

# Modeling Long-term Host Cell-*Giardia lamblia* Interactions in an *in vitro* 3D Cell Culture System

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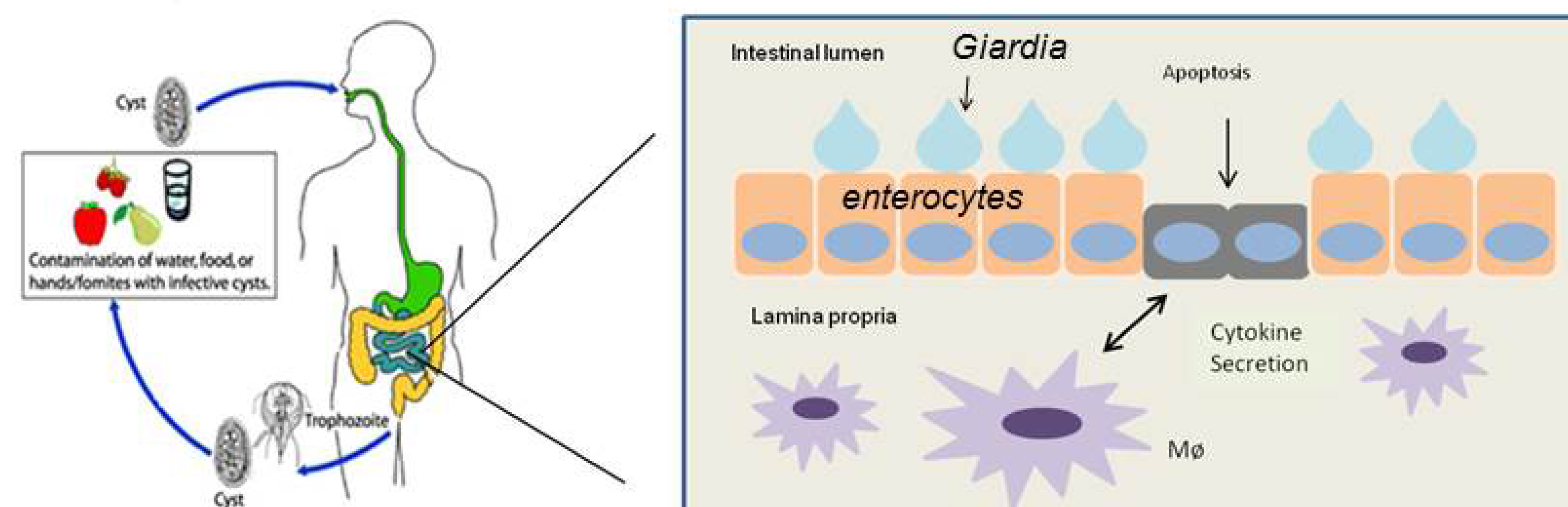


## BACKGROUND AND HYPOTHESIS

The flagellated protozoan parasite, *Giardia lamblia*, is the most common cause of diarrheal disease in humans, wildlife, and domestic animals. Parasite transmission typically occurs following ingestion of infectious cysts in contaminated food and/or water. Trophozoites preferentially colonize the small intestine by attaching to the gastrointestinal epithelial barrier (Figure 1). Infected hosts can display a wide range of symptom presentation. The majority are asymptomatic and exhibit few signs and symptoms of infection. Other infected individuals display abdominal cramping, nausea, bloating, vomiting, and diarrhea.

Epithelial cells exposed to *Giardia in vitro* exhibit actin rearrangement, tight junction disruption, increased intestinal permeability, apoptosis, and secrete chemotactic cytokines. However, these monoculture environments do not accurately reflect the three-dimensional nature of the gastrointestinal tract (Figure 1). Few studies have assessed how innate immune cells, such as macrophages, potentially contribute to disease pathology and parasite control or clearance. Additionally, long-term culturing of *Giardia* trophozoites with epithelial cells *in vitro* is problematic due to the microaerophilic nature of the parasite. Since *Giardia* infections can span several days to weeks, establishing an *in vitro* model that allows for co-incubation of host epithelial and immune cells with *Giardia* trophozoites over several days would greatly contribute to the understanding of late infection interactions.

Our model utilizes transwell inserts to co-culture a human epithelial cell line (Caco-2) and a murine peritoneal macrophage cell line (IC-21) in a manner that represents the apical-basolateral orientation of the small intestine (Figure 2). This model allows for assessment of cell-cell interactions between the epithelium and immune cells of the lamina propria through cytokines secretion, isolation of individual cell populations for signaling studies, and long-term *Giardia* effects spanning 21 days.



**Figure 1. *Giardia* life cycle.** Trophozoites colonize the small intestine where they attach to epithelial cells inducing apoptosis. In the gastrointestinal tract, enterocytes and macrophages communicate with each other through cytokine secretion.

## METHODS

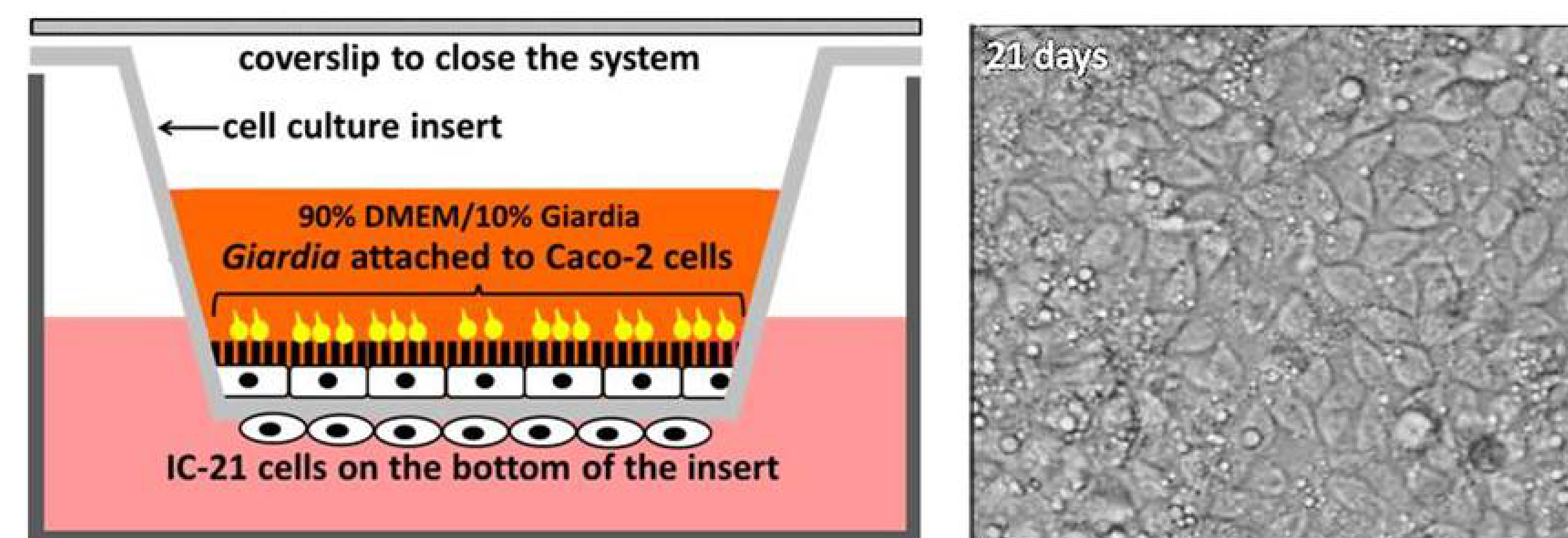
**Cell culture:** IC-21 macrophages were plated on the bottom of 24-well inserts. After 24 hours, the inserts were placed in a 24-well plate containing RPMI. Caco-2 cells were plated on top of the insert in DMEM. After 3 days, *Giardia* trophozoites (WBC6) (100,000 total parasites) were added to the insert in 90% DMEM/ 10% *Giardia* media. The cells were fed by removing half of the media and then adding fresh media to the well every other day until 5 days post infection. After 5 days, unattached parasites were eliminated by completely removing all the media and replenishing with fresh media every day.

**Cytokine array:** Conditioned medium was collected from the co-culture at 5 days and analyzed using the RayBio® Human Cytokine Antibody Array (AAH-CYT-1-2).

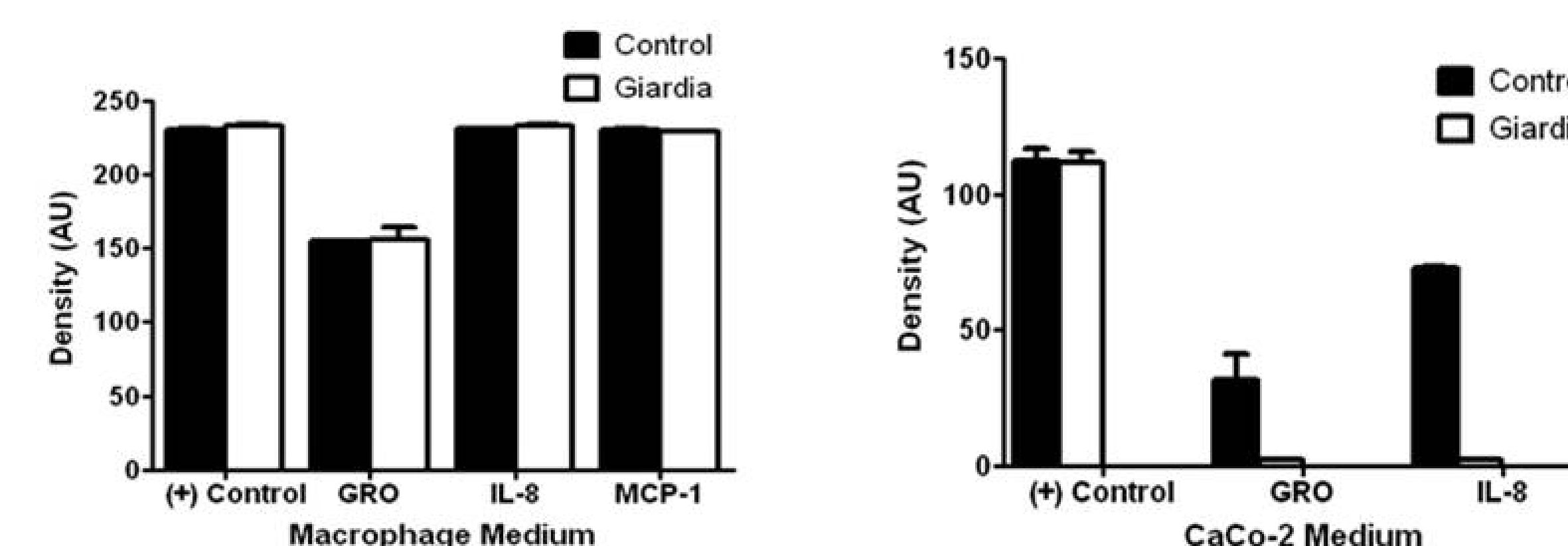
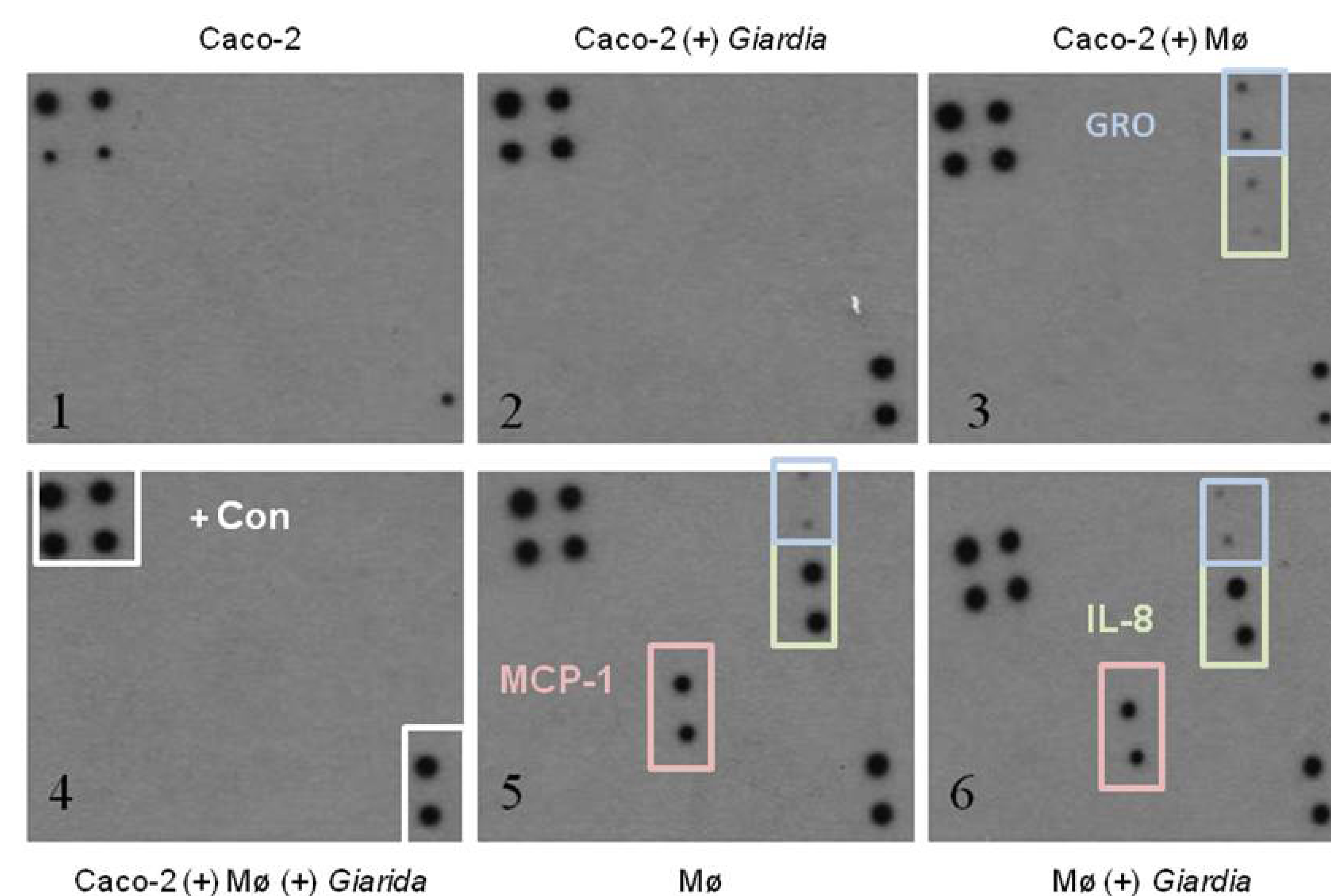
**Cell number assay:** Following 5 days of incubation in the co-culture, Caco-2 cells were stained with methylene blue. The eluted color was read at 595nm.

**Caspase 3 activity:** At 5 days, Caco-2 cell lysates were analyzed using the Abcam caspase-3 assay kit (ab39401) according to the manufacturer's instructions.

## RESULTS

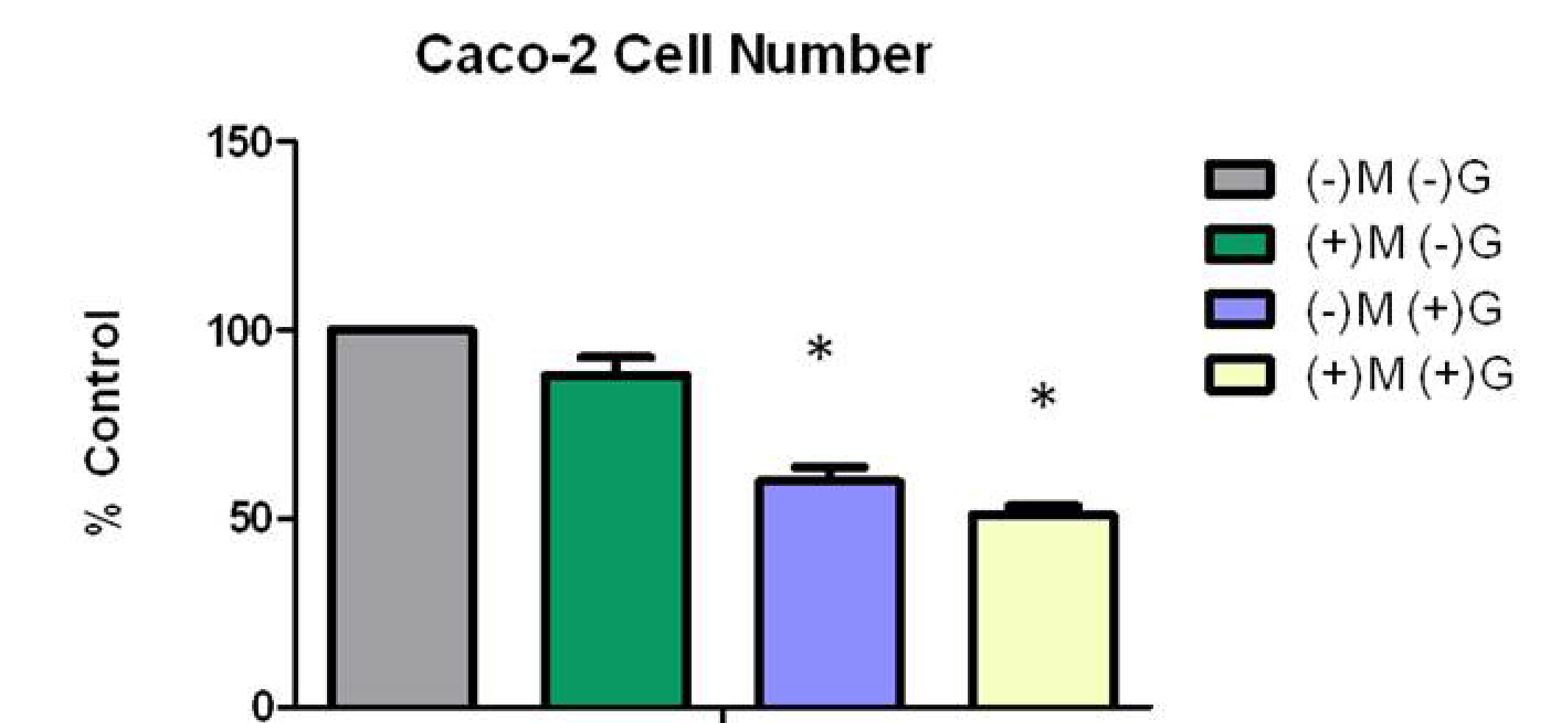


**Figure 2. Experimental design of the co-culture system.** Caco-2 cells are grown inside 24-well inserts in DMEM. IC-21 macrophages are plated at on the bottom of the inserts in RPMI media. After 72 hours, *Giardia* trophozoites are added to the inserts in 90% DMEM/ 10% *Giardia* media. Inserts are filled to the top with media and sealed with a round coverslip. Cultures were extended for 21 days.

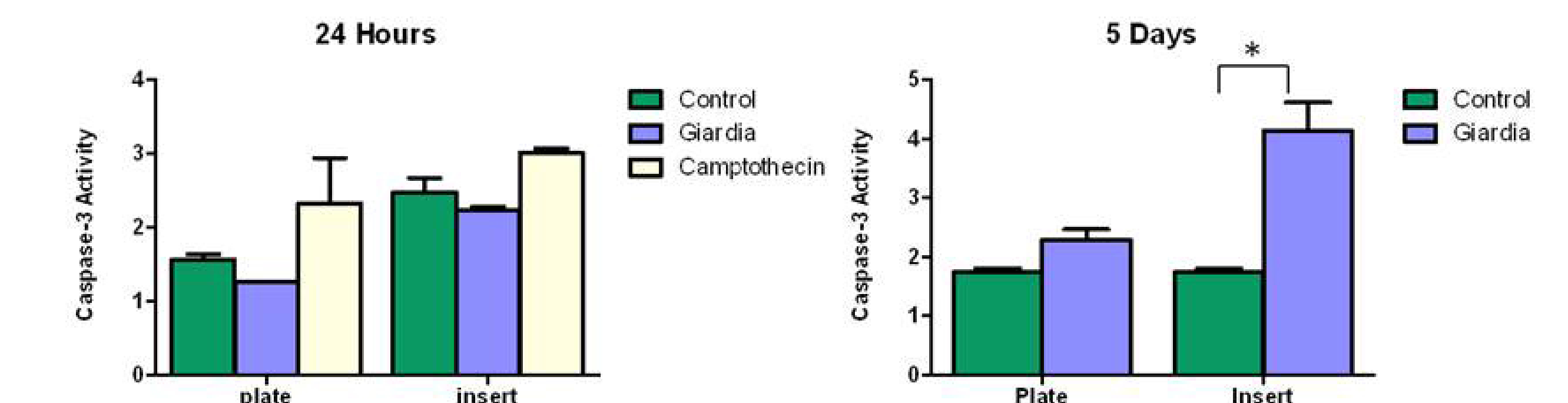


**Figure 3. Cytokine profile of the co-culture at 5 days.** Macrophages secreted the chemotactic cytokines MCP-1, IL-8, and GRO in both the presence and absence of *Giardia* trophozoites. The presence of macrophages in the co-culture induced secretion of IL-8 and GRO from Caco-2 cells. *Giardia* abolished the secretion of cytokines from Caco-2 cells indicating the parasite can potentially modulate the host immune response.

## RESULTS, continued



**Figure 4. Cell number assay of Caco-2 cells following 5-day incubation with *Giardia*.** Macrophages did not induce Caco-2 cell proliferation. *Giardia* significantly decreased Caco-2 cell number following 5 days of incubation. ( $p < 0.0001$ )  $n = 3$



**Figure 5. Caspase-3 activity of Caco-2 cells exposed to *Giardia* in plate and insert culture conditions.** Caspase-3 is a marker for apoptosis. *Giardia* did not increase caspase-3 activation at 24 hours in either the plate or insert conditions. At 5 days, the presence of trophozoites significantly induced apoptosis in Caco-2 cells through the activation of caspase-3 in the insert culture, but not the plate environment. ( $p < 0.05$ )  $n = 3$

## SUMMARY AND CONCLUSIONS

- *Giardia* trophozoites were successively cultured in the co-culture model for 21 days.
- Caco-2 cells display a differential cytokine profile when co-cultured with macrophages.
- *Giardia* trophozoites can abolish the secretion of pro-inflammatory cytokines from gastrointestinal epithelial cells.
- *Giardia* decreases Caco-2 cell number at 5 days in both the absence and presence of macrophages.
- *Giardia* induces apoptosis of Caco-2 cells through caspase-3 activation in a time dependent manner.
- Caspase-3 activation in Caco-2 cells was higher in the insert culture when compared to the plate environment.



Estrano Lab



Cole Lab