pregnancies; 3) at term, the median maternal plasma sCD40L concentration is higher in women in labor than those not in labor.

The interaction between CD40-CD40 ligand system, humoral immunity and inflammatory cells

CD40L (also known as CD154 [27, 77], TRAP [7], and gp39 [93]) is a 33 kD transmembrane protein [6, 45, 79] that is expressed on platelets upon their activation [52, 53] as well as on CD4⁺ T cells [6, 45, 57, 79, 119, 132]. The structure of CD40L is homologous to that of tumor necrosis factor (TNF)- α [57]. Together, with its receptor CD40 (a 48 kD transmembrane protein that is structurally homologous to the TNF receptor) [10, 57], CD40L exerts pro-inflammatory activity [6, 10, 19, 45, 52, 57, 79, 80, 115, 124, 130, 132, 135].

The CD40-CD40L system was first identified on activated T cells, where it plays an important role in B cell activation and isotype switching from IgM to IgG [6, 45, 57, 79, 132]. A mutation in the CD40L gene on the X chromosome was reported in X-linked immunodeficiency with hyper-IgM syndrome [5, 29, 36, 87, 91]. This syndrome is characterized by recurrent infections, increased susceptibility to neoplasms, and a high mortality rate [83]. Further support for the role of CD40L in T cell-dependent humoral immunity is derived from the results of the CD40L knockout mice model. These mice display a selective deficiency in humoral immunity with lower basal serum immunoglobulin isotype concentrations and undetectable IgE [81]. Moreover, these mice fail to mount a secondary antigen-specific response to immunization with a thymus-specific antigen, suggesting that CD40L is required for T cell-dependent antibody response [21, 105, 121, 136].

In addition to its expression on T cells, CD40 is expressed by monocytes macrophages and dendritic cells [14, 16, 47, 69, 133], and its interaction with CD40L leads to the synthesis of pro-inflammatory cytokines, such as interleukin (IL)-1 [134], IL-6 [111, 127], and TNF- α [134]. Furthermore, CD40L expression has been reported on eosinophils, mast cells, and basophils; the latter two cell types induce IgE production by B cells through the activation of the CD40 receptor by CD40L [39–42]. Collectively, these reports support the important role of CD40-CD40L system in humoral immunity, inflammation, and allergic reactions.

The role of the CD40-CD40L system in platelet activation and their pro-inflammatory effect

Platelets store CD40L and express it on their membranes upon activation [52, 54]. Shortly after its expression, CD40L is cleaved by CD40 and released into the plasma [53]. Platelets are the source of more than 90% of soluble CD40L [52], which is regarded as a marker for platelet activation [54, 85, 107].

The CD40L that is expressed upon the platelet membrane participates in many processes mediated by platelets, including the following: 1) proinflammatory response in vascular endothelial cells through their CD40 receptor leads to an increased endothelial cell expression of adhesion molecules (E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1) [52]; as well as secretion of cytokines, such as monocyte chemoattractant protein (MCP)-1, IL-18 and IL-6, through nuclear factor kappa B (NF κ B) activation [52]; and 2) soluble CD40L propagates a procoagulant phenotype by inducing tissue factor expression and down-regulating thrombomodulin expression by human umbilical vein endothelial cells (HUVEC) [123] and weak induction of tissue factor expression on whole blood monocytes [84].

Soluble CD40L enhances platelet activation by increasing: 1) P-selectin expression [20, 63] and platelet degranulation [63]; 2) reactive oxygen and nitrogen species generation; [20] and 3) platelet aggregation and platelet-leukocyte conjugation [20]. CD40L appears to be an $\alpha_{IIb}\beta_3$ ligand that is necessary for the stability of arterial thrombi [4]. Indeed, CD40L^{-/-} mice had a lower platelet density in their thrombi, which was associated with a delayed vessel occlusion, frequent thrombi rupture, and embolization that were not observed in the wild type mice [4]. Moreover, the administration of recombinant sCD40L normalized clot formation in the CD40L^{-/-} mice [4]. Thus, the major source of plasma sCD40L are activated platelets. Soluble CD40L is a key player in the pro-inflammatory effect of activated platelets. Moreover, this chemokine sustains platelet activation and participates in their pro-thrombotic activity.

The changes in sCD40L during normal pregnancy and its possible role in parturition

The findings that median maternal plasma sCD40L concentration is higher in pregnant than in non-pregnant women, as well as in those in labor at term compared to those not in labor are novel. Pregnancy [58, 60] and labor [126] have previously been associated with platelet activation, and our results are in accord with these reports. However, a previous study [104] reported that non-pregnant women have a higher mean serum sCD40L concentration than women with a normal pregnancy. Differences in sample size, study population, and method of sCD40L measurement may explain the discrepancy between the studies.

The report that CD40 is expressed in the human reproductive tract [71] further supports the concept that platelets may play an active role in parturition. Indeed, the perivascular expression of CD40 receptors was reported in the endometrium, myometrium, and uterine cervix [71]. The latter also had strong CD40 expression in the basal but not in the surface epithelium [71]. The low basal CD40 expression reported in fibroblasts of the myometrium, endometrium, and uterine cervix increased after

treatment with interferon γ (IFN γ) [71]. The treatment of these fibroblasts with CD40L and IFN γ induced a several-fold increase in their production of IL-6, IL-8 and MCP-1. This effect, however, was not observed with CD40L treatment alone [71].

Activated platelets induce COX-2 gene expression in endothelial cells through CD40L activity [97]. In addition, incubation of HUVEC cells with Jurkat D1.1 CD40L⁺ cells increased their COX-2 expression and prostaglandin E₂ (PGE₂) and PGI₂ secretion [31]; the latter was also increased by the exposure of HUVEC to recombinant soluble CD40L. Moreover, this study demonstrated that the CD40-CD40L-mediated induction of IL-6 in HUVEC is COX-2 dependent [31]. A similar effect on COX-2 activity and higher PGE₂ production by the CD40-CD40L system was also reported in human lung fibroblasts [140].

The induction of COX-2 expression in the reproductive tract by the CD40-CD40L system can be a mechanism by which activated platelets participate in term and preterm parturition. Indeed, cyclooxygenase is the rate-limiting step for prostaglandins and thromboxane A₂ production [100], and COX-2, an isoenzyme of cyclooxygenase, has been implicated in the mechanism of parturition through its effect on the synthesis of prostaglandins especially PGE₂ [17, 30, 35, 46, 49, 55, 66, 72, 118, 125]. Thus, the induction of COX-2 expression by the CD40-CD40L system in the non-pregnant uterus, cervix, and endometrium may be of importance during pregnancy and labor, since COX-2 expression may lead to the increased synthesis of prostaglandins and pro-inflammatory cytokines by fibroblasts in the reproductive tract, which in turn promotes uterine contractility and cervical ripening.

Platelet activation and pregnancy complications

There is a solid body of evidence supporting platelet activation in patients with preeclampsia and fetal growth restriction [1, 9, 11, 13, 38, 48, 50, 59, 62, 64, 65, 74, 75, 82, 86, 88, 96, 99, 102, 108, 116, 128, 137]. Indeed, elevated maternal plasma and platelet expression of markers for platelet activation, including P-selectin (CD62p) [48, 68, 74, 75, 86, 128, 137, 138], Annexin V [34, 50, 128], and sCD40L concentrations [2, 78, 95, 104], were reported in patients with these pregnancy complications. However, maternal platelet activation in women with preterm labor has not been studied extensively. It has been reported that patients with PTL had higher amniotic fluid concentrations of platelet activating factor than patients without labor at term [56], and that this chemokine was associated with lipopolysaccharide-induced PTL and delivery in mice [33]. In a study of platelet activation in the maternal circulation, patients with PTL had a higher mean platelet factor 4 and beta-thromboglobulin plasma concentrations than patients with term labor [117].

Our finding of a higher median maternal plasma sCD40L concentration in patients with preterm labor is novel and suggestive of an increased maternal platelet activation in patients with PTL regardless of the presence of IAI or previous episodes of vaginal bleeding. This may result from the following underlying mechanisms: 1) increased thrombin generation in patients with PTL [18] may lead to platelet activation and, subsequently, to the shedding of sCD40L into the plasma. Of note, similar to our findings, the concentrations of TAT complexes were not significantly different in the presence of IAI or vaginal bleeding [18]. 2) In addition, the increased maternal leukocyte activation among patients with PTL, was not associated with the presence of IAI [43]. This suggests that maternal systemic inflammation may activate T cells which could bind platelets through the CD40 receptor and activate them, leading to a further enhancement of the inflammatory process and an increased release of CD40L from the activated platelets into the maternal plasma.

The association between CD40L, increased COX-2 expression [31, 97], and prostaglandin generation [31] suggests that CD40L may participate in the process of labor. However, the lack of correlation between the admission-to-delivery interval and the maternal plasma concentrations of sCD40L, as well as the fact that patients with PTL and IAI had a significantly shorter admission to delivery interval than the other PTL subgroups, suggest that this may not be the case. Nevertheless, sCD40L may have local activity in the endometrium, myometrium, and uterine cervix [71] that is not reflected in the maternal circulation.

Conclusions

Preterm labor is associated with an elevated maternal platelet activation that can be induced by increased thrombin generation or maternal leukocyte activation. Our findings suggest that platelet activation occurs in preterm labor and, therefore, there is a potential role for anti-platelet agents (dietary or pharmacologic) in the treatment or prevention of this condition.

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References

- [1] Ahlawat S, Pati HP, Bhatla N, Fatima L, Mittal S. Plasma platelet aggregating factor and platelet aggregation studies in pre-eclampsia. *Acta Obstet Gynecol Scand*. 1996; 75:428–31.

- [2] Alacacioglu I, Ozcan MA, Piskin O, Yuksel F, Alacacioglu A, Demirkan F, et al. Increased concentration of soluble CD40 ligand in preeclampsia. *Clin Appl Thromb Hemost*. 2007;13:201–5.
- [3] Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. *Obstet Gynecol*. 1996;87:163–8.
- [4] Andre P, Prasad KS, Denis CV, He M, Papalia JM, Hynes RO, et al. CD40L stabilizes arterial thrombi by a beta3 integrin – dependent mechanism. *Nat Med*. 2002;8:247–52.
- [5] Apoil PA, Kuhlein E, Robert A, Rubie H, Blancher A. HIGM syndrome caused by insertion of an AluYb8 element in exon 1 of the CD40LG gene. *Immunogenetics*. 2007;59:17–23.
- [6] Armitage RJ, Fanslow WC, Strockbine L, Sato TA, Clifford KN, Macduff BM, et al. Molecular and biological characterization of a murine ligand for CD40. *Nature*. 1992;357:80–2.
- [7] Aruffo A, Hollenbaugh D, Wu LH, Ochs HD. The molecular basis of X-linked agammaglobulinemia, hyper-IgM syndrome, and severe combined immunodeficiency in humans. *Curr Opin Hematol*. 1994;1:12–8.
- [8] Ayhan A, Akkok E, Urman B, Yarali H, Dundar S, Kirazli S. Beta-thromboglobulin and platelet factor 4 levels in pregnancy and preeclampsia. *Gynecol Obstet Invest*. 1990;30:12–4.
- [9] Bagamery K, Landau R, Kvell K, Graham J. Different platelet activation levels in non-pregnant, normotensive pregnant, pregnancy-induced hypertensive and pre-eclamptic women. A pilot study of flow cytometric analysis. *Eur J Obstet Gynecol Reprod Biol*. 2005;121:117–8.
- [10] Banchereau J, Bazan F, Blanchard D, Briere F, Galizzi JP, van KC, et al. The CD40 antigen and its ligand. *Annu Rev Immunol*. 1994;12:881–922.
- [11] Bar J, Zosmer A, Hod M, Elder MG, Sullivan MH. The regulation of platelet aggregation in vitro by interleukin-1beta and tumor necrosis factor-alpha: changes in pregnancy and in pre-eclampsia. *Thromb Haemost*. 1997;78:1255–61.
- [12] Bloom AL. Physiology of blood coagulation. *Haemostasis*. 1990;20 (Suppl 1):14–29.
- [13] Bodis J, Torok A, Tinneberg HR. Hypothesis of preeclampsia requires inclusion of the role of platelets. *Am J Obstet Gynecol*. 1997;177:243–4.
- [14] Bolacchi F, Carbone M, Capozzi M, Ventura L, Cepparulo M, Niutta P, et al. Effect of different activation stimuli on the cytokine response of human macrophages to CD40L. *Cytokine*. 2001;16:121–5.
- [15] Brass LF, Zhu L, Stalker TJ. Minding the gaps to promote thrombus growth and stability. *J Clin Invest*. 2005;115:3385–92.
- [16] Brossart P, Grunebach F, Stuhler G, Reichardt VL, Mohle R, Kanz L, et al. Generation of functional human dendritic cells from adherent peripheral blood monocytes by CD40 ligation in the absence of granulocyte-macrophage colony-stimulating factor. *Blood*. 1998;92:4238–47.
- [17] Brown NL, Alvi SA, Elder MG, Bennett PR, Sullivan MH. Regulation of prostaglandin production in intact fetal membranes by interleukin-1 and its receptor antagonist. *J Endocrinol*. 1998;159:519–26.
- [18] Chaiworapongsa T, Espinoza J, Yoshimatsu J, Kim YM, Bujold E, Edwin S, et al. Activation of coagulation system in preterm labor and preterm premature rupture of membranes. *J Matern Fetal Neonatal Med*. 2002;11:368–73.
- [19] Chakrabarti S, Blair P, Freedman JE. CD40-40L signaling in vascular inflammation. *J Biol Chem*. 2007;282:18307–17.
- [20] Chakrabarti S, Varghese S, Vitseva O, Tanriverdi K, Freedman JE. CD40 ligand influences platelet release of reactive oxygen intermediates. *Arterioscler Thromb Vasc Biol*. 2005;25:2428–34.
- [21] Chirmule N, Tazelaar J, Wilson JM. Th2-dependent B cell responses in the absence of CD40-CD40 ligand interactions. *J Immunol*. 2000;164:248–55.
- [22] Ciferska H, Horak P, Hermanova Z, Ordeltova M, Zadrzil J, Tichy T, et al. The levels of sCD30 and of sCD40L in a group of patients with systemic lupus erythematoses and their diagnostic value. *Clin Rheumatol*. 2007;26:723–8.
- [23] Cipollone F, Ferri C, Desideri G, Paloscia L, Materazzo G, Mascellanti M, et al. Preprocedural level of soluble CD40L is predictive of enhanced inflammatory response and restenosis after coronary angioplasty. *Circulation*. 2003;108:2776–82.
- [24] Danese S, Katz JA, Saibeni S, Papa A, Gasbarrini A, Vecchi M, et al. Activated platelets are the source of elevated levels of soluble CD40 ligand in the circulation of inflammatory bowel disease patients. *Gut*. 2003;52:1435–41.
- [25] Danese S, Sans M, Scaldaferri F, Sgambato A, Rutella S, Cittadini A, et al. TNF-alpha blockade down-regulates the CD40/CD40L pathway in the mucosal microcirculation: a novel anti-inflammatory mechanism of infliximab in Crohn's disease. *J Immunol*. 2006;176:2617–24.
- [26] Danese S, Scaldaferri F, Vetrano S, Stefanelli T, Graziani C, Repici A, et al. Critical role of the CD40 CD40L ligand pathway in regulating mucosal inflammation-driven angiogenesis in inflammatory bowel disease. *Gut*. 2007;56:1248–56.
- [27] Davi G, Ferroni P. CD40-CD40L interactions in platelet activation. *Thromb Haemost*. 2005;93:1011–2.
- [28] Del CI, Cruz MA, Zhang H, Lopez JA, fshar-Kharghan V. Platelet activation leads to activation and propagation of the complement system. *J Exp Med*. 2005;201:871–79.
- [29] DiSanto JP, Bonnefoy JY, Gauchat JF, Fischer A, de Saint BG. CD40 ligand mutations in X-linked immunodeficiency with hyper-IgM. *Nature*. 1993;361:541–3.
- [30] Dong YL, Gangula PR, Fang L, Yallampalli C. Differential expression of cyclooxygenase-1 and -2 proteins in rat uterus and cervix during the estrous cycle, pregnancy, labor and in myometrial cells. *Prostaglandins*. 1996;52:13–34.
- [31] Dongari-Bagtzoglou AI, Thienel U, Yellin MJ. CD40 ligation triggers COX-2 expression in endothelial cells: evidence that CD40-mediated IL-6 synthesis is COX-2-dependent. *Inflamm Res*. 2003;52:18–25.
- [32] Elovitz MA, Baron J, Phillippe M. The role of thrombin in preterm parturition. *Am J Obstet Gynecol*. 2001;185:1059–63.
- [33] Elovitz MA, Wang Z, Chien EK, Rychlik DF, Phillippe M. A new model for inflammation-induced preterm birth: the role of platelet-activating factor and Toll-like receptor-4. *Am J Pathol*. 2003;163:2103–11.
- [34] Erez O, Hallak M, Lubet A, Maymon E, Mazor M, Tomer A. Platelets activation marker as predictor of severe preeclampsia in patients with history of preeclampsia. *Am J Obstet Gynecol*. 2001;185:S175.

- [35] Erkinheimo TL, Saukkonen K, Narko K, Jalkanen J, Ylikorkala O, Ristimäki A. Expression of cyclooxygenase-2 and prostanoid receptors by human myometrium. *J Clin Endocrinol Metab.* 2000;85:3468–75.
- [36] Facchetti F, Appiani C, Salvi L, Levy J, Notarangelo LD. Immunohistologic analysis of ineffective CD40-CD40 ligand interaction in lymphoid tissues from patients with X-linked immunodeficiency with hyper-IgM. Abortive germinal center cell reaction and severe depletion of follicular dendritic cells. *J Immunol.* 1995;154:6624–33.
- [37] Falco A, Romano M, Iapichino L, Collura M, Davi G. Increased soluble CD40 ligand levels in cystic fibrosis. *J Thromb Haemost.* 2004;2:557–60.
- [38] Felfernig-Boehm D, Salat A, Vogl SE, Murabito M, Felfernig M, Schmidt D, et al. Early detection of preeclampsia by determination of platelet aggregability. *Thromb Res.* 2000;98:139–46.
- [39] Gauchat JF, Aubry JP, Mazzei G, Life P, Jomotte T, Elson G, et al. Human CD40-ligand: molecular cloning, cellular distribution and regulation of expression by factors controlling IgE production. *FEBS Lett.* 1993;315:259–66.
- [40] Gauchat JF, Henchoz S, Mazzei G, Aubry JP, Brunner T, Blasey H, et al. Induction of human IgE synthesis in B cells by mast cells and basophils. *Nature.* 1993;365:340–3.
- [41] Gauchat JF, Henchoz S, Fattah D, Mazzei G, Aubry JP, Jomotte T, et al. CD40 ligand is functionally expressed on human eosinophils. *Eur J Immunol.* 1995;25:863–5.
- [42] Gauchat JF, Mazzei G, Life P, Henchoz S, Peitsch MC, Aubry JP, et al. Human CD40 ligand: molecular cloning, cellular distribution and regulation of IgE synthesis. *Res Immunol.* 1994;145:240–4.
- [43] Gervasi MT, Chaiworapongsa T, Naccasha N, Blackwell S, Yoon BH, Maymon E, et al. Phenotypic and metabolic characteristics of maternal monocytes and granulocytes in preterm labor with intact membranes. *Am J Obstet Gynecol.* 2001;185:1124–9.
- [44] Goules A, Tzioufas AG, Manousakis MN, Kirou KA, Crow MK, Routsias JG. Elevated levels of soluble CD40 ligand (sCD40L) in serum of patients with systemic autoimmune diseases. *J Autoimmun.* 2006;26:165–71.
- [45] Graf D, Korthauer U, Mages HW, Senger G, Kroczeck RA. Cloning of TRAP, a ligand for CD40 on human T cells. *Eur J Immunol.* 1992;22:3191–4.
- [46] Gross G, Imamura T, Vogt SK, Wozniak DF, Nelson DM, Sadovsky Y, et al. Inhibition of cyclooxygenase-2 prevents inflammation-mediated preterm labor in the mouse. *Am J Physiol Regul Integr Comp Physiol.* 2000;278:R1415–23.
- [47] Hakkinen T, Karkola K, Yla-Herttuala S. Macrophages, smooth muscle cells, endothelial cells, and T-cells express CD40 and CD40L in fatty streaks and more advanced human atherosclerotic lesions. Colocalization with epitopes of oxidized low-density lipoprotein, scavenger receptor, and CD16 (Fc gammaRIII). *Virchows Arch.* 2000;437:396–405.
- [48] Halim A, Kanayama N, el ME, Nakashima A, Bhuiyan AB, Khatun S, et al. Plasma P selectin (GMP-140) and glyocalicin are elevated in preeclampsia and eclampsia: their significances. *Am J Obstet Gynecol.* 1996;174:272–7.
- [49] Hanna N, Bonifacio L, Weinberger B, Reddy P, Murphy S, Romero R, et al. Evidence for interleukin-10-mediated inhibition of cyclo-oxygenase-2 expression and prostaglandin production in preterm human placenta. *Am J Reprod Immunol.* 2006;55:19–27.
- [50] Harlow FH, Brown MA, Brighton TA, Smith SL, Trickett AE, Kwan YL, et al. Platelet activation in the hypertensive disorders of pregnancy. *Am J Obstet Gynecol.* 2002;187:688–95.
- [51] Hellgren M. Hemostasis during normal pregnancy and puerperium. *Semin Thromb Hemost.* 2003;29:125–30.
- [52] Henn V, Slupsky JR, Grafe M, Anagnostopoulos I, Forster R, Muller-Berghaus G, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature.* 1998;391:591–4.
- [53] Henn V, Steinbach S, Buchner K, Presek P, Kroczeck RA. The inflammatory action of CD40 ligand (CD154) expressed on activated human platelets is temporally limited by coexpressed CD40. *Blood.* 2001;98:1047–54.
- [54] Hermann A, Rauch BH, Braun M, Schror K, Weber AA. Platelet CD40 ligand (CD40L) – subcellular localization, regulation of expression, and inhibition by clopidogrel. *Platelets.* 2001;12:74–82.
- [55] Hertelendy F, Rastogi P, Molnar M, Romero R. Interleukin-1beta-induced prostaglandin E2 production in human myometrial cells: role of a pertussis toxin-sensitive component. *Am J Reprod Immunol.* 2001;45:142–7.
- [56] Hoffman DR, Romero R, Johnston JM. Detection of platelet-activating factor in amniotic fluid of complicated pregnancies. *Am J Obstet Gynecol.* 1990;162:525–8.
- [57] Hollenbaugh D, Grosmaire LS, Kulas CD, Chalupny NJ, Braesch-Andersen S, Noelle RJ, et al. The human T cell antigen gp39, a member of the TNF gene family, is a ligand for the CD40 receptor: expression of a soluble form of gp39 with B cell co-stimulatory activity. *EMBO J.* 1992;11:4313–21.
- [58] Holmes VA, Wallace JM, Gilmore WS, McFaul P, Alexander HD. Soluble P-selectin levels during normal pregnancy: a longitudinal study. *Br J Obstet Gynecol.* 2002;109:997–1002.
- [59] Holthe MR, Lyberg T, Staff AC, Berge LN. Leukocyte-platelet interaction in pregnancies complicated with preeclampsia. *Platelets.* 2005;16:91–7.
- [60] Holthe MR, Staff AC, Berge LN, Lyberg T. Different levels of platelet activation in preeclamptic, normotensive pregnant, and nonpregnant women. *Am J Obstet Gynecol.* 2004;190:1128–34.
- [61] Hung DT, Vu TK, Wheaton VI, Ishii K, Coughlin SR. Cloned platelet thrombin receptor is necessary for thrombin-induced platelet activation. *J Clin Invest.* 1992;89:1350–3.
- [62] Hutt R, Ogunniyi SO, Sullivan MH, Elder MG. Increased platelet volume and aggregation precede the onset of preeclampsia. *Obstet Gynecol.* 1994;83:146–9.
- [63] Inwald DP, McDowall A, Peters MJ, Callard RE, Klein NJ. CD40 is constitutively expressed on platelets and provides a novel mechanism for platelet activation. *Circ Res.* 2003;92:1041–8.
- [64] Janes SL, Goodall AH. Flow cytometric detection of circulating activated platelets and platelet hyper-responsiveness in pre-eclampsia and pregnancy. *Clin Sci (Lond).* 1994;86:731–9.
- [65] Janes SL, Kyle PM, Redman C, Goodall AH. Flow cytometric detection of activated platelets in pregnant women prior to the development of pre-eclampsia. *Thromb Haemost.* 1995;74:1059–63.
- [66] Johansen B, Rakkestad K, Balboa MA, Dennis EA. Expression of cytosolic and secreted forms of phospho-

- lipase A(2) and cyclooxygenases in human placenta, fetal membranes, and chorionic cell lines. *Prostaglandins Other Lipid Mediat.* 2000;60:119–25.
- [67] Kahn ML, Nakanishi-Matsui M, Shapiro MJ, Ishihara H, Coughlin SR. Protease-activated receptors 1 and 4 mediate activation of human platelets by thrombin. *J Clin Invest.* 1999;103:879–87.
- [68] Karalis I, Nadar SK, Al YE, Blann AD, Lip GY. Platelet activation in pregnancy-induced hypertension. *Thromb Res.* 2005;116:377–83.
- [69] Kato T, Hakamada R, Yamane H, Nariuchi H. Induction of IL-12 p40 messenger RNA expression and IL-12 production of macrophages via CD40-CD40 ligand interaction. *J Immunol.* 1996;156:3932–8.
- [70] Kimura K, Tsuda H, Kwangseok Y, Tamura N, Kanai Y, Kobayashi S. Study of plasma levels of soluble CD40 ligand in systemic lupus erythematosus patients who have undergone plasmapheresis. *Ther Apher Dial.* 2005;9:64–8.
- [71] King AE, Kelly RW, Critchley HO, Malmstrom A, Sennstrom M, Phipps RP. Cd40 expression in uterine tissues: a key regulator of cytokine expression by fibroblasts. *J Clin Endocrinol Metab.* 2001;86:405–12.
- [72] Kniss DA. Cyclooxygenases in reproductive medicine and biology. *J Soc Gynecol Investig.* 1999;6:285–92.
- [73] Komura K, Sato S, Hasegawa M, Fujimoto M, Takehara K. Elevated circulating CD40L concentrations in patients with systemic sclerosis. *J Rheumatol.* 2004;31:514–9.
- [74] Konijnenberg A, Stokkers EW, van der Post JA, Schaap MC, Boer K, Bleker OP, et al. Extensive platelet activation in preeclampsia compared with normal pregnancy: enhanced expression of cell adhesion molecules. *Am J Obstet Gynecol.* 1997;176:461–9.
- [75] Konijnenberg A, van der Post JA, Mol BW, Schaap MC, Lazarov R, Bleker OP, et al. Can flow cytometric detection of platelet activation early in pregnancy predict the occurrence of preeclampsia? A prospective study. *Am J Obstet Gynecol.* 1997;177:434–42.
- [76] Koutroubakis IE, Theodoropoulou A, Xidakis C, Sfiridaki A, Notas G, Kolios G, et al. Association between enhanced soluble CD40 ligand and prothrombotic state in inflammatory bowel disease. *Eur J Gastroenterol Hepatol.* 2004;16:1147–52.
- [77] Langer F, Ingersoll SB, Amirkhosravi A, Meyer T, Siddiqui FA, Ahmad S, et al. The role of CD40 in CD40L- and antibody-mediated platelet activation. *Thromb Haemost.* 2005;93:1137–46.
- [78] Laskowska M, Laskowska K, Leszczynska-Gorzela B, Oleszczuk J. sCD40 ligand determined in maternal and umbilical cord blood in pregnancies complicated by preeclampsia with and without intrauterine growth retardation. *Gynecol Obstet Invest.* 2007;64:8–13.
- [79] Lederman S, Yellin MJ, Krichevsky A, Belko J, Lee JJ, Chess L. Identification of a novel surface protein on activated CD4+ T cells that induces contact-dependent B cell differentiation (help). *J Exp Med.* 1992;175:1091–101.
- [80] Lee Y, Lee WH, Lee SC, Ahn KJ, Choi YH, Park SW, et al. CD40L activation in circulating platelets in patients with acute coronary syndrome. *Cardiology.* 1999;92:11–6.
- [81] Lei XF, Ohkawara Y, Stampfli MR, Mastruzzo C, Marr RA, Snider D, et al. Disruption of antigen-induced inflammatory responses in CD40 ligand knockout mice. *J Clin Invest.* 1998;101:1342–53.
- [82] Leiberman JR, Hagay ZJ, Mazor M, Aharon M, Nathan I, Dvilansky A. Plasma and urine beta-thromboglobulin in severe preeclampsia. *Arch Gynecol Obstet.* 1988;243:165–8.
- [83] Levy J, Espanol-Boren T, Thomas C, Fischer A, Tovo P, Bordigoni P, et al. Clinical spectrum of X-linked hyper-IgM syndrome. *J Pediatr.* 1997;131:47–54.
- [84] Lindmark E, Tenno T, Siegbahn A. Role of platelet P-selectin and CD40 ligand in the induction of monocytic tissue factor expression. *Arterioscler Thromb Vasc Biol.* 2000;20:2322–8.
- [85] Lip GY, Patel JV, Hughes E, Hart RG. High-sensitivity C-reactive protein and soluble CD40 ligand as indices of inflammation and platelet activation in 880 patients with nonvalvular atrial fibrillation: relationship to stroke risk factors, stroke risk stratification schema, and prognosis. *Stroke.* 2007;38:1229–37.
- [86] Lok CA, Nieuwland R, Sturk A, Hau CM, Boer K, Vanbavel E, et al. Microparticle-associated P-selectin reflects platelet activation in preeclampsia. *Platelets.* 2007;18:68–72.
- [87] Lopez-Granados E, Temmerman ST, Wu L, Reynolds JC, Follmann D, Liu S, et al. Osteopenia in X-linked hyper-IgM syndrome reveals a regulatory role for CD40 ligand in osteoclastogenesis. *Proc Natl Acad Sci USA.* 2007;104:5056–61.
- [88] Loudon KA, Broughton PF, Heptinstall S, Fox SC, Mitchell JR, Symonds EM. Platelet reactivity and serum thromboxane B2 production in whole blood in gestational hypertension and pre-eclampsia. *Br J Obstet Gynaecol.* 1991;98:1239–44.
- [89] Ludwiczek O, Kaser A, Tilg H. Plasma levels of soluble CD40 ligand are elevated in inflammatory bowel diseases. *Int J Colorectal Dis.* 2003;18:142–7.
- [90] Luscher EF, Weber S. The formation of the haemostatic plug – a special case of platelet aggregation. An experiment and a survey of the literature. *Thromb Haemost.* 1993;70:234–7.
- [91] Macchi P, Villa A, Strina D, Sacco MG, Morali F, Brugnani D, et al. Characterization of nine novel mutations in the CD40 ligand gene in patients with X-linked hyper IgM syndrome of various ancestry. *Am J Hum Genet.* 1995;56:898–906.
- [92] Marcus AJ, Safier LB. Thromboregulation: multicellular modulation of platelet reactivity in hemostasis and thrombosis. *FASEB J.* 1993;7:516–22.
- [93] Marshall LS, Aruffo A, Ledbetter JA, Noelle RJ. The molecular basis for T cell help in humoral immunity: CD40 and its ligand, gp39. *J Clin Immunol.* 1993;13:165–74.
- [94] Mause SF, von HP, Zerneck A, Koenen RR, Weber C. Platelet microparticles: a transcellular delivery system for RANTES promoting monocyte recruitment on endothelium. *Arterioscler Thromb Vasc Biol.* 2005;25:1512–8.
- [95] Mellembakken JR, Solum NO, Ueland T, Videm V, Aukrust P. Increased concentrations of soluble CD40 ligand, RANTES and GRO-alpha in preeclampsia – possible role of platelet activation. *Thromb Haemost.* 2001;86:1272–6.
- [96] Missfelder-Lobos H, Teran E, Lees C, Albaiges G, Nicolaides KH. Platelet changes and subsequent development of pre-eclampsia and fetal growth restriction in women with abnormal uterine artery Doppler screening. *Ultrasound Obstet Gynecol.* 2002;19:443–8.
- [97] Mosheimer BA, Kaneider NC, Feistritz C, Djanani A, Sturn DH, Patsch JR, et al. CD40-ligand-dependent induction of COX-2 gene expression in endothelial cells

- by activated platelets: inhibitory effects of atorvastatin. *Blood Coagul Fibrinolysis*. 2005;16:105–10.
- [98] Mustard JF. The relationship between the structure of a thrombus or a hemostatic plug and the mechanisms involved in its formation. *Thromb Diath Haemorrh*. 1968;28 (Suppl):57–64; discussion 75–95:57–64.
- [99] Myatt L, Miodovnik M. Prediction of preeclampsia. *Semin Perinatol*. 1999;23:45–57.
- [100] Needleman P, Turk J, Jakschik BA, Morrison AR, Lefkowitz JB. Arachidonic acid metabolism. *Annu Rev Biochem*. 1986;55:69–102.
- [101] Nijm J, Wikby A, Tompa A, Olsson AG, Jonasson L. Circulating levels of proinflammatory cytokines and neutrophil-platelet aggregates in patients with coronary artery disease. *Am J Cardiol*. 2005;95:452–6.
- [102] Norris LA, Gleeson N, Sheppard BL, Bonnar J. Whole blood platelet aggregation in moderate and severe preeclampsia. *Br J Obstet Gynaecol*. 1993;100:684–8.
- [103] O'Sullivan BP, Linden MD, Frelinger AL III, Barnard MR, Spencer-Manzon M, Morris JE, et al. Platelet activation in cystic fibrosis. *Blood*. 2005;105:4635–41.
- [104] Oron G, Ben-Haroush A, Hod M, Orvieto R, Bar J. Serum-soluble CD40 ligand in normal pregnancy and in preeclampsia. *Obstet Gynecol*. 2006;107:896–900.
- [105] Oxenius A, Campbell KA, Maliszewski CR, Kishimoto T, Kikutani H, Hengartner H, et al. CD40-CD40 ligand interactions are critical in T-B cooperation but not for other anti-viral CD4+ T cell functions. *J Exp Med*. 1996;183:2209–18.
- [106] Pamuk GE, Vural O, Turgut B, Demir M, Umit H, Tezel A. Increased circulating platelet-neutrophil, platelet-monocyte complexes, and platelet activation in patients with ulcerative colitis: a comparative study. *Am J Hematol*. 2006;81:753–9.
- [107] Patel JV, Lim HS, Nadar S, Tayebjee M, Hughes EA, Lip GY. Abnormal soluble CD40 ligand and C-reactive protein concentrations in hypertension: relationship to indices of angiogenesis. *J Hypertens*. 2006;24:117–21.
- [108] Pekonen F, Rasi V, Ammala M, Viinikka L, Ylikorkala O. Platelet function and coagulation in normal and preeclamptic pregnancy. *Thromb Res*. 1986;43:553–60.
- [109] Phipps RP, Kaufman J, Blumberg N. Platelet derived CD154 (CD40 ligand) and febrile responses to transfusion. *Lancet*. 2001;357:2023–4.
- [110] Prasad KS, Andre P, Yan Y, Phillips DR. The platelet CD40L/GP IIb-IIIa axis in atherothrombotic disease. *Curr Opin Hematol*. 2003;10:356–61.
- [111] Ramshaw IA, Ramsay AJ, Karupiah G, Rolph MS, Mahalingam S, Ruby JC. Cytokines and immunity to viral infections. *Immunol Rev*. 1997;159:119–35.
- [112] Redline RW, Heller D, Keating S, Kingdom J. Placental diagnostic criteria and clinical correlation – a workshop report. *Placenta*. 2005;26 (Suppl A):S114–7.
- [113] rocha-Pinango CL, Ojeda A, Lopez G, Garcia L, Linares J. beta-thromboglobulin (beta-TG) and platelet factor 4 (PF4) in obstetrical cases. *Acta Obstet Gynecol Scand*. 1985;64:115–20.
- [114] Romero R, Snyder E, Scott D, Oyarzun E, Hobbins JC, Duffy TP. Beta-thromboglobulin during normal pregnancy, labor, and puerperium. *Am J Perinatol*. 1988;5:109–12.
- [115] Russo S, Bussolati B, Deambrosio I, Mariano F, Camussi G. Platelet-activating factor mediates CD40-dependent angiogenesis and endothelial-smooth muscle cell interaction. *J Immunol*. 2003;171:5489–97.
- [116] Saleh AA, Bottoms SF, Farag AM, Dombrowski MP, Welch RA, Norman G, et al. Markers for endothelial injury, clotting and platelet activation in preeclampsia. *Arch Gynecol Obstet*. 1992;251:105–10.
- [117] Saleh AA, Gerbasi FR, Mammen EF, Farag A. Increased platelet activation in preterm labor. *Thromb Res*. 1992; 65:475–7.
- [118] Sawdy R, Pan H, Sullivan M, Bennett P. Effect of selective vs. non-selective cyclo-oxygenase inhibitors on fetal membrane prostaglandin synthesis. *J Obstet Gynaecol*. 2003;23:239–43.
- [119] Schonbeck U, Libby P. The CD40/CD154 receptor/ligand dyad. *Cell Mol Life Sci*. 2001;58:4–43.
- [120] Schonbeck U, Varo N, Libby P, Buring J, Ridker PM. Soluble CD40L and cardiovascular risk in women. *Circulation*. 2001;104:2266–8.
- [121] Shepherd DM, Kerkvliet NI. Disruption of CD154:CD40 blocks generation of allograft immunity without affecting APC activation. *J Immunol*. 1999;163:2470–7.
- [122] Sixma JJ, Wester J. The hemostatic plug. *Semin Hematol*. 1977;14:265–99.
- [123] Slupsky JR, Kalbas M, Willuweit A, Henn V, Kroczeck RA, Muller-Berghaus G. Activated platelets induce tissue factor expression on human umbilical vein endothelial cells by ligation of CD40. *Thromb Haemost*. 1998;80:1008–14.
- [124] Steinhilbl SR. Platelets as mediators of inflammation. *Hematol Oncol Clin North Am*. 2007;21:115–21.
- [125] Stjernholm-Vladic Y, Stygar D, Mansson C, Masironi B, Akerberg S, Wang H, et al. Factors involved in the inflammatory events of cervical ripening in humans. *Reprod Biol Endocrinol*. 2004;2:74.
- [126] Suarez CR, Gonzalez J, Menendez C, Fareed J, Fresco R, Walenga J. Neonatal and maternal platelets: activation at time of birth. *Am J Hematol*. 1988;29:18–21.
- [127] Sugiura T, Kawaguchi Y, Harigai M, Takagi K, Ohta S, Fukasawa C, et al. Increased CD40 expression on muscle cells of polymyositis and dermatomyositis: role of CD40-CD40 ligand interaction in IL-6, IL-8, IL-15, and monocyte chemoattractant protein-1 production. *J Immunol*. 2000;164:6593–600.
- [128] Tomer A. Platelet activation as a marker for *in vivo* prothrombotic activity: detection by flow cytometry. *J Biol Regul Homeost Agents*. 2004;18:172–7.
- [129] Tygart SG, McRoyan DK, Spinnato JA, McRoyan CJ, Kitay DZ. Longitudinal study of platelet indices during normal pregnancy. *Am J Obstet Gynecol*. 1986;154: 883–7.
- [130] Urbich C, Dernbach E, Aicher A, Zeiher AM, Dimmeler S. CD40 ligand inhibits endothelial cell migration by increasing production of endothelial reactive oxygen species. *Circulation*. 2002;20:106:981–6.
- [131] Vakkalanka RK, Woo C, Kirou KA, Koshy M, Berger D, Crow MK. Elevated levels and functional capacity of soluble CD40 ligand in systemic lupus erythematosus sera. *Arthritis Rheum*. 1999;42:871–81.
- [132] van Kooten C, Banchereau J. CD40-CD40 ligand. *J Leukoc Biol*. 2000;67:2–17.
- [133] van KC, Banchereau J. Functions of CD40 on B cells, dendritic cells and other cells. *Curr Opin Immunol*. 1997;9:330–7.
- [134] Vecchiarelli A, Retini C, Pietrella D, Monari C, Kozel TR. T lymphocyte and monocyte interaction by CD40/CD40 ligand facilitates a lymphoproliferative response and killing of *Cryptococcus neoformans* in vitro. *Eur J Immunol*. 2000;30:1385–93.

- [135] Xu H, Arnaud F, Tadaki DK, Burkly LC, Harlan DM, Kirk AD. Human platelets activate porcine endothelial cells through a CD154-dependent pathway. *Transplantation*. 2001;72:1858–61.
- [136] Yin D, Ma L, Shen J, Byrne GW, Logan JS, Chong AS. CTLA-41g in combination with anti-CD40L prolongs xenograft survival and inhibits anti-gal ab production in GT-Ko mice. *Am J Transplant*. 2002;2:41–7.
- [137] Yoneyama Y, Suzuki S, Sawa R, Kiyokawa Y, Power GG, Araki T. Plasma adenosine levels and P-selectin expression on platelets in preeclampsia. *Obstet Gynecol*. 2001;97:366–70.
- [138] Yoneyama Y, Suzuki S, Sawa R, Miura A, Doi D, Otsubo Y, et al. Plasma nitric oxide levels and the expression of P-selectin on platelets in preeclampsia. *Am J Obstet Gynecol*. 2002;187:676–80.
- [139] Zeller JA, Lenz A, Eschenfelder CC, Zunker P, Deuschl G. Platelet-leukocyte interaction and platelet activation in acute stroke with and without preceding infection. *Arterioscler Thromb Vasc Biol*. 2005;25:1519–23.
- [140] Zhang Y, Cao HJ, Graf B, Meekins H, Smith TJ, Phipps RP. CD40 engagement up-regulates cyclooxygenase-2 expression and prostaglandin E2 production in human lung fibroblasts. *J Immunol*. 1998;160:1053–7.

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