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Research brief

Cytokine response of human THP-1 macrophages to *Trichomonas tenax*

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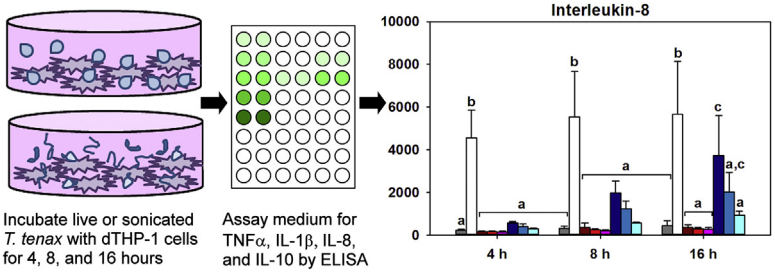
HIGHLIGHTS

- Human THP-1 macrophages were incubated with live or sonicated *Trichomonas tenax* for 4–16 h.
- Live *T. tenax* did not induce TNFα, IL-1β, IL-8, or IL-10 synthesis by THP-1 cells.
- The highest concentration of *T. tenax* lysate stimulated IL-8 synthesis after 16 h.

GRAPHICAL ABSTRACT



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ABSTRACT

Trichomonas tenax is a protozoan that inhabits the oral cavity of humans, most often those with poor oral hygiene. Although *T. tenax* is widely considered a commensal, recent studies have suggested a pathogenic role for the protozoan in persons with periodontitis. Here we investigated the capacity of *T. tenax* to induce pro-inflammatory cytokine secretion in human macrophages, with the idea that elicitation of inflammation may be one mechanism by which *T. tenax* contributes to oral pathology. Human THP-1 cells differentiated to the macrophage phenotype (dTHP-1) were incubated with live or sonicated *T. tenax* at trophozoite:dTHP-1 ratios of 1:5, 1:10, and 1:20. Culture media removed from the wells after 4, 8, and 16 h of stimulation were assayed by ELISA for tumor necrosis factor alpha, interleukin-1 beta, interleukin-8, and the immunoregulatory cytokine interleukin-10. Live *T. tenax* trophozoites failed to induce production of any of the cytokines tested, regardless of trophozoite:dTHP-1 cell ratio or length of co-incubation. *T. tenax* lysates stimulated interleukin-8 synthesis, but only after 16 h of incubation at the 1:5 trophozoite:dTHP-1 cell ratio. These results suggest that pro-inflammatory cytokine synthesis by human macrophages in direct response to *T. tenax* contributes little to oral pathology.

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1. Introduction

Trichomonas tenax (O.F. Müller) is a flagellated, aerotolerant, anaerobic protozoan that colonizes the human oral cavity, where it is found in and around diseased gums and teeth (Hersh, 1985). It has a world-wide distribution, with a prevalence that varies greatly among nations, *e.g.*, 4–53% in Germany, 10% in England, and 16–30% in the United States (summarized by Honigberg and Burgess, 1994). Although often described as a commensal organism, *T. tenax* has been implicated as an etiological agent of periodontitis (Feki et al., 1981; Kurnatowska et al., 2004; Marty et al., 2015). Periodontitis is the most common cause of tooth loss in the world (Darveau, 2010). It is an inflammatory process directed by cytokines produced by host cells in response to oral microorganisms (Silva et al., 2015). Cytokines induce the loss of epithelial attachment, connective tissue, and alveolar bone (Sahingur and Yeudall, 2015). The current study examined the ability of *T. tenax* trophozoites to stimulate pro-inflammatory cytokine production

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by macrophages, information that may be relevant to treatment decisions for persons colonized by the protozoan.

Pro-inflammatory cytokines implicated in periodontitis include interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα), both of which promote bone and tooth resorption by stimulating osteoclast development and production of matrix metalloproteinases (Assuma et al., 1998). Equally important is interleukin-8 (IL-8), a potent chemoattractant for neutrophils that generate reactive oxygen species and tissue-damaging proteolytic enzymes (Jayaprakash et al., 2015). Human cells produce IL-1β, TNFα, and IL-8 in response to the pathogenic trichomonad *Trichomonas vaginalis* (Fichorova et al., 2006; Fiori et al., 2013; Ryu et al., 2004; Seo et al., 2014). Thus, it is reasonable to speculate that *T. tenax* may likewise stimulate production of IL-1β, IL-8, and TNFα, and in this way contributes to the progression of periodontitis.

In this study, we incubated live or sonicated *T. tenax* trophozoites with human THP-1 cells differentiated to the macrophage phenotype (Schwende et al., 1996; Tsuchiya et al., 1982), and then measured IL-1β, IL-8, and TNFα released by the THP-1 cells into the culture medium. We also included interleukin-10 (IL-10) in our analysis, since both *T. vaginalis* and *Tritrichomonas foetus* can stimulate its production, and because the immunoregulatory activity of IL-10 may protect the protozoans from other elements of the immune system (Fichorova, 2009; Song et al., 2015; Vilela and Benchimol, 2013). Differentiated THP-1 (dTHP-1) cells were chosen as responder macrophages based on their ability to alter cytokine synthesis when challenged with such protozoans as *Trichomonas vaginalis* (Fiori et al., 2013), *Toxoplasma gondii* (Quan et al., 2013), *Plasmodium falciparum* (Kumaratilake et al., 1997), *Entamoeba histolytica* (Campbell et al., 2000), and *Leishmania* spp. (Cheekatla et al., 2012). The ability of dTHP-1 cells to synthesize TNFα, IL-1β, IL-8, and IL-10 after appropriate stimulation is well documented (Sun et al., 2014).

2. Materials and methods

2.1. Cell lines and culture conditions

THP-1 cells and the Hs-4:NIH strain of *T. tenax* were purchased from the American Type Culture Collection (ATCC) (Manassas, Virginia) and maintained according to ATCC recommendations. THP-1 cells were cultured at 37 °C under 5% CO_2 in complete RPMI-1640 medium supplemented with 10% fetal bovine serum and 0.05 mM 2-mercaptoethanol. Trophozoites were cultured at 35 °C in borosilicate glass screw-cap tubes in modified LYI *Entamoeba* medium supplemented with 15% adult bovine serum and 2% Diamond's vitamin solution.

2.2. Stimulation of differentiated THP-1 macrophages

THP-1 stimulation experiments were modeled after those described by Fiori et al. (2013). THP-1 cells were seeded into 24-well tissue culture plates at 2.5×10^5 cells/ml in 0.5 ml/well of complete RPMI medium containing 50 ng/ml phorbol 12-myristate 13-acetate (PMA) to induce differentiation to the macrophage phenotype. Differentiation was allowed to proceed over the next 48 h, after which the medium was replaced with complete RPMI containing no PMA. The cells were rested undisturbed for 48 h, followed by twice-daily medium changes for the next 5 days to remove background cytokine levels induced by PMA treatment. The dTHP-1 cells were then incubated with the following stimuli in serum-free RPMI medium: 1 μg/ml *Escherichia coli* 0127:B8 lipopolysaccharide (LPS) (positive control for cytokine induction); live *T. tenax* at trophozoite:dTHP-1 ratios of 1:5, 1:10, and 1:20; sonicated *T. tenax* trophozoites prepared in serum-free RPMI at the same ratios used for live cells; and serum-free RPMI medium containing no added stimulus (negative control for cytokine induction). Culture supernatants were collected after 4, 8, and 16 h of stimulation and stored at −80 °C until analyzed.

2.3. Measurement of cytokines by enzyme-linked immunosorbent assay (ELISA)

Human TNFα, IL-1β, IL-8, and IL-10 were measured in the culture supernatants by two-site capture ELISA, using commercial DuoSet monoclonal antibody kits (R&D Systems, San Diego, California) according to the manufacturer's instructions. Cytokines of known concentrations were included on each ELISA plate to enable construction of standard curves from which cytokine concentrations in the culture supernatants were calculated. A one-way analysis of variance (ANOVA) was used to determine whether means were significantly different among groups. Post-hoc analysis was performed using the Holm-Sidak multiple comparison test at an overall significance of 0.05.

3. Results and discussion

This study yielded little evidence to support the hypothesis that *T. tenax* contributes to oral pathology by directly inducing a pro-inflammatory cytokine response in macrophages. The ELISA results pooled from three (TNFα, IL-8, IL-10) or four (IL-1β) independent experiments for each cytokine are presented in Fig. 1. Incubation of dTHP-1 cells in serum-free RPMI medium containing no added stimulus resulted in negligible production of the four cytokines over the 16 h assay period (Fig. 1A–D). Culture of dTHP-1 cells with 1 μg/ml LPS induced significantly higher levels of TNFα (Fig. 1A) and IL-8 (Fig. 1D) by 4 h of stimulation, and of IL-1β (Fig. 1B) and IL-10 (Fig. 1C) by 8 h of stimulation, than the RPMI control ($P < 0.05$). Live *T. tenax* trophozoites failed to induce cytokine levels that were significantly different from the RPMI control, regardless of which cytokine, incubation time, or trophozoite:dTHP-1 cell ratio was tested (Fig. 1A–D). *T. tenax* lysates stimulated significantly higher levels of IL-8 than the RPMI control after 16 h of incubation at the 1:5 trophozoite:dTHP-1 cell ratio (Fig. 1D, $P < 0.05$). At 16 h, IL-8 concentrations (mean ± standard deviation) were 438 ± 234 pg/ml in the RPMI control, 5661 ± 2467 pg/ml in the LPS control, and 3723 ± 1873 pg/ml in the supernatant of THP-1 cells incubated with *T. tenax* lysate. Lower lysate concentrations and shorter incubation times did not induce IL-8 levels that were significantly different from those induced by RPMI.

The results we obtained with *T. tenax* differed somewhat from those obtained by Fiori et al. (2013), who induced cytokine synthesis in dTHP-1 cells by incubation with the *T. vaginalis* G3 strain. *T. tenax* lysates stimulated only IL-8 secretion, while *T. vaginalis* lysates stimulated production of TNFα and IL-1β in addition to IL-8. Live *T. tenax* trophozoites did not stimulate secretion of any cytokine examined herein, whereas live *T. vaginalis* stimulated measurable levels of IL-1β, but no TNFα or IL-8. Neither *T. tenax* nor *T. vaginalis* induced IL-10 production by dTHP-1 cells, regardless of whether the protozoans were added to dTHP-1 cultures as live trophozoites or lysates. Differences in the abilities of these two so-called “human-specific” trichomonads to stimulate cytokine synthesis in human macrophages were not wholly unexpected, given their phylogenetic and host-range differences. *T. tenax* and *T. vaginalis* are more closely related to the avian species *T. gallinae* and *T. stableri*, respectively, than to each other (Maritz et al., 2014). Moreover, *T. tenax* can infect both dogs (Cielecka et al., 2000; Szczepaniak et al., 2016) and cats (Cielecka et al., 2000) in addition to humans, demonstrating its expanded host range compared

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