New Adhesion Mechanism in Giardia: Role of the Ventrolateral Flange in the Attachment of Trophozoites to Rough and Porous Surfaces

S.L. Erlandsen,* A.P. Russo,** and J.N. Turner**

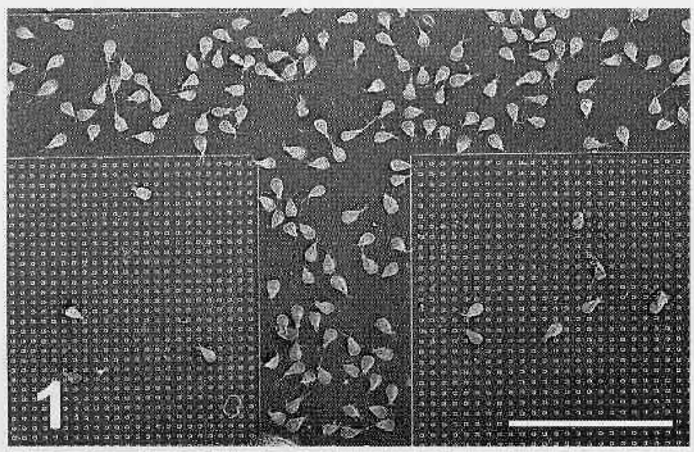
University of Minnesota Medical School

***New York State Department of Health, Wadsworth Center*

stan@mail.ahc.umn.edu

The protozoan parasite *Giardia*, an intestinal flagellate, has evolved an unique attachment organelle called the ventral adhesive disk (VAD). This attachment organelle mediates attachment of the trophozoite to the microvillous border (MVB) of intestinal absorptive cells, and production of lesions via attachment are thought to lead to diarrheal disorder characteristic of giardiasis [1]. The VAD has contractile proteins arranged around it's circumference and it has been suggested that contraction of this area might function like a purse-string suture. The resemblance of the VAD to a suction cup has led to the hypothesis that suction or a negative pressure produced under the VAD by a grasping action might produce the adhesive force regulating attachment. To test whether or not the generation of a negative pressure is necessary for attachment, experiments were designed in which the substratum was fabricated to have an uneven surface, or the substratum was composed of different arrangement of pores. In both cases negative pressure cannot be generated since the entire VAD cannot interact with the substratum due to its uneven or porous surface.

Recently we have reported the use of a model utilizing microfabrication of a substrate containing closely-spaced pillars (2). In real-time interference reflexion video microscopy, the ventrolateral flange, a cytoplasmic rim located around the periphery of the VAD, has been observed to form transient interactions with the substratum that dynamically change from focal to close contacts in seconds or less. To test the hypothesis that the VLF may mediate attachment separate from the VAD, altered topographical substrates consisting of arrays of columnar pillars were created through photolithography and micromolding of polystyrene in a polysilicone mold [3]. *Giardia* lamblia were grown overnight at 37°C, and cells washed in Hank's balanced saline before incubation of trophozoites for 5 minutes at 37°C to allow attachment to polystyrene chips containing arrays of columnar pillars. Distance (edge-edge separation) between arrays of pillars (1 µm high, 2 µm wide) ranged from 1 to 5 µm, a distance that would enable the pillar columns to prevent direct attachment of the VAD (~5 µm diameter) to the substratum. Cells were aldehyde fixed, postfixed in osmium, and critical point dried. Attachment of trophozoites to polystyrene substrates was evaluated using a Hitachi S-4700 field emission SEM operated at 2 keV.



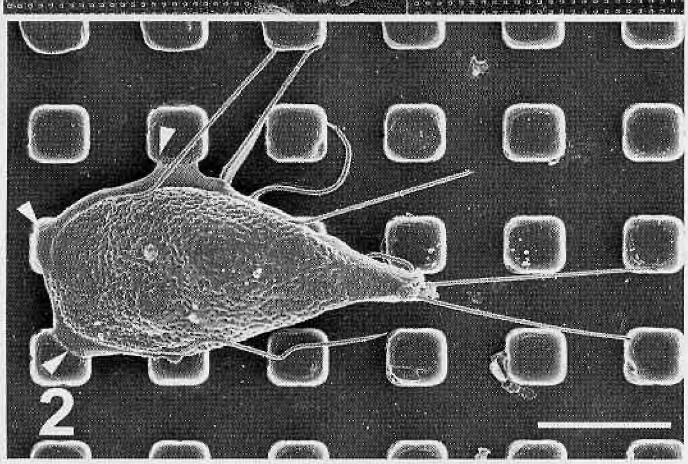


Figure 1. SEM of polystyrene substratum showing pattern of adhesion of G. lamblia trophozoites. Attachment of trophozoites occurs in greater number on flat topographical surfface than on arrays of microfabricated pillars. Magnification bar, 100 µm.

Figure 2. SEM of individual G. lamblia trophozoite adhering to top surface of pillars. Observe interaction of VLF (arrowheads) fusing with the tops of pillars. Interpillar spacing is 2 µm, which prevents the VAD from reaching the surface and generating a negative pressure under the VAD. Magnification bar, 5 µm.

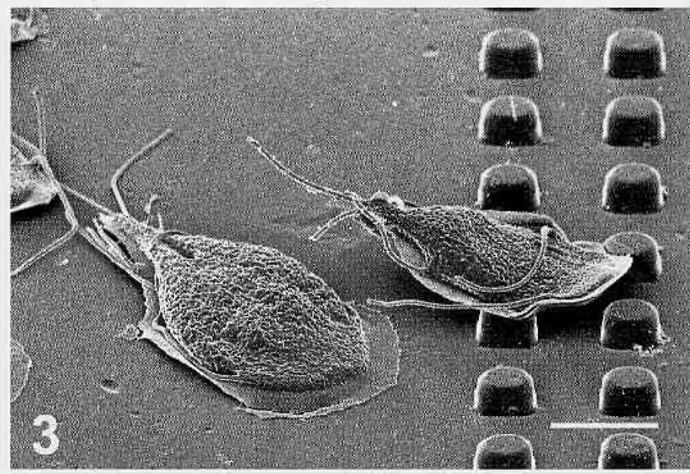
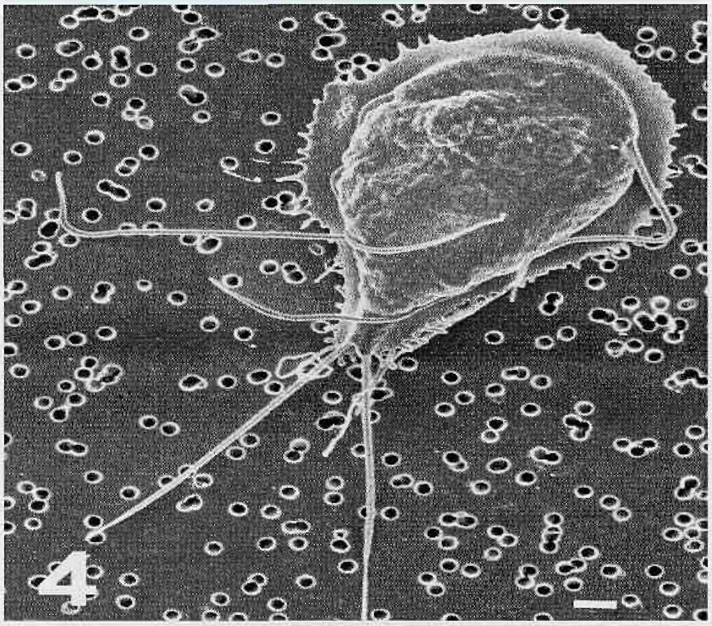


Figure 3. SEM of polystyrene substratum showing attachment of G. lamblia trophozoites via the VAD to the topographically flat surface (arrow) and adhesion of a trophozoite to the tops of the pillar array (arrowhead). The pillar array has a spacing of 2.5 µmas compared to the 5 µm diameter of the VAD and thus prevents direct interaction of the VAD with the substratum. Adhesion is mediated by fusion of the ventrolateral flange with the tops of the pillars.. Magnification bar, 4 µm.

Giardia trophozoites showed a high degree of adhesion to topographically flat surface of the polystyrene chips (Figure 1) and attachment appeared to be mediated by the VAD. Trophozoite attachment to topographical arrays of pillars was greatly reduced, but was not eliminated. Examination of trophozoites adhering to pillar arrays revealed attachment of the VLF to the tops of the pillars (Figures 2 & 3). Because pillar spacing prevented "suction-like" attachment of the VAD, adhesion appeared to be mediated by interaction of the VLF with the top surface of the pillars. This new mechanism of adhesion may provide new insights on transient interactions of trophozoites with the MVB.



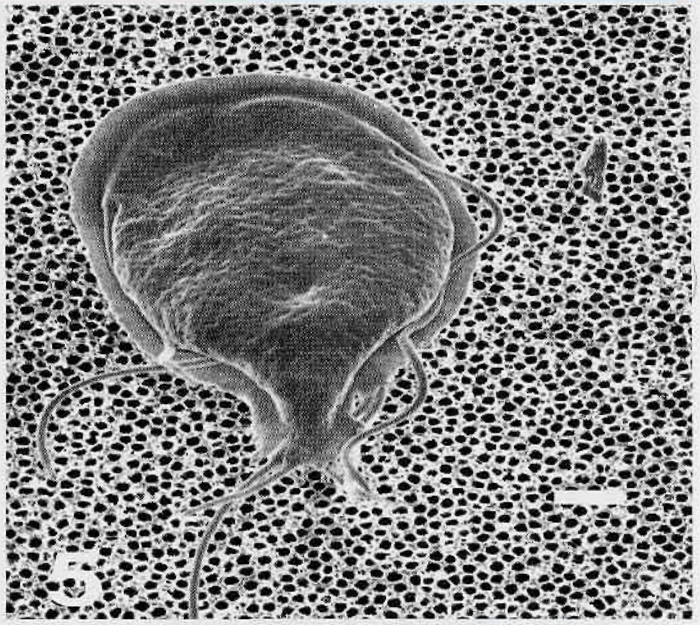


Figure 4. SEM of individual G. lamblia trophozoite adhering to surface of a nucleopore filter with 0.2 µm pores. Observe that spacing of the pores makes it impossible for the trophozoite to generate a negative pressure under the VAD, yet the trophozoite adheres to the surface of the filter. Magnification bar, 1 µm.

Figure 5 SEM of G. lamblia trophozoite attached to a highly porous anopore filter (alumina matrix) with 0.2 µm pores. The anopore filter has a much greater porosity per µm2 than the nucleopore filter. Again, the porous nature prevents generation of a negative pressure. Magnification bar, 1 µm.

In a second experiment, porous filters were utilized to examine the attachment of trophozoites. Small pore filters were chosen that would counteract the formation of a negative pressure by having multiple pores located within the area of the VAD. Polycarbonate, cellulose, and anopore filters possessing pore sizes of 0.2 - 0.4 microns were tested in an adhesion model where trophozoites are allowed to attach in a similar manner to the model described above. The number and the spacing of pores in the filter made it impossible for a negative pressure to be generated under the VAD. Examination of the polycarbonate filter (Figure 4) or the anopore filter (alumina matrix; Figue 5) clearly reveals that *Giardia* trophozoites can attach to these porous surfaces. SEM has clearly demonstrated that the membrane of the ventrolateral flange can directly fuse with the tops of the microfabricated pillar arrays, and it is likely the the adhesive quality of the VLF is responsible for adhesion to the thin substratum separating the pores in the filters.

The discovery of the adhesive nature of the VLF raises interesting questions as to how this might affect trophozoite attachment in vivo. The trophozoite swims in a helical fashion, much resembling the rotation of a falling leaf. Because the ventrolateral flange surrounds the VAD and it is highly flexible, it is possible that the VLF is the first part of the trophozoite to encounter the microvillous border of intestinal epithelial cells. The interaction of the flange with the epithelial cell surface may allow for a transient interaction the enables the trophozoite to orient itself so that the VAD can be brought into intimate contact with the cell surface of the host. Once it has this orientation, the edge or lateral crest of the VAD then penetrates the microvillous border and undergoes contraction permitting the VAD to form the tight adherence that produces the characteristic lesion or scar seen in the microvillous border that is the mirror-image of the VAD (1,2). ■

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References

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JOKES:

Q: What is the difference between a Quantum Theorist and a Beauty Therapist?

A: The Quantum Theorist uses Planck's Constant as a foundation, whereas the Beauty Therapist uses Max Factor.

Q: What's the difference between Max Factor and a Quantum Theorist?

A: Max Factor has models that work.

A fellow accidentally ingested some alpha-L-glucose and discovered that he had no ill effects.

Apparently he was ambidextrose.

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