bind to *P. falciparum (15, 16)*, reduced affinity for LFA-1, and no apparent affinity for fibrinogen *(16)*. Important insights into the function of ICAM-1 may be obtained from future investigations of relationships in additional individuals homozygous and heterozygous for K29M ICAM-1.

In summary, previous studies exploring sICAM-1 as a marker for cardiovascular and other diseases may need to be reevaluated in light of the demonstration that commercial sICAM-1 ELISAs vary markedly in their ability to recognize this ICAM-1 variant, which is common (20–35% allele frequency) in African-American populations.

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Detection of Fetal DNA and RNA in Placenta-Derived Syncytiotrophoblast Microparticles Generated in Vitro,

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Fetal DNA and RNA can be readily detected in maternal plasma samples *(1–4)*. Most of this material appears to be of placental origin *(5)*, and it appears to be in a predominantly cell-free form *(2)*, whereas circulatory mRNA is membrane-encapsulated *(6)*.

Pregnancy is associated with the release of microparticles by the syncytiotrophoblast membrane into the maternal circulation *(7)*. These particles, frequently termed STBM, are released by turnover of the syncytiotrophoblast monolayer covering the entire villous tree *(8–11)*. This process of normal physiologic syncytiotrophoblast turnover involves the release of apoptotic material into the maternal circulation by the extrusion of syncytial knots and the associated release of STBM *(8–11)*. The amount of material that is released by apoptotic shedding of syncytial knots (and STBM) is several grams per day *(9)*, and the circulating concentrations are increased significantly in preeclampsia *(11)*.

STBM particles have been suggested to evoke the mild maternal inflammatory response accompanying normal pregnancies *(12)*, and increased release has been proposed to play a role in the etiology of preeclampsia by triggering maternal endothelial cell damage *(13, 14)*.

As these particles are difficult to detect and prepare from maternal blood samples, use is frequently made of in vitro-prepared particles to study their physiologic activity *(13)*. In this context, we have recently extensively examined three different modes of STBM preparation: mechanical dissection of fresh placental villous tissues; in vitro

cultures of villous explants; and perfusion of single placental cotyledons *(15)*.

All three preparations lead to the production of STBM as confirmed by the presence of the syncytiotrophoblast-specific protein placental alkaline phosphatase, physiologic activity on human endothelial cell cultures, and their morphology, as seen by scanning electron microscopy *(15)*.

Intrigued by the seemingly parallel increased release of STBM and circulatory fetal nucleic acids in preeclampsia *(2, 7, 14, 16, 17)* and its potential relationship to the placental distress associated with the disorder, we examined whether these two events may be more intimately associated. For this reason, we examined whether fetal nucleic acids are physically associated with STBM.

In our study, after the receipt of informed consent and Institutional Review Board approval, we prepared, by the three previously described methods *(15)*), STBM from placentas from normal full-term pregnancies in which healthy males were delivered. In brief, villous explants were cultured in a 1:1 mixture of DMEM and Ham's F-12 medium (Gibco Invitrogen Life Technologies) supplemented with 10 g/L antimycotics and antibiotics (Gibco Invitrogen Life Technologies), 100 mL/L fetal calf serum, 25 kIU/L heparin (Roche Diagnostics), 50 kIU/L aprotinin (Fluka Chemicals), and 2 mmol/L $MgSO_4$ for 72 h at 37 °C in 5% CO_2, after which the culture supernatant was collected and stored at −70 °C. Mechanically dissected STBM were prepared by washing villous tissue three times in phosphate-buffered saline (PBS) containing 100 mmol/L $CaCl_2$, after which the tissue was manually dissected and rinsed overnight at 4 °C in 100 mL of 0.15 mol/L NaCl supplemented with 10 g/L antimycotics and antibiotics. After rinsing, the tissues were discarded, and the supernatant was collected and stored at −70 °C. For the collection of STBM from placental perfusion, the intervillous space (maternal compartment) of a single cotyledon was perfused with an in vitro system, using a medium composed of NCTC-135 tissue culture medium diluted with Earle's buffer (1:1) with added glucose (1.33 g/L), dextran 40 (10 g/L), 40 g/L bovine serum albumin, heparin (2.5 kIU/L), and clamoxyl (250 mg/L). The perfusates from the intervillous space were collected and stored at −70 °C.

STBM from these three preparations were harvested by a three-step centrifugation procedure at 4 °C: 1000*g* for 10 min, 10 000*g* for 10 min, and 70 000*g* for 90 min. The final pellet, containing the STBM, was washed once with PBS, resuspended in 1 mL of sterile PBS containing 50 g/L sucrose, and stored at −70 °C until use.

We examined the presence of fetal DNA and RNA in these STBM. The amount of fetal DNA was measured by a TaqMan® real-time PCR assay for a Y-chromosome-specific sequence (*SRY*) *(17)*, whereas the presence of fetal mRNA was quantified by a similar quantitative reverse transcription-PCR (RT-PCR) assay for the corticotropin-releasing hormone (*CRH*) gene, which is known to be expressed in the placenta *(18)*.

The protein content in each STBM preparation was quantified with the advanced protein assay reagent (Cytoskeleton). DNA was extracted from STBM by use of the High Pure PCR Template Preparation Kit (Roche Diagnostics). Total RNA was isolated using High Pure RNA Isolation Kit (Roche Diagnostics) and eluted in 50 μL of elution buffer. cDNA was reverse-transcribed from 500 ng of total RNA by use of a commercial reverse transcription system (Promega).

Table 1. Concentrations of fetal DNA and *CRH* mRNA in STBM prepared by villous explant culture, mechanical dissection, and placental perfusion.[a]

STBM preparation method	Copies/mg of STBM	
	DNA (*SRY*)	mRNA (*CRH*)
Villous explant culture		
Median	94 530.5	4986
Range	9444–158 135	1232–10 500
Mechanical dissection		
Median	2593	121 144.5
Range	307–7745	17 868–331 607
Placental perfusion		
Median	620	222 352.5
Range	268–2975	98 190–275 981

[a] Six placentas were used for each STBM preparation. Fetal DNA (*SRY* locus) and mRNA (*CRH*) concentrations were determined by real-time PCR and real-time RT-PCR, respectively.

Real-time quantitative PCR and real-time quantitative RT-PCR were used for all DNA and mRNA quantifications as described previously *(17, 19, 20)*. The real-time PCR and real-time RT-PCR reactions were set up according to the manufacturer's instructions (Applied Biosystems) in a reaction volume of 25 μL. Each sample was analyzed in duplicate, and the corresponding calibration curve was run in parallel with each analysis. Absolute concentrations of *CRH* mRNA and *SRY* DNA were expressed as copies/mg of STBM.

Our analysis showed that all STBM preparations contained both fetal DNA and mRNA, although the concentrations of each of these fetal analytes differed in the three preparations (Table 1). In this regard, the highest concentration of fetal DNA was detected in STBM prepared by in vitro villous explant cultures (Fig. 1A), whereas the highest concentration of fetal CRH RNA was present in STBM obtained by perfusion of a placental cotyledon (Fig. 1B).

Although we took great care to harvest as many of the STBM as possible by the use of high-speed ultracentrifugation, we were still able to detect considerable amounts of fetal DNA in the STBM-free supernatant of villous explant preparations. The amounts of fetal DNA in the supernatants cleared by ultracentrifugation were approximately fourfold higher than those in the matching STBM preparations. Provided that these results can be extrapolated to the release of fetal DNA into maternal plasma, then it is possible that the major proportion of circulatory fetal DNA may exist in a completely particle-free form. On the other hand, very little CRH mRNA was detected in the STBM-free villous explant supernatants (~10% of that

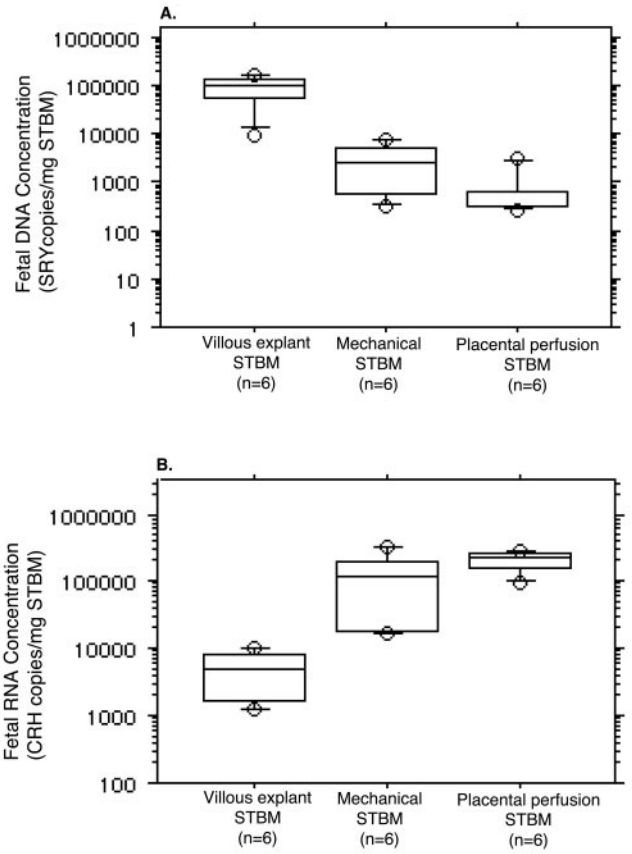

Fig. 1. Box-plots of fetal DNA and mRNA concentrations in STBM prepared by villous explant culture, mechanical dissection, and placental perfusion.

Fetal DNA (*SRY* locus; *A*) and mRNA (*CRH*; *B*) concentrations were determined by real-time PCR and real-time RT-PCR, respectively, and are represented as copies/mg of STBM. Six placentas were used for each STBM preparation. The *line inside each box* represents the median value; the *limits of the boxes* represent the 75th and 25th percentiles; the *error bars* indicate the 10th and 90th percentiles; and ○ indicate outliers.

present in the STBM preparation). Again, provided that the observations we have made with in vitro-generated STBM correspond to the in vivo situation, then it is possible that fetal mRNA species may be largely associated with membrane particles, as has been reported previously *(6)*. It is also likely, that these few mRNA species present in the cleared culture supernatants are associated with very small microparticles that are not effectively harvested by high-speed ultracentrifugation.

In our study, STBM prepared by placental perfusion may be regarded as being the closest representatives of those generated under normal physiologic conditions in that here STBM are collected directly from the intervillous space, the site where they would typically enter the maternal circulation. The presence of fetal DNA and mRNA species in all three STBM preparations, particularly in those obtained by perfusion of the maternal compartment of the placenta under near-physiologic conditions, implies that cell-free fetal nucleic acids may similarly be associated with STBM in vivo. This facet, however, needs to be confirmed by the analysis of STBM isolated from maternal blood samples, currently a technically demanding undertaking.

The difference we observed in fetal DNA and mRNA content in the three STBM preparations may be attributable to the manner in which these particles are generated, in that those obtained by perfusion or in vitro culture are generated predominantly by apoptotic cell turnover, in contrast to STBM isolated by mechanical disruption, in which release of STBM may involve necrotic pathways *(15)*.

In this context it is worth noting that the release of STBM differs in normal pregnancy compared with preeclampsia *(9, 21)*. In normal pregnancy, the shedding of placental particles occurs continuously as part of the self-renewal of the syncytiotrophoblast monolayer, a process that involves apoptosis of the aged nuclei and fusion of cytotrophoblast cells *(9)*. In preeclampsia, this process is altered in that syncytiotrophoblast apoptosis rates are dramatically increased, which has been suggested to contribute to the increased release of STBM, possibly by apo-necrotic pathways *(21)*.

Therefore, provided that circulatory fetal nucleic acids are indeed associated with STBM in vivo, then it is possible that the analysis of the fetal DNA and RNA content of STBM in the maternal circulation in normal and pathologic pregnancies may yield new insights into the underlying mechanisms leading to their release by the syncytiotrophoblast. Furthermore, if this proviso concerning the presence of fetal nucleic acids with STBM in vivo is true, then it may also provide a new strategy for the enrichment of these fetal analytes from maternal blood samples.

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High Ischemia-Modified Albumin Concentration Reflects Oxidative Stress But Not Myocardial Involvement in Systemic Sclerosis, *Didier Borderie,[1†*] Yannick Allanore,[2†] Christophe Meune,[3] Jean Y. Devaux,[3] Ohvanesse G. Ekindjian,[1] and André Kahan[2]* (Departments of [1] Biochemistry A, [2] Rheumatology A, and [3] Nuclear Medicine, Paris V University, Assistance Publique-Hôpitaux de Paris, Cochin Hospital, Paris, France; † these authors contributed equally to this work; * address correspondence to this author at: Service de Biochimie A, 27 rue du faubourg Saint-Jacques, 75014 Paris, France; fax 33-1-5841-1585, e-mail didier.borderie@cch.ap-hop-paris.fr)

Systemic sclerosis (SSc) is a connective tissue disease characterized by widespread vascular lesions and fibrosis of the skin and internal organs. In SSc, vasospasm causes frequent episodes of reperfusion injury and free-radical-mediated endothelial disruption. Primary myocardial involvement is far more common than initially suspected on clinical grounds *(1–5)* and affects survival rates because it is associated with a poor prognosis *(6, 7)*. Myocardial fibrosis is thought to occur secondarily to repeated focal ischemia in the coronary microcirculation as a result of abnormal vasoreactivity, with or without associated structural vascular disease *(4, 5)*. The early and accurate identification of cardiac involvement is therefore of paramount clinical importance.

The concentration of ischemia-modified albumin (IMA), as measured by the albumin cobalt binding test (Ischemia Technologies, Inc.), is a new marker to rule out transient myocardial ischemia *(8, 9)*. This test measures the binding of exogenous cobalt to the NH_2 terminus of human albumin. In the presence of myocardial ischemia, structural changes occur in the NH_2 terminus of albumin, rapidly reducing its capacity to bind transition metal ions after an ischemic event *(10)*.

We assessed the accuracy of the albumin cobalt binding test for detecting ischemia in SSc patients and investigated the roles of myocardial ischemia and peripheral oxidative stress in this condition. We also considered carbonyl residues and advanced oxidation protein products (AOPP) as factors indicative of protein oxidation.

We included consecutive patients hospitalized for systematic follow-up who fulfilled the American Rheumatism Association preliminary criteria for SSc. The exclusion criteria were pregnancy; symptoms of heart failure, including class III or IV dyspnea (New York Heart Association); venous distension and recent major lower limb edema; pulmonary arterial hypertension (systolic arterial pressure >40 mmHg and/or mean artery pressure >25 mmHg, determined by echocardiography); severe pulmonary involvement (forced vital capacity or carbon monoxide diffusing capacity <50% of the predicted normal value); renal involvement (creatinine concentration > 106 μmol/L); or severe disease complications such as cancer or gangrene. At the time of the study, none of the patients was taking medication for cardiac or vascular disease. If previously treated with vasodilators, patients were asked to stop taking these drugs 3 days before admission. This interruption period corresponds to five times the half-life of calcium channel blockers and angiotensin-converting enzyme. All patients gave informed consent for all procedures, and the study was approved by the local ethics committee (Paris, Cochin).

We assessed the following in all patients: blood cell count, Westergren erythrocyte sedimentation rate, serum creatinine concentration, and anti-centromere and anti-topoisomerase I antibody concentrations. The concentration of high-sensitivity C-reactive protein was measured by immunoturbidimetry on a Roche modular PP instrument using the CRP latex Tina-quant® assay (Roche Diagnostics). Pulmonary involvement was assessed by computed tomography scan, forced vital capacity, and the ratio of carbon monoxide diffusion capacity to hemoglobin concentration. Pulmonary arterial systolic pressure was determined by Doppler echocardiography at rest. The thickness of the skin was quantified on a scale of 0–3, by use of the modified Rodman skin scoring technique, for each of 17 body surface areas *(11, 12)*.

All patients underwent thallium-201 myocardial single-photon-emission computerized tomography at rest, using a gamma camera (Starport 400AT; General Electric) interfaced with an ADAC computer (DPS 3300). Myocardial perfusion was assessed semiquantitatively by two expe-