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Trypanosoma brucei brucei: A comparison of gene expression in the liver and spleen of infected mice utilizing cDNA microarray technology

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ABSTRACT

Trypanosoma brucei brucei, the infectious agent of the disease known as Nagana, is a pathogenic trypanosome occurring in Africa, where it causes significant economic loss to domesticated livestock. Although many studies on the histopathology of organs of mice infected with *T. b. brucei* have been reported, little work has been done regarding gene expression in these organs in infected mice. In this paper, we describe the use of cDNA microarray to determine gene expression profiles in the liver and spleen of mice infected with *T. b. brucei* (STIB 920) at peak parasitaemia (12 days after infection). Our results showed that a total of 123 genes in the liver and 389 genes in the spleen were expressed differentially in *T. b. brucei* infected mice. In contrast, however, in an acute infection in mice caused by *Trypanosoma brucei evansi*, a species genetically related to *T. b. brucei*, 336 genes in the liver and 190 genes in the spleen were expressed, differentially, indicating that the liver of mice was more affected by the acute *T. b. evansi* infection whilst the spleen was more affected by the subacute *T. b. brucei* infection. Our results provide a number of possible reasons why mice infected with *T. b. evansi* die sooner than those infected with *T. b. brucei*: (1) mice infected with *T. b. evansi* may need more stress response proteins to help them pass through the infection and these are probably excessively consumed; (2) proliferating cell nuclear antigen was more down-regulated in the liver of mice infected with *T. b. evansi*, which indicated that the inhibition of proliferation of hepatocytes in mice infected with *T. b. evansi* might be more severe than that in *T. b. brucei* infection; (3) more hepatocyte apoptosis occurred in the mice infected with *T. b. evansi* and this might be probably the most important reason why mice died sooner than those infected with *T. b. brucei*. Studies of the changes in the gene expression profile in the liver and spleen of mice infected with *T. b. brucei* may be helpful in understanding the mechanisms of pathogenesis in Nagana disease at the molecular level. By comparing the gene profiles of the liver and spleen of mice infected with *T. b. brucei* with *T. b. evansi*, we have identified a number of factors that could explain the differences in pathogenesis in mice infected with these two African trypanosomes.

1. Introduction

Trypanosoma brucei is a widely distributed haemoflagellate protozoan parasite found in many countries of Africa and is transmitted by tsetse flies of the genus *Glossina* to a broad range of mammal species, varying from waterbuck and hartebeest to lions and hippopotamus and including domesticated livestock (Hoare, 1972). Among the three subspecies of *T. brucei*, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* cause the debilitating disease called 'sleeping sickness' in humans while *T. b. brucei* causes the disease called 'Nagana' in domestic mammals and this species is considered to be the most virulent in domestic animals among the three subspecies of *T. brucei* (Hoare, 1972; Ochiogu et al., 2008). Thousands of domestic and wild mammals suffer from *T. b. brucei* infection each year in endemic regions, which causes serious economic loss (Hoare, 1972; Troeberg et al., 2000).

The liver is one of the most important visceral organs affected by pathogens, including *T. b. brucei*. Many pathological changes such as hepatauxe, congestion, haemorrhagic lesions, cellular infiltration in the portal tract and fatty degeneration of hepatocytes, and hepatocellular degeneration have been reported in animals infected with *T. b. brucei* (Fiennes, 1970; Murray et al., 1973; Morrison et al., 1981a; Anosa and Kaneko, 1984). Anosa and Kaneko (1984) observed that the necrosis of the hepatocytes caused by

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T. brucei infection in deer mice affected only a small fraction of cells. They inferred that the increased breakdown of red blood cells and intense antigenic stimulation were related to the changes in the Kupffer's cell and perivascular accumulation of lymphocytes, respectively. Extensive damage of the spleen was also observed in the animals infected with the *brucei*-group of trypanosomes (Sadun et al., 1973; Brown and Losos, 1977; Barrowman and Roos, 1979; Morrison et al., 1981b; Anosa and Kaneko, 1984; Ngeranwa et al., 1993; Li et al., 2009). An intense proliferative response, particularly the B-dependent follicular areas, was observed and was accompanied by a dramatic increase in the number of plasma cells in the splenic red pulp. Furthermore, focal haemorrhage, deposition of fibrin, necrosis, and infiltration of polymorphonuclear leukocytes were also found during the progression of *T. brucei* in the spleen of infected mice. The most severe of these changes was observed in the peripheral follicular areas (Fiennes, 1970; Murray et al., 1973; Morrison et al., 1981b; Anosa and Kaneko, 1984). The pathological changes in the liver and spleen of infected animals indicated that *T. b. brucei* infection induces severe destructive and irreversible changes that lead to death.

It is known that the liver is one of the key organs responsible for immunological elimination of murine trypanosome infections (Albright et al., 1990). Among the lymphatic tissues, the spleen is one of the most important organs and serves as a first line of defense in response to parasitic invasion (Biswas et al., 2001). Although the clinical and pathological manifestations of *T. b. brucei* have been widely reported (Fiennes, 1970; Murray et al., 1973; Morrison et al., 1981a,b; Anosa and Kaneko, 1984), there is limited information about the changes that occur at the molecular level in the immunological organs of mice infected with *T. b. brucei* and the reasons why mice with *Trypanosoma brucei evansi* infection died sooner than those infected with *T. b. brucei*. Therefore, the purpose of the present study is: (1) to examine the effects of *T. b. brucei* infection on the liver and spleen of mice at the molecular level to further understand the mechanisms of pathogenesis details in *T. b. brucei* infection; (2) to determine the reason(s) why mice infected with *T. b. evansi* died sooner than those infected with *T. b. brucei* by comparison of the results from other studies on *T. b. evansi* infection (Li et al., 2009).

2. Materials and methods

2.1. Animals and trypanosome infection

Twelve male BALB/c mice (approximately 6–8 weeks old) were purchased from the Experimental Animals Center of Sun Yat-Sen (Zhongshan) University, Guangzhou and were maintained in an air-conditioned animal room at 25 °C with free access to water and food under 12 h light/dark cycles. After 1 week acclimation, mice were randomly allocated into two groups ($n = 6$). One group was infected with 5×10^2 *T. b. brucei* STIB 920, a virulent strain which can cause mice die at about 5–6 weeks depending on the number of parasites inoculated, by intraperitoneal injection as described by Rasooly and Balaban (2004) and the parasitaemia was monitored daily from 3 days after infection by examining wet-smears of tail-tip blood with a haematocytometer under a microscope. The second group of mice was maintained uninfected as control.

2.2. Sample preparations for microarray analysis

All mice were euthanized by CO_2 inhalation 12 days after infection when the parasitaemia of the infected mice reached a peak level (>10^8 cells organisms/ml). The liver and spleen were removed from each mouse and stored in liquid nitrogen as soon as possible.

2.3. Microarray hybridization and data analysis

Microarray analysis was carried out by the United Gene Holdings, Ltd., Shanghai, China as described by Li et al. (2009). Data from the hybridization experiments were viewed as a normalized ratio (Cy5/Cy3) in which significant deviations from 1 (no change) are indicative of increased ($\geqslant$2.0) or decreased ($\leqslant$0.5) levels of gene expression in infected mice relative to control (non-infected) mice. Finally, genes were categorized into GeneOntology (GO) (http://www.geneontology.org).

2.4. Quantitative real-time RT-PCR for microarray data validation

To verify the microarray data, several genes, such as heat shock protein 70 (Hsp70), proliferating cell nuclear antigen (Pcna), Caspase3 and the housekeeping gene glyceraldehyde-3-phophate dehydrogenase (GAPDH) from different categories were chosen for quantitative real-time PCR analyses (Table 2). Quantitative real-time RT-PCR was carried out as described by Li et al. (2009).

2.5. Immunohistochemistry of Hsp70 and Pcna in liver and spleen sections

Immunohistochemistry was conducted on the liver and spleen specimens removed from the mice on day 12 post-infection with *T. b. brucei* and from non-infected (control) mice with a mouse monoclonal antibody to Hsp70 (dilution 1:300; Santa Cruz, CA, USA) or Pcna (dilution 1:300; Santa Cruz). The signal was detected with a horseradish peroxidase detection system using DAB (Sigma, MO, USA). Sections were examined microscopically after the specific staining and photographs were taken using a digital image-capture system (Olympus, Tokyo, Japan). We counted 30 fields in the immunohistochemistry of every molecule. Significant differences (*P*-values) were determined by comparison of means by Student's *t*-test using SPSS 11·0 software (SPSS Inc., Chicago, USA).

2.6. TUNEL detection

Our results from the microarray analysis indicated that *T. b. brucei* infection could significantly induce apoptosis in the liver but not in the spleen of infected mice. Therefore, apoptosis in the livers of trypanosome infected and non-infected (control) mice was further analyzed using a commercial kit (GENMED, Shanghai, China) based on the TdT-mediated dUTP-digoxigenin nick end labelling (TUNEL) of apoptotic cells, according to the manufacturer's instructions. Sections were examined microscopically after staining and photographs were taken using a digital image-capture system (Olympus). We counted 30 fields of every image in the TUNEL analysis and significant differences (*P*-values) were determined by comparison of means by Student's *t*-test using SPSS 11·0 software (SPSS Inc., Chicago, USA).

3. Results

3.1. Gene expression profile changes in the liver and spleen of mice infected with T. b. brucei

The genes that showed differential expression in the liver and spleen of mice infected with *T. b. brucei* were sorted into 17 groups according to their functions (Fig. 1). Of the total of 14,000 genes on the microarray chip, 44 genes with GO annotation were up-regulated and 79 were down-regulated in livers of the infected mice after 12 days of infection, in comparison with the controls. Transport-related genes represented the largest functional group among the altered genes in the liver and spleen of infected mice