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Cytokine gene expression and pathology in mice experimentally infected with different isolates of *Trypanosoma evansi*

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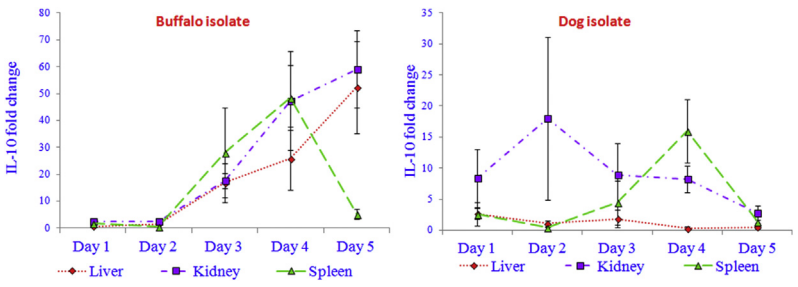
HIGHLIGHTS

- First report of IL-2, IL-4, IL-10 and IL-12 gene expression in *T. evansi* infection.
- IL-10 cytokine in *T. evansi* infection is important and mediated by parasite factors.
- Increment in IL-6, TNF-α and IFN-γ in mice tissues at different time points.
- Buffalo isolate of *T. evansi* seems to be more pathogenic than dog isolate in mice.

GRAPHICAL ABSTRACT



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ABSTRACT

Aim of the present study was to assess the cytokine gene expression in liver, kidney and spleen and histopathological changes in mice infected with buffalo and dog isolates of *Trypanosoma evansi*. Forty-four Swiss albino mice was divided into eleven groups of four mice each and injected subcutaneously with 1×10^5 trypanosomes of buffalo and dog isolate to twenty mice each, four mice served as control. Mice were examined for clinical signs, blood smear for trypanosome counts. Blood for PCR, liver, kidney, spleen, heart, lung, testis and abdominal muscle for histopathology and liver, kidney, spleen for cytokine gene expression studies, were collected. Mice showed dullness, lethargy, hunched back, sluggish movements on D4 and D5 in buffalo and dog isolate, respectively. Parasite count in blood varied between the two isolates of *T. evansi*. By PCR, trypanosome DNA was detected on D1 and D2 for buffalo and dog isolate, respectively. Splenomegaly was observed in mice infected with buffalo isolate but not with dog isolate. Histopathological changes were observed in liver, kidney, spleen and heart of mice but no changes in testis and abdominal muscles. Blood vessels of liver, heart, lung showed presence of trypanosomes in mice infected with buffalo isolate but not for dog isolate. Cytokine gene expression of IL-2, IL-4, IL-6, IL-12, TNF-α and IFN-γ increased in liver, kidney and spleen in both these isolates. However, the buffalo isolate exhibited pronounced increase in cytokine gene expression when compare to dog isolate of *T. evansi*. Anti-inflammatory cytokine gene IL-10 showed 50–60 and 10–20 folds increment in buffalo and dog isolates, respectively. This is the first report of IL-4, IL-6, IL-10 and IL-12 cytokine changes in mice infected with *T. evansi*. A variation in pathogenicity between buffalo and dog isolates was recorded indicating buffalo isolate of *T. evansi* remained more pathogenic in mice.

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1. Introduction

Trypanosomosis is an economically important disease in many developing countries and affecting a wide range of domestic and wild species of animals. *Trypanosoma (T.) evansi* is also highly pathogenic to laboratory animals like rat, mice and rabbits (Singla et al., 2003). Several human trypanosomosis infection by *T. evansi* have been reported recently from India (Truc et al., 2013). Disease progression and the clinical, haematological and pathological aspects of the *Trypanosoma evansi* in the host varies depending on the isolate's virulence, host susceptibility and epizootic conditions. Visceral forms of *T. evansi* have been reported in heart, optic lobes, cerebrum, liver, kidney and lungs (Singla et al., 2001). Also during the infection, an increase in the level of total protein, alpha-2-globulin, beta-globulin and gamma-globulin has been reported and related to host immune response (Costa et al., 2010). The immune system is involved in infection control and activates different cellular mechanisms, in which cytokines play an important role. Cytokines are low molecular weight proteins which act as intercellular mediators involved in many biological processes such as inflammation, fibrosis, angiogenesis, cell growth, cell proliferation and immune response (Tizard, 2002). Lymphocytes in the blood produces interferon-gamma (IFN-γ) in response to the *T. evansi* antigens which in turn activates the macrophages and increase their ability to phagocytise the parasites. The activated macrophages induce the production of inflammatory cytokines such as IL-1, IL-6 and TNF-α playing an important role in the replication of the parasite as well as in the host immune response (Magez et al., 2007). Cytokines, in addition to their well documented roles, are referred to have suppressing activity on erythropoiesis (Cançando and Chiattone, 2002) and having key role in anaemia associated with inflammation (Noyes et al., 2001). Unlike other trypanosomosis, information about the immunological mechanisms in *T. evansi* infection is limited. Further, different isolates of the *T. evansi* has not been compared for their pathogenicity and an attempt was made in this study. There is no data regarding the cytokine gene expression in liver, kidney and spleen of mice such as IL-2, IL-4, IL-6, IL-12, TNF-α, IFN-γ and anti-inflammatory cytokine IL-10, in *T. evansi* infection. The present study was aimed to provide information about the cytokine gene expression in liver, kidney, spleen, and pathology of mice infected with buffalo and dog isolates of *T. evansi*.

2. Materials and methods

2.1. Experimental animals

Forty four male Swiss albino mice (inbred) approximately 6–8 weeks of age weighing 25–30 gm were procured from National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad, India and used in the present study. Mice were housed in individually ventilated cages (IVC) and fed with pellet rodent feed and purified water *ad libitum*. The animal house room temperature and humidity were maintained at 23 ± 2° C, and 50–70%, respectively. The animal experiments were approved by Institutional Animal Ethics Committee of NIVEDI, Bengaluru (CPCSEA Registration No. 881/03/ac/05/CPCSEA) for using experimental animals in this study and carried out as per Committee for the Purpose of Supervision and Experiments on Animal (CPCSEA) guidelines, Ministry of Environment, Forests and Climate change, Government of India, New Delhi.

2.2. Trypanosoma evansi isolate

Different isolates of *T. evansi* such as buffalo and dog were maintained in the Parasitology Laboratory, NIVEDI, Bengaluru, India was used in the present study. The different isolates of *T. evansi* were propagated in mice as mentioned earlier (Sengupta et al., 2010, 2012, 2014) and purified using diethyl amino ethyl (DEAE) cellulose (DE.52-Whatman) column (Lanham and Godfrey, 1970). Counting of the trypanosomes was done as per method described previously (Janeen et al., 1972).

2.3. Animal infection

Forty four Swiss albino mice were divided in to eleven groups of four mice each and five groups of mice per isolate and one control group of four mice. Each mice in the control group were given sterile PBS through subcutaneous route. Twenty mice per isolate were infected with buffalo and dog isolate of *T. evansi* (maintained in mice) by injecting each with 1×10^5 trypanosomes by subcutaneous route to simulate the natural infection of animals. The dose of trypanosomes were fixed based on our experience from previous studies (Sengupta et al., 2010, 2012, 2014). All the mice were examined for the presence of trypanosomes by examination of blood smear on 1–5 days post infection (D1-D5). The mice were observed for development of clinical signs and four mice per isolate was sacrificed each day by using overdose of anaesthesia from D1-D5.

2.4. Blood smear examination and trypanosome counts

Blood was collected from intracardiac route, smear prepared on grease free glass slide and stained with Giemsa's stain. The stained slides were examined under light microscopy and counted the trypanosomes per high power field (100× magnification). The magnitude of infection was expressed as the number of trypanosomes per high power field.

2.5. DNA extraction and PCR detection of trypanosomes

Blood was collected in dipotassium EDTA coated vacutainer tubes and genomic DNA was isolated by using QIAmp DNA mini kit (Qiagen, USA) and followed the DNA extraction as per the kit manufacturer's instructions. The presence of *T. evansi* DNA in blood from D1 to D5 was done by using two primers DITRY F and DITRY R with accession number EF495337 and followed the PCR protocol as reported (Sengupta et al., 2010).

2.6. Pathology

At the time of sacrifice the gross examination of visceral organs were done and liver, kidney, spleen, heart, lung, testis and abdominal muscles were collected in 10% buffered formalin. The formalin fixed tissues were processed, embedded in paraffin, 5 μm tissue sections were prepared using rotatory microtome and stained with haematoxylin and eosin (Bancroft and Stevens, 1996). After staining, the sections were dehydrated in increasing ethanol concentrations, cleared in xylene, mounted in DPX and were examined under light microscope for histological changes. The criteria for scoring pathological changes in various organs were considered as reported earlier (Krishnamoorthy et al., 2007).

2.7. Cytokines gene expression

The liver, kidney and spleen tissues were collected in RNA later® solution (Ambion, USA) and stored in deep freezer at −80° C until used. The RNA extraction was carried out from liver, kidney and spleen tissues by using RNA extraction kit RNeasy mini kit (Qiagen, USA). The RNA concentration was measured (NanoDrop C2000,