# Fibronectin Attachment to Different Stages of *Trypanosoma cruzi*[1]

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**Abstract**: Fibronectin attachment to different stages of *Trypanosoma cruzi* of different virulence derived from a single strain was studied using enzyme-immunohistochemical staining. Fibronectin selectively attached to the kinetoplast region of glutaraldehyde fixed trypomastigote and epimastigote, and there was no difference in staining intensity between parasites of different virulence. However, fibronectin attached to the whole cell surface of amastigote as well as the kinetoplast region but in lesser degree. Furthermore, only the amastigote was less deeply stained when the fibronectin treatment was omitted from the entire staining procedures.

*Key words*: *Trypanosoma cruzi*, trypomastigote, developmental stages, fibronectin

## INTRODUCTION

The penetrating mechanism of *Trypanosoma cruzi* trypomastigote into mammalian cells is important for parasites to persist and spread infection in animals. Parasites at the trypomastigote stage leave destructed host cells and invade new cells. At the first step of penetration, trypomastigotes seem to recognize special glycoproteins on the cell surface, especially glycoproteins containing N-acetyl-glucosamine (Henriquez *et al.*, 1981; Crane and Dvorak, 1982; Piras *et al.*, 1983; Katzin and Colli, 1983). Recently, Ouaissi *et al.*, (1984) reported that fibronectin on fibroblast cell surface might act as a recognition site for attachment of the parasites. They used mainly glutaraldehyde-fixed trypomastigotes and human plasma fibronectin for binding assay. Using living cells, Wirth and Kierszenbaum (1984) also found that fibronectin enhanced macrophage association with invasive forms (trypomastigotes) of *T. cruzi*. In the present experiment, we attemped to examine the difference of fibronectin binding in different stages of *T. cruzi* and between trypomastigotes of different virulence.

## MATERIALS AND METHODS

### *Parasites*

The strains of different virulence derived from the Tulahuen strain were used.

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Trypomastigote: Trypomastigotes of high and low virulence developed in mouse fibroblast cell culture system (Hermosura *et al.*, 1985) were purified through CM-cellulose column (Kanbara and Nakabayashi 1983).

Epimastigotes: Culture forms consisting more than 99% of epimastigotes in the modified NNN medium were used. Because of long maintenance in culture since 1971 they have lost virulence.

Amastigotes: Amastigotes of high and low virulence grown in mouse fibroblast cell culture system and separated from ruptured cells were roughly collected by centrifugation at 120 *g* for 5 min.

*Human fibronectin*

The human plasma fibronectin obtained by Gelatin-Sepharose 4B (Pharmacia) affinity chromatography was kindly supplied by Dr. Oishi, the Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University.

*Antiserum*

Commercial preparations of Antiserum to Human-Fibronectin from rabbit (Behring Institute, West Germany) and Peroxidase conjugated IgG Fraction Goat Anti-Rabbit IgG (Heavy and Light Chains Specific) (Capper, USA) were used.

*Location of fibronectin binding site of parasites*

We followed the methods described by Ouaissi *et al.* (1984), except for the use of peroxidase-conjugated IgG fraction of goat anti-rabbit IgG serum instead of fluorescein-conjugated sheep anti-rabbit $\gamma$-chain antiserum. Briefly, each sample of parasites was fixed in 0.25% glutaraldehyde in phosphate buffered saline (PBS), pH 7.2, and dried on slides. The samples were incubated in 2% bovine serum albumin in PBS for 1 h, washed with PBS three times, incubated in fibronectin solution (5 $\mu$g/ml) in PBS for 1 h, washed with PBS three times, incubated in rabbit antiserum to human fibronectin diluted 1 : 20 in PBS for 30 min, washed with PBS three times, incubated in peroxidase-conjugated goat antiserum to rabbit IgG diuted 1 : 80 in PBS for 30 min, washed with PBS three times, incubated in substrates (5 mg of 3,3'-diaminobenzidine tetrahydrochloride and 0.1 ml of 1% $H_2O_2$ in 10ml of 0.05 M Tris-buffer pH 7.5) for 15 min, washed in distilled water three times, dried and observed under a microscope. As control experiments, firstly normal rabbit serum was used instead of anti-human fibronectin rabbit serum and secondly the fibronectin treatment was omitted from the entire procedures.

## RESULTS

As shown in Photo. 1, fibronectin bound not only trypomastigotes but also epimastigotes at the kinetoplast region. Judging from the staining intensity, there was no difference of binding affinity between parasites of different virulence. However, some difference was found among developmental stages, that is, fibronectin could adhere to the

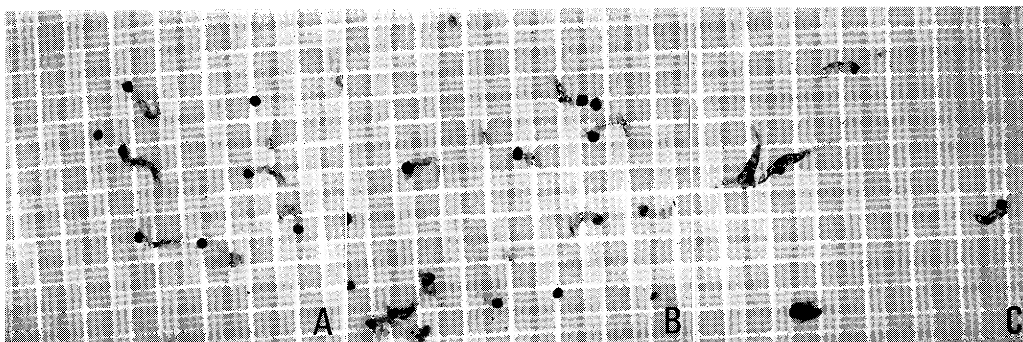


Photo 1. Fibronectin attachment to virulent trypomastigotes (A), low virulent trypomastigotes (B) and epimastigotes (C). The kinetoplast region was selectively stained by enzyme-immunohistochemical method after fibronectin treatment, but was not stained when fibronectin treatment was omitted or when anti-human fibronectin rabbit serum was replaced by normal rabbit serum.

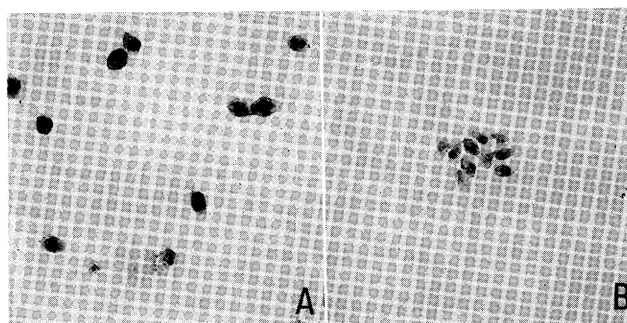


Photo 2. Fibronectin attachment to amastigotes (A). Whole cell surface was less deeply stained than the kinetoplast region. This staining was observed in lesser degree when the fibronectin treatment was omitted from the entire assay course (B).

whole cell surface of amastigote as well as the kinetoplastic region although in lesser degree (Photo. 2A). When fibronectin treatment was omitted from the entire procedure or normal rabbit serum was used instead of rabbit anti-human fibronectin serum, trypomastigote and epimastigote were not stained. However, amastigote was slightly stained when the entire procedure was performed without fibroncetin treatment (Photo. 2B).

## DISCUSSION

Fibronectin selectively attached to the kinetoplast region of parasites at all stages that were fixed in 0.25% glutaraldehyde but this specific attachment was not clearly seen when living parasites were used for fibronectin binding assay. The specificity of this reaction was supported by the following results that the nonspecific adhesion of protein was prevented by the previous treatment of fixed parasites with bovine serum albumin, that the nonspe-

cific staining was not seen when fibronectin was omitted from the entire assay procedure and that the use of normal rabbit serum instead of anti-human fibronectin rabbit serum could not stain the parasites. However, only the amastigote showed different pattern of binding assay. The whole cell surface of amastigote was stained as well as the kinetoplast region although in lesser degree and again this staining was also found less deeply when fibronectin treatment was omitted from the entire assay procedure. This means that the surface substances on amastigote cell contain antigenically similar material to human fibronectin, or bind fibronectin derived from host fibroblasts or calf serum, to which polyclonal antibodies to human fibronectin react extensively. These materials may be attached to the cell surface because of its adhesive character given by protein-polysaccharide complex which was suggested to exist on the amastigote cell surface (Kanbara *et al.*, 1983, 1985). Concerning the functional specificity of the kinetoplast region as a fibronectin receptor as suggested by Ouaissi *et al.* (1984), it is doubtful because fibronectin attachment is neither specific to invasive form (trypomastigote) nor related to virulence of trypomastigote. However, it cannot be denied that the location change of a kinetoplast accompanying morphological change enables the function of the fibronectin binding site to work, because only the trypomastigote posseses the kinetoplastic region at the posterior end of a body. At the moment, we cannot understand why the kinetoplast region selectively attach fibronectin. More exact information on its biochemical quality and its morphological character using immunohistochemical electronmicroscopy will be required.

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*Trypanosoma cruzi* の異なる発育期原虫に対するフィブロネクチンの結合

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最近 *Trypaosoma cruzi* のトリポマスティゴート型原虫の宿主細胞への侵入時の細胞認識物質としてフィブロネクチンが注目されている．私達は単一原虫株（Tulahuen 株）由来で病原力の異なる二つの株から得た，異なる発育期の原虫に対するフィブロネクチンの結合性を調べた．グルタルアルデヒドにて固定した原虫を牛アルブミンにて非特異蛋白結合部をおおった後，人フィブロネクチンを働かせ，その結合状態を酵素免疫化学的方法にて検出した．トリポマスティゴートおよびエピマスティゴート型原虫は，キネトプラスト存在領域にて選択的にフィブロネクチンを結合した．一方アマスティゴート原虫はこの領域だけでなく細胞表面全体が程度が低いが染色された．更に反応系からフィブロネクチン処理を抜いても軽度の染色が認められた．またいずれの型においても病原力による染色の差は認められなかった．これらの結果はキネトプラスト領域の生化学的特異性を示すが，トリポマスティゴートの細胞侵入時の細胞認識機構との関連性については疑いをなげかける．

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