IL-10 release by bovine epithelial cells cultured with *Trichomonas vaginalis* and *Tritrichomonas foetus*

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Trichomonas vaginalis *and* Tritrichomonas foetus *are parasitic protists of the human and bovine urogenital tracts, respectively. Several studies have described the cytotoxic effects of trichomonads on urogenital tract epithelial cells. However, little is known about the host cell response against trichomonads. The aim of this study was to determine whether* T. foetus *and* T. vaginalis *stimulated the release of the cytokine interleukin (IL)-10 from cultured bovine epithelial cells. To characterise the inflammatory response induced by these parasites, primary cultures of bovine oviduct epithelial cells were exposed to either* T. vaginalis *or* T. foetus*. Within 12 h after parasite challenge, supernatants were collected and cytokine production was analysed. Large amounts of IL-10 were detected in the supernatants of cultures that had been stimulated with* T. foetus*. Interestingly,* T. vaginalis *induced only a small increase in the release of IL-10 upon exposure to the same bovine cells. Thus, the inflammatory response of the host cell is species-specific. Only* T. foetus *and not* T. vaginalis *induced the release of IL-10 by bovine oviduct epithelial cells.*

Key words: *T. vaginalis* - *T. foetus* - cytokines - host-cells - IL-10

Human and bovine trichomoniases are sexually transmitted diseases caused by the parasites *Trichomonas vaginalis* and *Tritrichomonas foetus*, respectively. These parasites are capable of causing severe vaginal, prostatic and urethral inflammation, which are linked to infertility and adverse pregnancy outcomes in their hosts (BonDurant 2005, Johnston & Mabey 2008). **T. vaginalis** infection typically elicits aggressive, local cellular immune responses that cause inflammation in the vaginal epithelium and ectocervix in women and in the urethra in men (Shafir et al. 2009). There are few studies that address the responses of host cells to *T. vaginalis* and *T. foetus* infection. Some reports have shown that the cytokines interleukin (IL)-8 and tumour necrosis factor (TNF)-α are secreted when host cells are exposed to specific molecules from *T. vaginalis* (Bastida-Corcuera et al. 2005, Fichorova et al. 2006). Even HeLa cells cultured with this parasite have been reported to secrete some cytokines (Chang et al. 2006).

To verify the response of host cells to trichomonad infection, a primary culture of bovine epithelial cells (BOECs) was grown to model host cells, following previously published procedures (Midlej et al. 2009). The JT strain of *T. vaginalis* (Fig. 1A) and the K strain of *T. foetus* (Fig. 1B) were used as models for human and bovine parasites, respectively. Parasites were cultivated in trypticase-yeast extract-maltose (TYM) medium (Diamond 1957) supplemented with 10% foetal bovine serum. Host cell cultures were exposed to a parasite:BOECS cell ratio of 5:1 for 12 h in 37ºC (Fig. 1C, D). Cells were equilibrated in incubation medium for 5 min at 37ºC (5% CO_2) prior to parasite addition. The medium contained two parts of complete DMEM (pH 7.2) and one part TYM medium (W/D 2:1) without serum. The incubation medium and temperature were chosen after testing parasite and host cell viabilities in several different conditions. The supernatants of cultures with parasites or without (controls) were collected and cytokine levels were quantified with specific kits for measuring IL-10 (Becton Dickinson, Franklin Lakes, NJ, USA), which were used according to the manufacturers' instructions. The analyses demonstrated that BOECs secrete a large amount of IL-10 after 12 h of stimulation with the bovine parasite, *T. foetus*. Compared to control cells, parasite-exposed cells released approximately nine-fold more IL-10 (Fig. 2). However, when the BOECs were exposed to the human parasite, *T. vaginalis,* the levels of IL-10 remained at baseline and increased only slightly compared to controls (Fig. 2).

Previous in vitro studies revealed that exposure to *T. vaginalis* stimulated IL-8 secretion in human neutrophils (Ryu et al. 2004), in human monocytes (Shaio et al. 1995) and in human vaginal cells (Singh et al. 2009). Moreover, these studies revealed that cytokine secretion was dependent on NF-κB. It was further demonstrated that the cytokines IL-2 and interferon (IFN)-γ were secreted by murine lymphocytes infected with *T. vaginalis* (Kirch et al. 2004). In addition, *T. vaginalis* also stimulated the secretion of TNF-α, IL-1β and IL-6 by human macrophages (Han et al. 2009).

In this study, increased IL-10 was detected in the supernatants of bovine host cell cultures exposed to *T. foetus*. It is important to mention that IL-10 was also detected in previous studies of dendritic cells and macrophages

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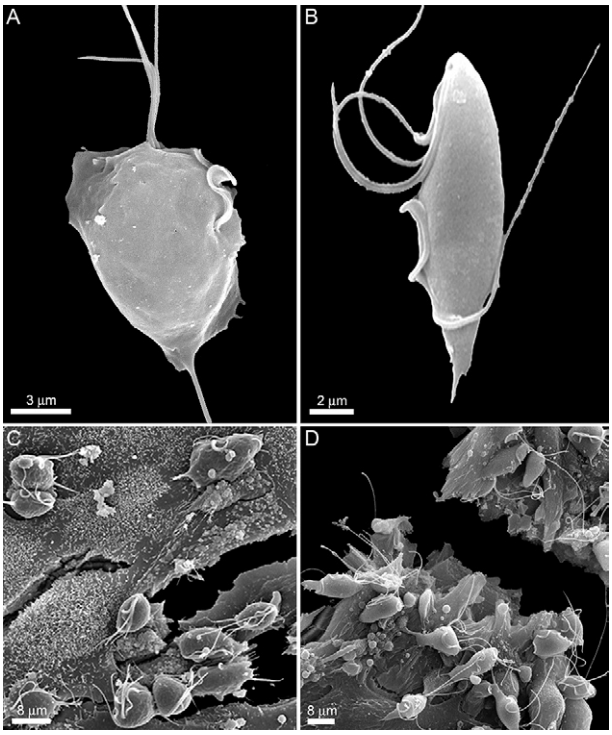

Fig. 1: scanning electron microscopy images of *Trichomonas vaginalis* JT (A) and *Tritrichomonas foetus* K (B). The parasites are adhered to bovine epithelial cells after 12 h of interaction. C: *T. vaginalis*; D: *T. foetus*.

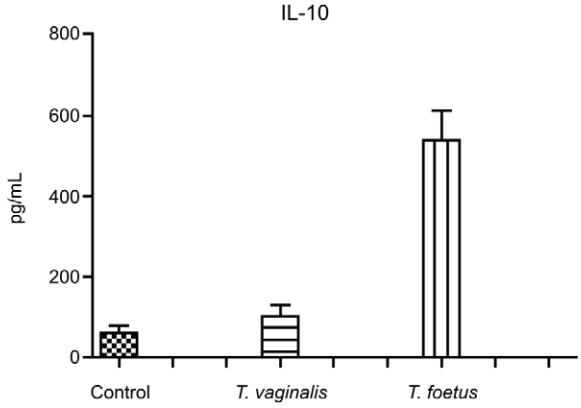

Fig. 2: interleukin (IL)-10 production by bovine epithelial cells before (control) and after interaction with *Trichomonas vaginalis* and *Tritrichomonas foetus*. Note that *T. foetus*, but not *T. vaginalis*, stimulates IL-10 production by bovine epithelial cells. These results represent the means and standard errors of the mean from triplicate cultures in pg/mL.

infected with *T. vaginalis* (Ryu et al. 2004, Scott et al. 2005). IL-10 is an important anti-inflammatory signal that is secreted by several cell populations involved in the immune response, such as macrophages, monocytes and B and T lymphocytes. Additionally, previous reports showed that IL-10 is secreted by immune cells infected with human immunodeficiency virus (HIV), thereby decreasing the number of mature dendritic cells and facilitating viral infection (Alter et al. 2010). These data are relevant because trichomonas-positive patients are more likely to acquire HIV (Moodley et al. 2002). It is hypothesised that this might occur because of the presence of IL-10, which could interfere with cellular defence against HIV entry. Previous studies have also demonstrated that IL-10 could suppress the production of IFN-γ, TNF-α, IL-1 and IL-8 (Moore et al. 2001).

Interestingly, IL-10 secretion was only detected when *T. foetus*, but not *T. vaginalis,* interacted with BOECs. Fichorova et al. (2006) reported that between the two species tested, only *T. vaginalis* LPG induced IL-8 secretion by human cells, thereby demonstrating that the host cell response depends on the species of trichomonad used.

Taken together, these data suggest that cytokine release does not occur when human cells are stimulated with bovine parasites or *vice versa* (i.e., when bovine cells are stimulated with human parasites). Thus, these results indicate that the host immune cell response is species-specific.

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