Rev. Inst. Med. trop. São Paulo
35(3):253-257, maio-junho, 1993

AGGREGATORY BEHAVIOUR OF PLATELETS INCUBATED WITH SUBCELLULAR FRACTIONS OF NORMAL AND CHAGASIC HUMAN SYNCYTIOTROPHOBLAST

A.R. EYNARD, S. MUÑOZ, L. RUIZ MORENO, M.E. PASQUALINI & S.P. de FABRO.

SUMMARY

The surface of human syncytiotrophoblast does not induce maternal blood platelet aggregation even though it is not an endothelium. It can be surmised that as occurs in endothelial injury the subcellular components of the syncytiotrophoblast may have pro-or antiaggregatory activity. During congenital Chagas' disease which is associated to trophoblast lesions, platelets may play a role in the development of *T. cruzi*-induced placentitis. In the present work the aggregatory behaviour of normal human blood platelets was recorded after their challenging with subcellular fractions of syncytiotrophoblast isolated from normal and chagasic women. Nuclear, Mitochondrial, Microsomal and Supernatant fractions isolated from normal and chagasic syncytiotrophoblast failed to induce *per se* any aggregatory reaction on platelets. When samples of platelet-rich plasma (PRP) were preincubated with normal and chagasic nuclear fractions and then stimulated with collagen at threshold level (CT-PRP) an inhibition of the aggregatory response was observed. Treatment of CT-PRP with normal and chagasic mitochondrial fractions induced inhibition of platelet aggregation whereas only chagasic fraction reduced latency time. Microsomal fraction from normal placentas showed no significant effects on platelet aggregation. It is concluded that subcellular fractions of normal human syncytiotrophoblast do not exhibit any effect on platelet aggregation, whereas those subcellular fractions enriched in intracellular membrane components isolated from chagasic placentas inhibit platelet aggregation.

KEY WORDS: Human congenital Chagas' disease; Human platelet aggregation; Trypanosoma cruzi; Chagas' disease; Placenta.

INTRODUCTION

Platelet aggregation occurs when contact with the subendothelium surface is made. They also promptly react when incubated with subcellular fractions of certain organs and tissues such as liver, myocardium, brain, skeletal muscle and kidney[15]. Even though the luminal surface of human syncytiotrophoblast is not considered a typical endothelium, maternal platelet aggregation does not occur.

The passage of *T. cruzi* from the blood of an infected pregnant woman to the fetus through the placenta may produce congenital Chagas' disease, in which widespread hemorraghic necrosis occurs[1,6].

Platelets play a role in the capture of *T. cruzi* from circulation[16]. Large areas of denudated trophoblastic villi and necrotic lesions had been reported in placental tissues from chagasic women[1] suggesting that platelet disbalance may play a role in the hemorraghic lesions described in this infectious disease. This study was performed to determine the aggregatory response of resting and threshold stimulated human platelets challenged with subcellular fractions of normal or chagasic human syncytiotrophoblast.

MATERIAL AND METHODS

Platelets were obtained from human blood of 28 volunteers of both sexes, 20-40 years old.

Address for correspondence: Dr. A.R. Eynard. II Catedra de Histologia e Instituto de Biologia Celular, Facultad de Ciencias Medicas; CONICET. Agencia Postal N. 4, 5000. Córdoba, ARGENTINA.

Blood was collected into 3.8% sodium citrate (9:1, v/v) and centrifugated to obtain platelet rich plasma (PRP) and platelet poor plasma (PPP). In order to detect any synergistic or inhibitory effect induced by the subcellular fractions, samples of 450µl of PRP were preincubated for 5 minutes with the corresponding subcellular fraction and then stimulated at threshold level with collagen (calf skin, Type I, Sigma Chemical Co.). The appropriate collagen concentrations were established through preliminary experiments in each volunteer as described elsewhere[14]. Negative and positive control samples were obtained by incubating PRP with phosphate buffered saline (PBS) or collagen, respectively. Aggregation studies were performed using a single channel aggregometer (CYBORG Electrónica, Argentina). The following parameters were studied: 1. The length of the lag time, recorded from the addition of the subcellular fraction until the onset of the first sharp pen deflection. 2. The maximal transmission (scale deflection), indicator of the completion of the aggregation expressed as a *percentage* of aggregation[5,11]. Data were evaluated by variance analysis (ANOVA). The significance level was established at $p<0.05$. *Placentae* were obtained after normal term delivery from 10 normal and 4 chagasic women. The pathological condition was diagnosed by immunofluorescence and Machado-Guerreiro tests. These women did not have clinical evidence of the disease.

Nuclear, mitochondrial, microsomal and supernatant fractions were obtained from isolated normal and chagasic syncytiotrophoblast by ultracentrifugation[8,14]. Purity of the fractions was evaluated by electron microscopy as previously described[2].

Samples of normal and chagasic fractions suspended in PBS and containing 135, 270 and 405µg/protein were added to 950µl aliquots of PRP and then stimulated or not with collagen as described above.

RESULTS

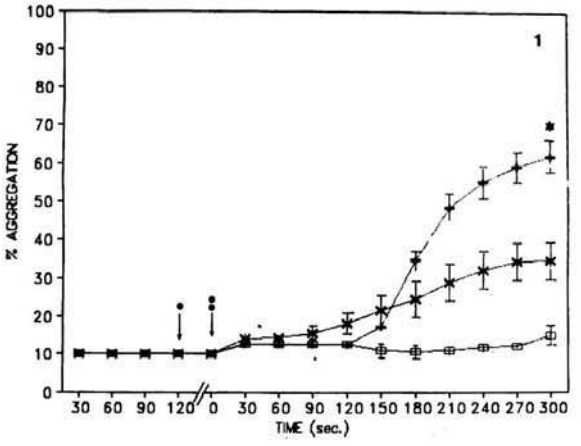
Compared with positive controls stimulated only with collagen, nuclear, mitochondrial, microsomal and supernatant fractions isolated from both normal and chagasic syncytiotrophoblast at the three concentrations studied failed to induce *per se* any aggregatory changes on normal resting platelet. As shown in figure 1 pretreatment with normal nuclear fraction upon CT-PRP induced inhibition of the *percent* of aggregation, being this effect more intense with the chagasic fraction, in comparison to controls stimulated only with collagen. Similar inhibition was recorded when normal and chagasic mitochondrial fractions were pre-added to CT-PRP samples whereas only chagasic fraction shortened the lag time period (fig. 2). Normal microsomal fraction induced an increase of the *percent* of aggregation of CT-PRP samples and the chagasic microsomal fraction exerted an inhibitory effect on the *percent* of aggregation of CT-PRP but the observed differences were on the limits of signification (fig. 3). No modifications were recorded on the lag time parameter with both fractions with respect to the positive controls. Preincubation of CT-PRP with normal and chagasic supernatant showed erratic behaviour; thus, the results were inconclusive.

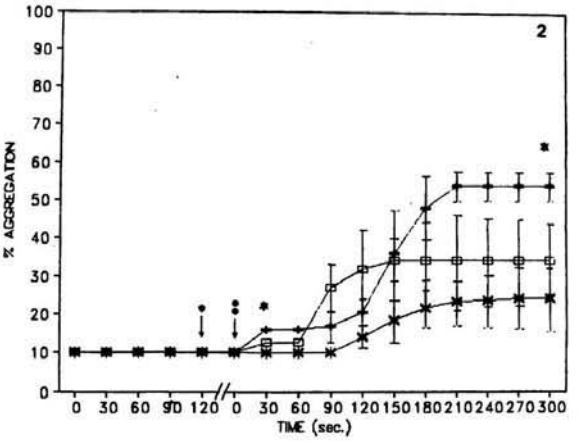
DISCUSSION

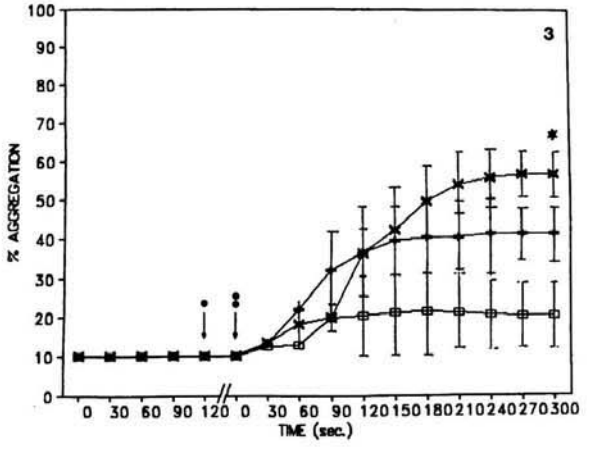
Platelets seem to be important in the removal of circulating *T. cruzi* from the blood stream[16]. Chronic tissue injury induced by nidation of *T. cruzi* in the trophoblast is accompanied by the recruitment of macrophages and leukocytes[1]. This fact could favor the activation of lipolytic enzymes and phospholipases[2], which in turn trigger the arachidonate cascade with a disbalanced production of vasoactive metabolites such as the pro-aggregatory thromboxane B2 and antiaggregatory hydroxyeicosatetraenoic acids[3,7,10].

Even though the subcellular fractions were isolated from human syncytiotrophoblast devoided of connective tissue cells, the remaining activity of hydrolitic enzymes and ADP could be responsible of the shortening of the lag time induced by the pre-addition of chagasic mitochondrial fractions on CT-PRP samples in the early phase of aggregation.

Subcellular mitochondrial and nuclear fractions isolated from normal placentae induced a significant inhibition of platelet activity. This inhibition was more marked when platelets were incubated with subcellular fractions obtained from chagasic placentae. The inhibition of the aggregation observed with most of the subcellular fractions, which were more evi-







Figures 1-3. Aggregatory response of collagen- stimulated human platelets to which nuclear (1), mitochondrial (2) or microsomal (3) fractions from normal or chagasic human syncytiotrophoblast had previously been added. Each experiment was run at least in duplicate and the results are given as mean ± SEM evaluated by variance analysis. References: control + (collagen); X stimulated with normal fractions of human syncytiotrophoblast; □ stimulated with chagasic syncytiotrophoblast; • addition of the subcellular fraction; : collagen; * significant at $p<0.05$.

dent in chagasic placentae, having a thick basal membrane[1,6] could be modulated by fibronectins and other antiaggregatory glycoproteins originated from this basal membrane. Fibronectin is a glycoprotein which inhibits the last stage of platelet aggregation but induces a shortening of the lag time[5,13,17] probably by the limitation of platelet-collagen[11] interaction.

Circulating epimastigotes of *T. cruzi* permeate syncytiotrophoblast inducing small multifocal cytological injuries which progress until the cell[1,6] disruption. These abnormalities expose intracellular membranes of human syncytiotrophoblast to the contact with both, platelets and circulating parasites, thus favoring the activation of the former. Indeed, platelets seem to be involved in the mechanisms of protection against *T. cruzi* since mice experimentally depleted of platelets were more susceptible to infection with *T. cruzi*[16]. In our experimental conditions microsomal fractions were enriched in plasma membrane whereas other internal membranes components such as endoplasmic reticulum were present to a lesser extent as indicated by their increase of the 5' nucleotidase and alkaline phosphatase activities and electron microscope observations[2,6,14]. It has been reported that in normal conditions the luminal border of the human syncytiotrophoblast is the main contributor of plama membrane to the microsomal fraction[19]. Although minor inhibitory effect of microsomal fraction on the aggregation were recorded when this fraction was isolated from chagasic women it may be concluded that those areas of the syncytiotrophoblast usually exposed to the maternal circulating platelets do not exert any detectable action on the aggregation. On the contrary, those subcellular fractions enriched in intracellular membrane components inhibit platelet aggregation. This effect was more evident in chagasic placentae suggesting that the modifications of membranes induced by *T. cruzi*[12] might be the reason for the demonstrated platelet functional abnormalities and might also be involved in the pathogenesis of chagasic placentitis.

RESUMO

Comportamento agregatório das plaquetas incubadas com frações subcelulares de sinciciotrofoblasto humano normal e chagásico.

A superfície do sinciciotrofoblasto humano não induz agregação das plaquetas maternas apesar de não ser um endotélio. Lesões endoteliais propiciam o aparecimento de agregados plaquetários, o que nos leva a questionar se os componentes subcelulares do sinciciotrofoblasto também poderiam propiciar eventos semelhantes. Na doença de Chagas congênita, que está associada a lesões a nível de trofoblasto, as plaquetas poderiam desempenhar algum papel no desenvolvimento da placentitis induzida pelo *T. cruzi*. Neste trabalho estudou-se o comportamento agregatório das plaquetas humanas normais expostas a frações subcelulares do sinciciotrofoblasto isolado de placentas de mulheres normais e chagásticas. As frações *Nuclear, Mitocondrial, Microsomal* e *Sobrenadante* isoladas do sincicitrofoblasto normal ou chagásico não induziram *per se* reação agregatória de plaquetas. Quando amostras de plasma rico em plaquetas (PRP) foram pré-incubadas com fração nuclear de placentas normais ou chagásicas e pós-estimuladas com doses limiares de colágeno (DLC-PRP) observou-se uma inibição da resposta agregatória. O tratamento de DLC-PRP com fração Mitocondrial de trofoblasto normal e chagásico também induziu inibição da agregação plaquetária porém somente a fração chagásica diminuiu o tempo de latência. A fração Microsomal das placentas normais não provocou diferenças significativas na agregação plaquetária. Conclui-se que as frações subcelulares do sinciciotrofoblasto humano normal não tem ação significativa na agregação plaquetária, enquanto que a incubação com frações subcelulares de placentas chagásicas, enriquecidas em componentes membranosos intracelulares, induziu a inibição da agregação plaquetária.

ACKNOWLEDGMENTS

This work was partialy supported by grants of CONICOR (Cordoba) and CONICET (Argentina) to Drs. ARE and SPF. We are indebted to Dr. Martha Gonzalez Cremer for critical reading of the manuscript and to Dra. Martha Romano for her collaboration in the obtention of some of the placentae.

REFERENCES

1. BITTENCOURT, A.L. - Congenital Chagas' disease. **Amer. J. Dis. Child., 130:97-103, 1976.**

2. CALDERON, R.O. & de FABRO, S.P. - **Trypanosoma cruzi.** Fusogenic ability of membranes from cultures of epimastigotes in interactions with human syncytiotrophoblast. **Exp. Parasit., 56:169-179, 1983.**

3. CHANG, J.; BLAZEK, E.; KREFT, A.F. & LEWIS, A.J. - Inhibition of platelet and neutrophil phospholipase A2 by hydroxy-eicosatetraenoic acids (HETEs). A novel pharmacological mechanism for regulating free-fatty acid release. **Biochem. Pharmacol., 34:1571-1575, 1985.**

4. EYNARD, A.R.; TREMOLI, E.; CARUSO, D.; MAGNI, F.; SIRTORI, C.R. & GALLI, G. - Platelet formation of 12-hydroxy eicosatetraenoic acid and thromboxane B2 is increased in type IIA hypercholesterolemic subjects. **Atherosclerosis, 60:61-66, 1986.**

5. EYNARD, A.R.; PASQUALINI, M.E. & ROVASIO, R.A. - Exogenous fibronectin modifies the aggregation of collagen stimulated human platelets. **Experientia, 46:680-682, 1990.**

6. FRETES, R. & de FABRO, S.P. - **Trypanosoma cruzi:** modification of alkaline phosphatase activity induced by tryponastigotes in cultured human placental villi. **Rev. Inst. Med. trop. S. Paulo, 32:** 403-408, 1990.

7. HAJJAR, D.P.; MARCUS, A.J. & ETINGIN, O.R. - Platelet - Neutrophil- smooth muscle cell interactions: lipoxygenase- derived mono- and dihydroxy acids activate cholesteryl ester hydrolysis by the cyclic AMP dependent protein kinase cascade. **Biochemistry, 28:8885-8891, 1989.**

8. KASPI, T. & NEBEL, L. - Isolation of syncytiotrophoblast from human term placenta. **Obstet. & Gynec., 43:549-557, 1974.**

9. LAGA, F.M.; DRISCOLL, S.G. & MUNRO, L. - Quantitative studies of human placenta. **Biol. Neonate, 23:231-259, 1973.**

10. LAGARDE, M.; BOUTILLON, M.M.; GUICHARDANT, M.; LELLOUCHE, J.P.; BEAUCOURT, J.P.; VANHOVE, A. & GREE, R. - Further studies on the anti-thromboxane A2 activity of mono- hydroxylated fatty acids. **Biochem. Pharmacol., 38:1863-1864, 1989.**

11. MOON, D.G.; KAPLAN, J.E. & MAZURKEWICZ, J.E. - The inhibitory effect of plasma fibronectin on collagen induced platelet aggregation. **Blood, 67:450-457, 1986.**

12. RUIZ-MORENO, L. & de FABRO, S.P. - Effect of **Trypanosoma cruzi** on the protein pattern of human syncytiotrophoblast. **Comun. biol., 8**:351-360, 1990.

13. SANTORO, S.A. - Inhibition of platelet aggregation by fibronectin. **Biochem. biophys. Res. Commun., 116**:135-140, 1983.

14. SCHNEIDER, N.O.; CALDERON, R.O. & de FABRO, S.P. - Isolation and characterization of cell membranes from human placenta. **Acta physiol. lat. amer., 31**: 283-289, 1981.

15. TSAO, C.H. - Platelet aggregation by rat liver. Evidence for ADP as major factor. **Amer. J. Path., 64**:501-512, 1971.

16. UMEKITA, L.F. & MOTA, I. - Role of platelets in the **in vivo** removal of **T. cruzi** from circulation. **Braz. J. med. biol. Res., 23**: 593-598, 1990.

17. VANDERPUYE, O.A. & SMITH, C.H. - Proteins of the apical and basal plasma membrane of the human placental syncytiotrophoblast. Immunochemical and electrophoretic studies. **Placenta, 8**:591-608, 1987.

Recebido para publicação em 16/09/1992
Aceito para publicação em 17/12/1992