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High-throughput dynamic analysis of differentially expressed genes in splenic dendritic cells from mice infected with *Schistosoma japonicum*

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ABSTRACT

Dendritic cells are the initiation and key point of immune response and play a role in immune regulation. So we explored the mechanisms involved in immune regulation of dendritic cells (DCs) against schistosomiasis using mice infected with *Schistosoma japonicum*. Splenic DCs from normal mice and mice with acute and chronic *S. japonicum* infection were sorted by flow cytometry. The numbers and functions of differentially expressed genes (DEGs) in DCs were determined by high-throughput analysis. All DEGs with transcription-level fold changes of ≥2 were selected and matched to corresponding genes in databases. Annotations and cluster analysis of DEGs were performed to compare differences between groups. Six important DEGs about immune regulation—*CD86*, *TLR2*, *DC-SIGN*, *Capase3*, *PD-L2*, and *IL*-7r were selected, and their transcription levels at different stages of schistosomisis were validated by qPCR. The Venn diagram of DEGs implied some genes are functional at all stages during *S. japonicum* infection, while others are only involved at certain stages. GO and KEGG pathway annotations indicated that these DEGs mainly belong to biological regulation, regulation of biological process, regulation of cellular process, antigen processing and presentation, cell adhesion molecules, cytokine–cytokine receptor interaction and Toll-like receptor signaling. Cluster analysis revealed immune regulation existed in splenic DCs. The results above indicated that the mechanisms underlying immune regulation to *S. japonicum* infection in mice are very complex. The present high-throughput dynamic analysis of DEGs in splenic DCs provides valuable insights into the molecular mechanisms underlying immune regulation in *S. japonicum* infection.

1. Introduction

Cercariae of *Schistosoma japonicum* that invade the final host percutaneously develop from skin-stage, lung-stage, and liver-stage schistosomula to form pairs; this is followed by maturation into adult worms that begin to produce eggs 24 days after infection [1–4]. The eggs require approximately 11 days to mature, and they are mainly deposited in the liver, where they cause pathological damage [5]. Secretions; excretions; shed surface membrane; and lysates after the death of schistosomula, adult worms, and eggs act as sources of antigens, which induce the host immune response or immune suppression. In particular, the miracidia within mature eggs continuously produce and release soluble egg antigen (SEA) into the blood circulation of hosts [6]. During the acute stage of *S. japonicum* infection, schistosome antigens induce Th1-dominantcell-mediated immune response in the host. At 8–12weeks post infection, the host enters the chronic infection stage, and SEA released by adult worms induces a shift from Th1-type cellular immunity to Th2-type cellular immunity [7–10]. Immune regulation facilitates the survival of parasites, simultaneously preventing severe impairment of host immunity [11].

Compared with macrophages and B cells, dendritic cells (DCs) possess the strongest antigen-presenting ability [12] and initiate the immune response. DCs play an important role in inducing the immune response and immune suppression [13,14]. Therefore, DCs are useful for studying the mechanisms underlying the immune regulation against schistosomiasis. However, to date, few studies have addressed the molecular mechanisms involved in these processes.

Microarrays enable the high-throughput analysis of large numbers of genes. Po-ching et al. (2011) reported the use of microarray technology in studying genes related to immune regulation in macrophages at different stages of *S. japonicum* infection [15].The microarray technique has been used to study the gene expres-

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sion profile of lipopolysaccharide-stimulated DCs induced by the *S. japonicum*-derived recombinant protein Sj16 [16]. High-throughput sequencing methods have been used to elucidate the molluscicidal mechanisms of a wettable power of niclosamide ethanolamine salt and the salt of quinoid-2′5-dichloro-4′-nitrosalicylanilide [17].

The present study aimed to gain a preliminary understanding of the roles of DCs in the immunity against *S. japonicum*, and to elucidate the underlying mechanisms. In particular, important functional molecules involved in the immune response and immune suppression in DCs were investigated.

2. Materials and methods

2.1. Animals and parasites

Specific pathogen-free C57BL/6 mice aged 6–10 weeks were purchased from the Center for Animal Experiment of Wuhan University. Miracidia-positive snails were obtained from the Hunan Institute of Schistosomiasis Prevention and Control, China. Mice were randomly divided into 3 groups with 6 mice each: the normal group (group N), mice infected with *S. japonicum* for 28 days (group I_{28}), and mice infected with *S. japonicum* for 63 days (group I_{63}). Group I_{28} represents mice at the acute infection stage, whereas group I_{63} represents mice at the chronic infection stage. According to methods described in previous studies [18], group I_{28} and group I_{63} mice were infected with 25 ± 1 cercariae of *S. japonicum* through the abdominal skin, while group N mice were subjected to the same treatment but without cercariae. The mice were euthanized on schedule, and spleens were removed for subsequent experiments. All animal experiments were approved by the Animal Ethics Committee of Wuhan University (Approval No.: 2015-0018).

2.2. Preparation of mouse splenic DCs

DCs were sorted by flow cytometry according to methods described in a previous study [19]with slight modifications. Mouse spleens were collected for the preparation of single-cell suspensions at a cell density of 1×10^6/mL. Then, 300 μL of the cell suspension was added to a 1.5-mL Eppendorf tube, to whichPE-Cy5-conjugated CD3e and FITC-conjugated CD11c (eBioscience, USA) were successively added. Armenian hamster IgG isotype control FITC (eBioscience, USA) was added to the isotype control tube. Each tube was incubated in the dark at 4 °C for 30 min and then washed 5 times with PBS. The samples obtained were used for sorting DCs by flow cytometry (FACSAria III, BD Biosciences, USA).

2.3. Whole-genome oligo microarray

Total RNA was extracted from the sorted DCs by Trizol reagent (Invitrogen, USA) and then purified with the RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. Next, the purity of RNA was assessed by NanoDrop ND-1000 (Nanodrop Technology, USA). Suitable RNA samples were selected as cDNA templates for the preparation of cRNA. Then, cRNA was purified and its purity re-assessed. Next, selected RNA samples were labeled with Cy-3 using the Quick Amp Labeling Kit with 1color (Agilent Technologies, USA) and scanned by the microarray scanner (Agilent Technologies).

2.4. Microarray analysis of differentially expressed genes

The Agilent Mouse 4 × 44 K Gene Expression Microarray v2, containing 39,000 mouse genes from the RefSeq, Ensembl, Unigene, GenBank, and RIKEN databases, was used in the study. Gene expression levels were first compared between any 2 groups to select differentially expressed genes (DEGs) as genes with fold changes of ≥2. These were then subjected to searches against the Gene Ontology (GO) database and Kyoto Encyclopedia of Genes and Genomes (KEGG) database, followed by cluster analysis. Microarray data have been submitted to Gene Expression Omnibus (GEO) under the accession number GSE79713.

2.5. Validation of 6 DEGs by real-time PCR

Total RNA was extracted from the sorted DCs by Trizol reagent and reverse transcribed into cDNA by a cDNA synthesis kit (Fermentas, Canada). Primers specific to *CD*, *TL*,*DC*, *Ca*, *PD*, *IL*,and *GAPDH* were designed based on their gene sequences, and the gene sequences obtained were stored in GenBank under accession numbers NM_019388, NM_011905, NM_133238, XM_017312543, NM_021396, XM_017316439, NM_008372. The genes were amplified by PCR using SYBR Green fluorescent dye (Takara, Japan) with a total reaction volume of 20 μL. The thermal cycling was as follows: 95 °C for 3 min; 39 cycles of 95 °C for 10s, 60 °C for 10s, and 72 °C for 15s; and finally, 95 °C for 10s.The cycle threshold (CT) values of each gene were normalized by the average value of GAPDH, and the fold change in the gene expression level was calculated by the $2^{-\triangle\triangle CT}$ method.

2.6. Statistical analysis

Student's *t*-test was used to analyze experimental data. The difference between 2 groups was considered significant and highly significant at $p < 0.05$ and $p < 0.01$, respectively.

3. Results

3.1. Purity of mouse splenic DCs sorted by flow cytometry

Purification efficiencies of $CD11c^+$DCs sorted from groups N, I_{28}, and I_{63}were 98.6%, 93.1%, 96.0%, respectively (Fig. 1). The purity of the DCs indicated their suitability for subsequent microarray experiments. The splenic DCs from 3 groups of mice sorted by flow cytometry were collected in 1.5-mL Eppendorf tubes for storage in a −80 °C freezer until use.

3.2. Venn diagram analysis

Both upregulated and downregulated genes with fold changes of ≥2 according to comparisons between any 2 groups of mouse DCs were defined as DEGs. The corresponding Venn diagrams are shown in Fig. 2a and b. The overlapping part represents the number of overlapping DEGs between groups, whereas the non-overlapping part represents the number of unique DEGs between groups. The analysis of these specific DEGs revealed that 760 and 795 genes were specifically upregulated and downregulated, respectively, in DCs of group I_{28} relative to those of group N; these genes were considered to be associated with the immune response during acute infection. Furthermore, 219 and 210 genes were specifically upregulated and downregulated, respectively, in DCs of group I_{63} relative to those of group N. Relative to group I_{28}, 640 and 725 genes were specifically upregulated and downregulated, respectively, in DCs of group I_{63}. DCs of both infected groups exhibited 622 common upregulated genes and 332 common downregulated genes compared with those of group N.

3.3. GO analysis

GO analysis involves classifications by biological process (BP), cell component (CC), and molecular function (MF). GO analysis revealed the number of genes with common annotations; the top