Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *86*, Suppl. III, 125-126, 1991

SERUM FIBRONECTIN PROMOTES THE *LEISHMANIA* INTERACTION WITH NEUTROPHILS AND MACROPHAGES

M. A. VANNIER-SANTOS; E. M. B. SARAIVA* & W. DE SOUZA

Departamento de Parasitologia e Biofísica Celular, Instituto de Biofísica Carlos Chagas Filho *Departamento de Imunologia, Instituto de Microbilogia, Universidade Federal do Rio de Janeiro, Ilha do Fundão, 21949 Rio de Janeiro, RJ, Brasil

In the last decade, extracellular matrix proteins, such as fibronectin (FN), were demonstrated to have remarkably dynamic roles in several biological phenomena including cell attachement, migration, differentiation, phagocytosis of complement or IgG opsonized particles (Reviewed in Ruoslahti, 1988, *Ann. Rev. Biochem., 57*: 375-413; Brown, 1986, *J. Leukoc. Biol., 39*: 579-594).

Conflicting results have been reported concerning the participation of FN in microorganism opsonization and subsequent destruction. FN was shown to bind to bacteria, including *Staphylococcus aureus*, without promoting their phagocytosis (Verbrugh et al., 1981, *Infect. Immun., 33*: 811-819; Proctor et al., 1982, *Blood, 59*: 681-687; Van De Water et al., 1983, *Science, 220*: 201-204). Other authors, however, report that FN alone can promote uptake and killing of *S. aureus* (Yang et al., 1988, *J. Infect. Dis., 158*: 823-830; Yonemasu et al., 1988, *Microbiol. Immunol., 32*: 795-805). Results on the extent of intracellular killing are also in disagreement. In studies using *Trypanosoma cruzi*, FN enhanced the association of both trypomastigote and amastigote forms with host cells (Wirth & Kierszenbaum, 1984, *J. Immunol., 133*: 460-464; Noisin & Villalta, 1989, *Infect. Immun., 57*: 1030-1034). It was also shown that *Leishmania* grown in absence of FN were deficient in their ability to attach to human monocytes (Wyler et al., 1985, *Infect. Immun., 49*: 305-311). Nevertheless, there is no available data on the participation of FN in the intracellular destruction of tripanosomatid parasites.

In this study we attempted to determine the role of FN in the ingestion and killing of *L. mexicana amazonensis* promastigotes by rat polymorphonuclear neutrophils and mouse peritoneal macrophages.

This work has been supported by CNPq, FINEP and CEPG-UFRJ.

Experiments using neutrophils were performed as in Pimenta et al., 1987. (*J. Submicrosc. Cytol., 19*: 387-395). Briefly, neutrophils were collected from the peritoneal cavity of *Rattus rattus*, 18-20 h after the inoculation of a 0.1% glycogen solution. The cells interacted in suspension in 2 ml of PBS (phosphate buffered saline) with or without 30 μg/ml human serum FN (Sigma) or 2% normal autologous serum. After interactions the cells were processed for conventional transmission electron microscopy.

Just a few neutrophils were found infected by the parasites in the absence of FN or normal serum. When infected this neutrophils generally displayed well preserved parasites (Fig. 1a). Most of the leukocytes were infected in the presence of FN but the parasites were almost always completely destroyed and sometimes even hardly identified (Fig. 1b). Interestingly these neutrophils presented many surface processes, sometimes still present in the endocytic vacuoles (Fig. 1b: arrows). Serum appeared to be less active in promoting parasite uptake and killing.

In order to test if FN could be important for the intracellular *Leishmania* destruction, we interacted this parasite with macrophages, their most common host cells.

Macrophage interactions were performed as described in Saraiva, et al., 1989, (*J. Cell Sci., 93*: 481-498), using a 10:1 parasite-macrophage ratio. The FN effect was approached comparing the interaction of promastigotes cultivated, for at least 3 passages, in medium supplemented with fetal calf serum (FCS) or FN-depleted FCS, as described by Engvall & Ruoslahti, 1977 (*Int. J. Cancer, 20*: 1-5). The percentages of infected cells were determined after 1 and 72 h of interaction.

Kinetical quantification of the *Leishmania*-macrophage interaction, revealed that FN was important not only for the parasite entry but also for

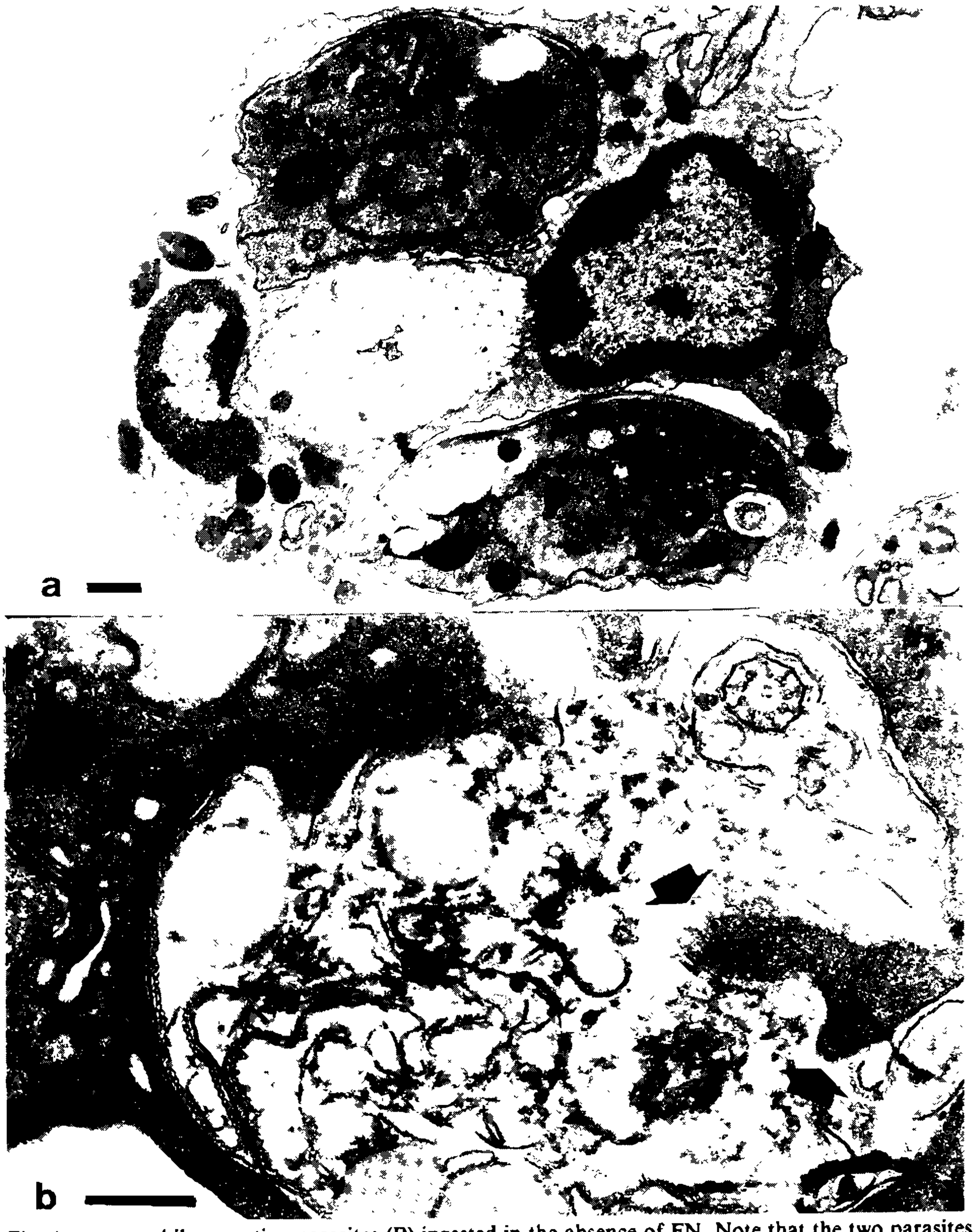

Fig. 1a: neutrophil presenting parasites (P) ingested in the absence of FN. Note that the two parasites are structurally preserved after the 60 min interaction. Fig. 1b: neutrophil presenting destroyed promastigote after FN treatment. Surface processes can be observed protruding to the endocytic vacuole lumen (arrows). Bars correspond to 0.5 μm.

its destruction. In experiments with FN we observed that both the percentual of infected macrophages and the number of parasites per macrophage were increased.

The parasite binding was increased by 112% after FN treatment, whereas the association of parasites grown in FN-depleted medium was about 53% of controls.

The percentual reduction of parasite numbers in controls, FN-depleted, FN-supplemented preparations, was respectively, 89; 63 and 84. Therefore we suggest that FN can modulate *Leishmania* invasion and survival in phagocytic cells.

ACKNOWLEDGMENTS

To Antônio Lisboa for the photographical work.