Placenta (2005), **26**, 789–795
doi:10.1016/j.placenta.2004.08.014

Placental Alkaline Phosphatase (PLAP) Enzyme Activity and Binding to IgG in Chagas' Disease

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Paper accepted 23 August 2004

Placentas and plasma from women with and without Chagas' disease and cultures of human placental villi with *Trypanosoma cruzi*, neuraminidase, phospholipase A_2 and phospholipase C were analyzed in order to verify if the alterations in placental alkaline phosphatase (PLAP) enzyme activity are caused by *T. cruzi* as observed in previous works. As IgG receptivity happens to be one of the proposed functions of PLAP, general IgG binding ability of the placentas treated with the mentioned enzymes, which are present on the parasite's surface, were also tested.

The phospholipases caused an increase of PLAP's enzyme activity in the supernatant of infected placentas and a decrease of enzyme activity in the membrane of cultured placentas, therefore suggesting the cleavage of PLAP by parasitic enzymes. Desialylation could also partially inhibit PLAP's enzyme activity in supernatant and membrane of placenta culture.

Placentas from healthy patients presented higher IgG receptivity than those from patients with Chagas' disease. In vitro infection of healthy placentas with *T. cruzi* caused no difference in IgG receptivity in placental sections with respect to controls but the phospholipases and neuraminidase increased the IgG receptivity of cultured placentas. The IgG transference index was higher for patients with Chagas' disease than for those without it. Although binding to IgG does not completely inhibit the enzyme activity of PLAP, it interferes with the enzyme activity of PLAP.

We concluded that the enzymes on the surface of *T. cruzi* trypomastigotes can not only affect PLAP's enzyme activity but also increase the IgG binding ability of the placenta and this can be related to the actions of neuraminidase–transsialidase, phospholipase A_2 and phospholipase C on the parasite surface. The modification of PLAP from women with Chagas' disease should be considered as a result of multiple factors.

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Keywords: *Trypanosoma cruzi*; Placenta; Neuraminidase; Phospholipase A_2; Phospholipase C; Placental alkaline phosphatase; IgG

INTRODUCTION

Placental alkaline phosphatase (PLAP, EC 3.1.3.1) is a GPI anchored protein on the surface of human placenta [1]. Its phenotype is determined by the fetal genotype in chromosome 2. PLAP presents great polymorphism in zymograms although this is not correlated with the newborn's sex, weight, blood type or placental weight, it might be related to spontaneous abortions [1–4] and be relevant in the mother–fetal interaction [5,6]. Although the biological role of PLAP is still unclear [1], PLAP was reported to be physiologically active toward phosphoetanolamine, inorganic pyrophosphate and pyridoxal 5′ phosphate in humans [7]. Another biological function suggested for PLAP is its role as an IgG acceptor. Transplacental transport of IgG could depend on the fetal genotype of PLAP [8–10].

PLAP was suggested to be one of the receptors used by the parasite *Trypanosoma cruzi* to trigger signal pathways that allow invasion into human trophoblasts [11]. As an intracellular parasite, *T. cruzi* must enter into the cytoplasm of a susceptible mammalian host cell where it replicates and completes its life cycle. The trypomastigote is the stage of the parasite at which it is able to invade mammalian cells. The invasive process requires a coordinated series of events at the host cell membrane surface [12,13].

Anchored to the trophoblast surface or released into plasma, PLAP could be susceptible to different enzymatic actions of *T. cruzi*. PLAP's enzyme activity decreases in the plasma of pregnant women with Chagas' disease when compared to non-infected women, especially at the third trimester of pregnancy [14–16]. Placentas from women with Chagas' disease and

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0143–4004/$–see front matter

normal placenta infected with *T. cruzi* trypomastigotes also demonstrated diminished PLAP activity in the syncytiotrophoblast [11,17,18]. The above-mentioned works concerning alterations in PLAP's enzyme activity suggested that IgG binding to the placenta, related to PLAP, could also be altered by the parasite.

PLAP contains many sialic acid residues, which could be important for its active site [19,20]. *T. cruzi* can recognize sialic residues from the surface of host cells during the invasion [12]. *T. cruzi* expresses a stage-specific transsialidase—neuraminidase which would sialylate its own surface and enable its entry into the host cell. PLAP's sialic residues could facilitate the entry of *T. cruzi* into the placenta [21—23].

Since phospholipases C and A_2 are reported to be present on the surface of trypomastigotes and are able to cleave GPI anchors [24—28], the GPI anchors of PLAP could be affected by them.

We measured PLAP's enzyme activity and binding to IgG on placentas from patients with Chagas' disease, and in cultures of human placenta with *T. cruzi*. We also compared the IgG transference in pregnant women with and without Chagas' disease.

We studied the changes of PLAP's activity and binding to IgG under the effects of phospholipase A_2, phospholipase C and neuraminidase, to simulate single action of some surface enzymes of the parasite, in order to find out a mechanism by which the parasite could affect PLAP. Phospholipase C is known to be able to remove the PLAP from syncytiotrophoblast [25,29,30]. There is no report about the effects of phospholipase A_2 on PLAP.

MATERIALS AND METHODS

Placenta from women with Chagas' disease

Seven placentas from women with Chagas' disease (serologically diagnosed) and 10 placentas from women with negative diagnosis of Chagas' disease at 38 to 40 weeks of gestation were obtained. All patients with Chagas' disease were chronical, as the microstrout technique performed in plasma samples looking for trypomastigotes were negative.

Samples of central villi were isolated, washed with PBS and fixed in formol 10% for in situ IgG binding assays and microscopical examinations. Plasma from each patient were collected and stored at −20 °C.

Parasites

Trypomastigotes from Tulahuen strain were used. They were isolated from Albino Swiss mouse blood following Andrews and Colli [12] by centrifuging blood at 100 *g* for 10 min and keeping it at 37 °C for 1 h. Pellet content was resuspended in M-199 media.

Placental cultures

Central villi of placental cotyledons from healthy women, without infection, were cultured at 37 °C, pH 7, in a final volume of 1.5 ml of M-199 culture medium with 0.1% penicillin and 0.01% streptomycin. Cultures were performed without FCS since it contains phospholipases. Previous detections for apoptosis molecules in the culture system were negative.

Placental villi were co-cultured with 1×10^5 bloodstream trypomastigotes (Tulahuen strain) of *T. cruzi* or with addition of different experimental factors: neuraminidase (2 U/ml, Sigma, N-3001), phospholipase A_2 (2 U/ml, Sigma P-9279) or phospholipase C (2 U/ml, Sigma P-7633).

Cultures were harvested after 24 h. Controls were maintained at same conditions without parasites or any factors considered in this study. A group of the harvested placental villi were processed for microscopical studies (histological examinations and in situ IgG binding) and the other group was homogenized for biochemical studies (enzyme activity). The culture media were also kept at −20 °C for biochemical studies, since it could be considered an analogue to patient's plasma.

The experiment was performed with each of the 10 placentas from women with negative Chagas' diagnosis.

Enzyme kinetics

Harvested placental villi were homogenized with an OMNI 1000 homogenizer. Five cycles of 10 s, high speed application were performed for each sample with 0.25 ml of washing buffer (PBS) per 30 mg of placental villi.

Samples were heated at 60 °C for 15 min in order to inactivate other alkaline phosphatases. Protein content was determined following Lowry et al. [31].

Alkaline phosphatase activity was determined following Messer et al. [32] by incubating 50 µl of sample at 37 °C in a media containing 2.5—20 mM of Na-p-nitrophenyl phosphate, 10 mM Cl_2Mg in 100 mM carbonate—bicarbonate buffer in a final volume of 550 µl. Reactions were stopped by addition of 2 ml of 0.1 N NaOH. *p*-nitrophenol was measured by absorbance at 405 nm.

Enzyme activity was expressed as µmol of *p*-nitrophenol produced per minute, per mg of protein. Lineaweaver-Burk Graphics were confectioned to estimate and visualize the modifications on kinetic parameters: Maximal Velocity (MV) and Michaelis—Menten Constant (K_M).

In situ IgG binding

Histological sections were pretreated with H_2O_2 (3%) for 15 min, rinsed with water and incubated with purified human gamma globulin (200 µg/ml in PBS) (courtesy of Laboratorio de Hemoderivados, Universidad Nacional de Córdoba) for 2 h at room temperature. After thorough washing and blockade with PBS—BFS, binding of IgG was detected by employing a polyclonal antibody conjugated with peroxidase anti-human IgG of goat origin (Sigma A-6029) conjugated with peroxidase. Peroxidase reaction was revealed preincubating the sections for 10 min with a diaminobenzidine solution [0.5 mg of