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Profiling of jejunum inflammatory gene expression during murine eimeriosis

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ABSTRACT

To understand the host-parasite relationship during coccidiosis it is necessary to identify the transcriptional profile of the local host. In this study, gene profiling in the mouse jejunum due to infection with *Eimeria papillata* was investigated using Agilent microarray technology. On day 5 post-infection, the characterization of infected and non-infected mice jejunum transcriptional response was compared. There was an increase in the level of tumour necrosis factor-α, nitrite/nitrate and nitric oxide synthase activity was observed following infection. Also, the activity of glutathione peroxidase was reduced from 86.5 to 38.2 mU/g. In addition, *E. papillata* infection was associated with an increase in the activities of both the mice alkaline phosphatase and lactate dehydrogenase. Moreover, experimental *E. papillata* infection in mice induced a significant elevation in protein carbonyl content, by about 70%. Agilent genome microarray detected 11 genes whose expression was up-regulated by more than 10-fold, and 30 genes whose expression was down-regulated by a similar amount five days after infection with *E. papillata*. The expression profiles of the Fas apoptotic inhibitory molecule 3(FAIM3), chemokine (*C*-X-C motif) receptor 5 (Cxcr5), succinate receptor 1 (SUCNR1), hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 3 (Hsd3b3) and cytochrome P450, family 2, subfamily b, polypeptide 9 (Cyp2*b*9) genes, arbitrarily selected from the microarray analysis, closely resemble the expressions determined by quantitative PCR. The data indicate that, *E. papillata* is associated with the induction of inflammatory response and with gene regulation in mice jejunum.

1. Introduction

Protozoan parasites of the genus Eimeria are known to be responsible for coccidiosis, a parasitic disease characterised by enteritis of varying severity, and associated with many structural and functional changes to their hosts leading to nutrition imbalance, disturbance in food digestion and absorbance, and finally decreased weight and low performance [1,2]. Economically, intestinal coccidiosis is considered to be a threat to the commercial rearing of animals including cattle, rabbits and poultry [3]. When used to infect the jejunum of mice, *Eimeria papillata* has been employed as a model to explore many pathological changes and metabolic changes in the infected mice [4].

Detailed studies of the molecular biology of host–parasite interactions could help us for better understanding to prevent and control parasitic diseases. In particular, in recent years, gene profiling technology has allowed scientists to acquire a much better understanding of the molecular processes concerned with health and disease by enabling the expression of numerous gene transcripts to be monitored in a single hybridization experiment [6,7].

In this regard, cDNA microarray technology has been intensively applied in the study of local host transcription profiles after intestinal *Eimeria* infection [2,8], as well as in the study of the impact of this infection upon other organs [9,10].

One of the advantages of cDNA microarray technology is that its ability to characterise host transcriptional responses, whether across the whole genome or on a tissue-specific basis, enables scientists to study complex transcriptional patterns, the understanding of which provides new insights to analyze intricate biological system. The host-parasite interactions during coccidiosis are a good example of the kind of contexts that can be thoroughly

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investigated using cDNA microarray technology in order to provide vital insights that can inform the development of new treatments to control these complex protozoan pathogens [8].

Accordingly, this study uses Agilent-one-colour microarray analysis to study gene expression in mice jejuna infected with *E. papillata*. Experimental microarray analysis results were also verified using quantitative real time PCR of the jejunal tissues.

2. Materials and methods

2.1. Mice and infection

The mice used in this study were 10–12 week old male Swiss albino mice, obtained from the animal facilities of King Saud University. All animals were fed a standard diet and water ad libitum. Our experiments were approved by the relevant government animal welfare authorities. Also, we followed internationally accepted rules on animal protection.

E. papillata oocysts were provided by Prof. Mehlhorn (University of Dusseldorf, Germany). The population of *E. papillata* from which the oocysts were drawn had been maintained in infected mice. The oocysts were collected from the faeces of these infected mice, and following the method described by Schito et al. [11], they were sterilized with sodium hypochlorite and washed several times with a sterile NaCl solution before being used for this experiment.

In this study ten mice were infected by oral inoculation with 1500 sporulated oocysts of *E. papillata* suspended in saline. Ten control mice were inoculated with saline alone. Subsequently, fresh faecal pellets were collected once a day from each of the mice and weighed. Following the method outlined in Dkhil et al. [12], the collected faecal pellets were first suspended in 2.5% (wt/vol) potassium dichromate, which was then diluted with a saturated saline solution to allow the oocysts to separate and float. A McMaster chamber was used to count the oocysts, with the results expressed as number of oocysts per gram of faeces [11].

2.2. Preparation of jejunal tissue

Five days after infection all the experimental mice (both infected and control) were sacrificed. The jejuna were removed from the mice using standard aseptic precautions and were cut into small pieces and washed in sterile physiological saline. The collected jejunal pieces (included the whole parts of serosa, muscularia, submucosa and mucosa), were then immediately weighed and homogenized with 50 mM Tris-HCl and 300 mM sucrose to give an ice-cold 50% (w/v) homogenate medium. This homogenate was then centrifuged at 3000×*g* for 20 min at 4 °C. The supernatant (10%) was rapidly frozen and stored at −80 °C until needed for the various experimental determinations.

2.3. Oxidative stress biomarkers

Oxidized proteins were determined in the jejunal homogenate by the estimation of protein carbonyl content through the formation of labelled protein hydrazone derivatives using 2, 4-dinitro phenyl hydrazine [13]. According to the manufacturer protocol (ALPCO Diagnostics, USA), both of TNF-α level and iNOs activity was determined in the jejuna homogenate using antigen antibody interaction in 96-microplate wells by immunosorbent assay.

Glutathione peroxidase activity within the jejunum was determined kinetically depending on the catalysing potential of GPx to reduce H_2O_2 and to oxidize reduced glutathione to form oxidized glutathione which in turn is reduced by glutathione reductase and NADPH to form $NADP^+$. This process results in decreased absorbance of the solution at 340 nm, which is directly proportional to the GPx activity [14].

The enzymatic activities of alkaline phosphatise (ALP) and lactate dehydrogenase (LDH) were determined via kinetic ultraviolet methods using commercially available kits (Biodiagnostic Company, Gizza, Egypt) following the methods set out in the manufacturer's manual.

2.4. Isolation of total RNA

In order to isolate the total RNA in the collected jejunal tissue, the frozen tissue was homogenized in liquid nitrogen and the total RNA was isolated with TRIzol (Sigma-Aldrich). An Agilent RNA 6000 Nano Kit was then used with the Agilent 2100 Bioanalyzer (Agilent Technologies) to determine the quality and integrity of the isolated RNA. The amount of RNA isolated was quantified by measuring the absorbance at 260 nm on the ND-1000 Spectrophotometer (NanoDrop Technologies) (the A_{260nm} measure).

2.5. Labelling of RNA and hybridization

Labelling and hybridization were performed as detailed in the protocol for one-Color Microarray-Based Gene Expression Analysis (version 5.5, part number G4140-90050). Specifically, the Agilent Low RNA Input Linear Amp Kit (Agilent Technologies, Palo Alto, California) was used to amplify and label 1 μg of total RNA in the presence of cyanine 3-CTP and cyanine 5-CTP (Perkin Elmer). Subsequently, a ND-1000 Spectrophotometer (NanoDrop Technologies) was used to measure the yields of cRNA along with the dye-incorporation rate.

Hybridization was undertaken with 825 ng of the cyanine 3 and cyanine 5 labelled cRNA hybridized to Agilent Whole Mouse Genome Oligo Microarrays closely following the procedure previously set out in Arandey Cortes et al. [15].

2.6. Scanning analysis

The agilent's Microarray Scanner System (Agilent Technologies, Palo Alto, California) was used to monitor the signals produced from the fluorescent hybridized microarrays. Again following the procedure in Arandey Cortes et al. [15] with the exception that the data returned by the Feature Extraction Software was analyzed using the Rosetta Resolver® gene expression data analysis system (Rosetta Bio software) rather than Agilent's GeneSpring GX program. The local signal of each spot was measured inside a 300-μm-diameter circle. The local background was determined as described in Dkhil et al. [16]. The latter was then subtracted from the former to give the net signal intensity and the ratio of Cy5 to Cy3.

2.7. Gene quantification

Real time PCR was performed as detailed previously [12]. cDNA was synthesized from the previously isolated RNA using a QuantiTect™ Reverse transcription kit (Qiagen). The QuantiTect™ SYBR® Green PCR kit (Qiagen) was then used for amplifications in the ABI Prism® 7500HT Sequence Detection System (AppliedBiosystems, Darmstadt, Germany) with gene-specific QuantiTect™ primers, also supplied by Qiagen (Hilden, Germany). PCR reactions were performed and evaluated as set out in Delic et al. [17].

2.8. Statistical analysis

Comparisons between groups were performed using SPSS software. Significant differences between groups were identified using the unpaired Student's *t*-test.