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A study of immunomodulatory genes responses to macrophages of *Schistosoma japonicum* infection during different stages by microarray analysis

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ABSTRACT

Macrophages initiate, modulate, and also serve as final effector cells in immune responses during the course of schistosomal infections. In this study, we investigated the gene expression profile and functional changes of macrophages in immune responses against the *Schistosoma japonicum* by microarray analysis. Hierarchical clustering analysis demonstrated that a significant switch in gene transformation associated with a type-1 response and linked with a type-2 cytokine phenotype occurs between 4.5 and 8 weeks post-infection. Moreover, the gene profiles at 3 later time-points following egg challenge were similar in complexity and magnitude. The data also showed that there were mostly inhibition of gene expression related TLR, IFN, MHC and TNFrsf at the switch between 4.5 and 8 weeks post-infection, It is suggested that these immunomodulatory genes may be down-regulated in defense against *S. japonicum* eggs and granuloma pathology. The induction of alternatively activated macrophage (AAMϕ) was important for dampening the inflammation in hepatic granulomas and contributing to a decrease in cytotoxicity. The gene expressions involved in repair/remodeling during liver fibrosis were also observed after egg production. Understanding the immune mechanisms associated with parasitic resistance, pathology of parasite infection, and parasite growth will provide useful insight on host–schistosome interactions and for the control of schistosomiasis.

1. Introduction

Schistosomiasis continues to be a serious worldwide public health problem (Chitsulo et al., 2000). *Schistosoma japonicum* causes the most severe pathological changes and the slowest immune resistance manifestation (Cheever et al., 1994; Freedman, 1997). It is estimated that several million people in China are infected every year and there is considerable economic loss due to infection of human and domestic animals (Zheng et al., 2012). During the course of infection, different stages of *S. japonicum* cause markedly varied patterns of immune responses, such as infective larvae-induced acute inflammatory responses in the early stage of infection, and parasitic eggs-induced granulomatous reaction in the later stage (Flores Villanueva et al., 1994; Freedman, 1997; Mountford and Trottein, 2004; Rumbley et al., 1998), in the mammalian hosts (Gryseels et al., 2006; Rollinson et al., 1987; Smithers and Terry, 1965). Secretory antigens produced from schistosoma and eggs are able to regulate certain chemokines to inhibit their interaction with host receptors and their biological activity (Fallon and Dunne, 1999; Hogg et al., 2003; Smith et al., 2005).

In recent years, the application of microarray analysis for studying the interaction and the gene expression modes between pathogenic invasion and host macrophages has obtained good results (Loke et al., 2002; Wells et al., 2003). Furthermore, there are several examples where DNA microarray have been successfully applied to detect the variations in host gene expression after parasite infection (Burke et al., 2010a, 2011; Chaussabel et al., 2003; Min et al., 2003; Perry et al., 2011; Reece et al., 2006;

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Rodriguez et al., 2004). However, only few studies have focused on the genomics related to schistosome-infected hosts, including discussions regarding immune polarization responses on tissue pathological changes and vaccine immunity, as well as the variations in the host CD4 cytogene expression profile during the period of infection (Hoffmann et al., 1999; Ji et al., 2003; Kane et al., 2004; Sandler et al., 2003). Recent array studies showed that many chemokine subsets were activated stepwise in the livers of mice infected with *S. japonicum*, and the expression profiles of gene clusters were significantly higher than that in the spleen. The differences of transcription factors were not only confirmed the distinctions between systemic and localized immune responses during the infection, but also exhibited these significant gene activations promoted the recruitment of neutrophils and macrophages to the liver, leading to the subsequent granulomatous inflammation, and hepatic fibrosis (Burke et al., 2010a,b). Microarray technology allows for accurate transcriptome detection and can be systematically applied to investigate the interaction between hosts and schistosomes.

Previous studies have revealed that the development of immunopathology in mouse during *S. japonicum* infection was from Th1 type to Th2 polarization after eggs generation (Oswald et al., 2001; Techau et al., 2007). However, immune responses are a series of complicated network system involving different pathways (Chiu et al., 2003; Morelli et al., 2000; Pearce and MacDonald, 2002; Stavitsky, 2004; Wilson et al., 2007) and in *S. japonicum* infection the immune responses are mainly regulated by the activated cell types of the innate immune responses, especially the macrophages (Chiu et al., 2003; Park et al., 2001; Sandler et al., 2003). Macrophage (Mϕ) plays the role of cause-and-effect in immune responses. Studies showed that the macrophage could further modulate the subsequent development of acquired immune responses due to variations in the cytokine microenvironment of peripheral tissues (Martinez et al., 2008; Medzhitov and Janeway, 2000; Stout et al., 2005). Different types of macrophages have different functions and specific molecular characteristics (Gordon and Taylor, 2005). Recent studies showed that the differentiation of macrophages mostly inclined toward the induction of AAMϕ to participate in modulating the host's immunity during the parasites chronic infection to develop into an advantageous environmental pattern for the existence of parasites (Kreider et al., 2007; Noel et al., 2004; Raes et al., 2007).

In this study, we aim to discuss the gene expression profile and functional switching of macrophages in immune responses, and the impact on the immunomodulation of the host against the *S. japonicum*. Therefore, the representative clusters of gene modulation of the host in different stages of infection would be screened and analyzed in the cellular molecular aspect, including the host defense against infection, control of disease pathology, and restraint of parasitic growth that will be helpful for future studies on schistosome immunology and transcriptomics.

2. Results

2.1. Parasitological and histological analysis

Mice used in this experiment were infected with 35 cercariae. At different time points after infection, spleens of sacrificed mice were collected for purification of $CD11b^+$ cells. Adult worms and liver tissue were also collected for analysis of worm burden and schistosome egg-induced pathogenesis. Adult worms of schistosome were first observed at 4 weeks post-infection (pi) (Fig. 1A). The sizes of spleen increased significantly from 4.5 weeks pi onwards (Fig. 1B and C) which was consistent with the granuloma formation and fibrosis in liver.

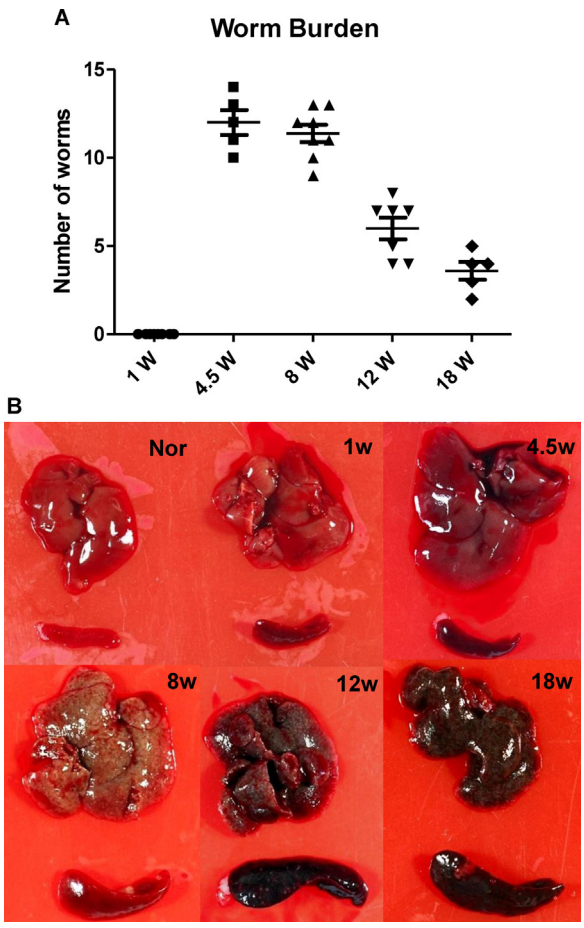

Fig. 1. Worm burden, spleen and liver pathology induced by schsitosome infection. (A) Adult worm burdens in each group were determined. (B) Spleen size and fibrotic pathology of liver increased over time following *S. japonicum* infection. (C) H&E-stained liver tissue section of non-infected or *S. japonicum*-infected mice at indicated post-infection time points. The arrows indicate the deposition site of egg. Magnification, ×400. (D) Analysis purification ratio of $CD11b^+$ cells among spleen cells of mice infected with *S. japonicum*. (D1) The purity of $CD11b^+$ cells in the experiment is above 95%. The data shown is a representative result; the number in the figure is the mean value of three independent experiments. (D2) $CD11b^+$ cells were also analyzed for CD11c and F4/80 expression on dot-blots. Numbers in the right upper and right lower quadrants show the cell frequency of the quadrant from total $CD11b^+$cells.

2.2. Flow cytometry

After infection with *S. japonicum*, the splenic $CD11b^+$ cells of C57BL/6 mice were purified by MASC. The $CD11b^+$ cell proportion was analyzed by flow cytometry, and samples with purity > 95% were then used to carry out the subsequent microarray experiments (Fig. 1D1). Besides the CD11b marker, we also examined the expression of F4/80, a transmembrane protein expressed on the surface of macrophages, and CD11c, a cell surface marker of dendritic cells, for verifying the cell type of the MACS-purified $CD11b^+$ cells. We found that F4/80 was highly expressed on the $CD11b^+$ cells, but CD11c were not (Fig. 1D2).

2.3. Microarray analysis

Determination of the proportion of splenic $CD11b^+$ cells and RNA quality. RNA quality is a critical factor influencing array analysis. After RNA extraction, the quality and integrity of the experimental specimen RNA were determined by measuring the A_{260}/A_{280} ratio; denaturing electrophoresis and microfluidic chip technology, respectively. All measured RNA specimens have two