NOTES

Interaction between Entamoeba histolytica and Intestinal Epithelial Cells Involves a CD44 Cross-Reactive Protein Expressed on the Parasite Surface

PATRICIA RENESTO,* PHILIPPE J. SANSONETTI, AND N. GUILLÉN

Unité de Pathogénie Microbienne Moléculaire, Institut National de la Santé et de la Recherche Médicale U389, Institut Pasteur, 75015 Paris, France

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This study shows that Entamoeba histolytica binds hyaluronic acid. The binding molecule was identified as an 80-kDa membrane protein and was recognized by anti-CD44 monoclonal antibodies. These data indicate that a CD44 cross-reacting adherence molecule is expressed on E. histolytica.

Amoebiasis, a human disease resulting from infection by Entamoeba histolytica, is a major public health problem. It represents the third most prevalent cause of parasitic death after malaria and schistosomiasis (17). Among the initial steps leading to symptomatic infection is the attachment of E. histolytica parasites to host intestinal epithelial cells. While different adhesion molecules have been identified on the surface of E. histolytica (1, 12, 15, 16), only the 260-kDa galactose- and N-acetyl-galactosamine-sensitive lectin is considered to play a crucial role in the interaction between the parasite and an epithelial cell (2, 10). We have attempted to identify other adhesive molecules involved in the interaction between E. histolytica parasites and epithelial cells. Experiments using the human intestinal cell line Caco-2, which has been described as a model for in vitro amoeba-cell interaction, were performed (6, 11).

Hyaluronidase inhibits E. histolytica adhesion to epithelial cells. To determine the extent of 260-kDa-lectin-dependent amoebic adhesion on Caco-2 epithelial cells, the number of [35S]methionine-labeled amoebae adhering to these cells was determined in the presence of N-acetyl-galactosamine and compared to the number obtained with untreated cells. As shown in Fig. 1A, even when a high concentration of this sugar (100 mM) was added to the cell suspension, 57.1 ± 6.3% of the amoebae remained associated with the epithelial monolayer (n = 7; P < 0.001), indicating that other molecules may participate in the adhesion. Hyaluronidase, an enzyme specific for hyaluronic acid (HA) (8), was then tested to determine its effect on the adhesive process. When epithelial cells were incubated for 30 min at 37°C with this enzyme and washed before addition of amoebae, we observed a significant inhibition of trophozoite adhesion (Fig. 1), which was maximal at 20 U of hyaluronidase (26.6% ± 3.4%; n = 6; P < 0.001) per ml. This effect is not only concentration dependent (Fig. 1A) but also time dependent (not shown), suggesting that a simple decrease in negative charges associated with the epithelial cells is not likely to intervene and argues rather in favor of an effect associated with the hyaluronidase enzymatic activity. The integrity of the Caco-2 monolayer indicated that the decrease in

* Corresponding author. Mailing address: Unité de Pathogénie Microbienne Moléculaire, Institut National de la Santé et de la Recherche Médicale U389, Institut Pasteur, 75015 Paris, France. Phone: (33) 01 40 61 32 47. Fax: (33) 01 45 68 89 53. E-mail: prenesto@pasteur.fr.

FIG. 1. Adhesion of E. histolytica parasites to epithelial cells. (A) [35S]methionine-amoebae, preincubated with N-acetyl-galactosamine (GalNac; 100 mM) or HA (5 mg/ml), were allowed to adhere for 30 min at 20°C on confluent Caco-2 monolayers, and results were compared with results with untreated trophozoites (control). In some experiments, epithelial cells were pretreated with hyaluronidase (Hyal.; 20 U/ml) before amoebae were added. (B) Concentration-dependent effect of hyaluronidase. Data are presented as means ± standard errors of the means of six to seven distinct experiments performed in triplicate. The + indicates that a P value is less than 0.05.
adhesion was not the consequence of a cytolytic effect of hyaluronidase against the epithelial monolayer. This was determined by observation by optical microscopy and by a $^{51}$Cr-release assay (1.9% ± 0.4% versus 2% ± 0.6% for untreated cells; $n$ = 3; $P > 0.1$). The inhibition of amoebic adhesion to Caco-2 cells by hyaluronidase indicated that HA might be involved. To investigate this hypothesis, the adhesion assay was performed with trophozoites that had been incubated for 20 min in the presence of exogenous HA before their loading on a Caco-2 monolayer. In contrast to the result mentioned above, HA failed to reduce the amount of radioactivity bound to epithelial cells, even when it was used at 5 mg/ml (Fig. 1A). In fact, optical microscopy observation revealed that HA induced the aggregation of amoebae (Fig. 2), thus suggesting the presence of a receptor for HA on the amoebic surface. This phenomenon results from the binding of molecules of HA to the sites located on the surfaces of two adjacent amoebae as previously described for macrophages (7) or lymphoma cell lines (9). In consequence, it can be hypothesized that, even if the number of amoebae directly interacting with Caco-2 cells was significantly lowered in the presence of HA, this effect could be...
attenuated by amoebae forming aggregates. In fact, inhibition should occur only if all HA receptors are saturated by exogenous HA. Because of its viscosity, high concentrations of HA could not be tested.

**Characterization and localization of a CD44 cross-reacting protein on E. histolytica.** Based on the above-described observations, affinity chromatography purification of a putative amoebic HA receptor was initiated. Amoebic lysates were prepared from subconfluent amoebae (4 × 10^7 cells) that had been washed twice to remove serum and resuspended in a lysis medium (5 × 10^5 amoebae/ml; 10 mM Tris-HCl, 150 mM NaCl, 2 mM MgCl₂, 1 mM CaCl₂, 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 6 μM leupeptin, [pH 8]). Following 1 h of incubation at 4°C, the cell suspension was centrifuged (10,000 g, 10 min) and the supernatant was applied to a CNBr-activated Sepharose 4B column covalently coupled to HA. Following extensive washings with 0.15 and 0.45 M NaCl, the bound material was eluted with a 1.5 M salt concentration in 10 mM Tris-HCl. Analysis of the obtained fractions by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis under reducing conditions showed that an 80-kDa protein remained bound to the affinity column (Fig. 3A). When purification was performed by using trophozoites previously labeled with ^[35]S][methionine, the same band was eluted from the affinity column. This result clearly indicated that the 80-kDa protein is an amoebic component. We then tested the reactivity of this protein against anti-CD44 monoclonal antibodies (MAbs), considering HA a glycosaminoglycan found ubiquitously as an extracellular matrix component, the principal ligand for CD44 (14). In addition, the principal isoform of CD44 present on hematopoietic cells and lymphocytes is a 80-kDa protein, which invade tissues through CD44 and amoebic trophozoites follow the same metastatic route towards the liver and other organs (5).

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**REFERENCES**


**FIG. 4.** Expression of CD44 cross-reacting protein on the surface of E. histolytica. Amoebae were stained with J-173 or Hermes-3 and then submitted to FACscan analysis. Fluorescence was measured from the total cell population incubated with the second Ab alone (control, tracings in white) or with anti-CD44 MAbs followed by the second anti-mouse-fluorescein isothiocyanate Ab (tracings in black). Data are representative of three distinct experiments.