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Regulation of Schistosome Egg Antigen Induced IL-4 Gene Transcription in Murine Schistosomiasis japonica

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Abstract

The transcription of IL-4 gene was investigated in the spleen of BALB/c mice infected with *Schistosoma japonicum* (*S. japonicum*). Spleens were removed at 0, 3, 5, 8, 10, and 12 wk after infection and the spleen cells were incubated in the presence of SEA or ConA. The extracted RNA was analyzed for IL-4 mRNA by reverse transcription PCR (RT-PCR). No detectable IL-4 mRNA RT-PCR products was found in SEA or ConA treated spleen cells from uninfected, or 3 wk infected mice, whereas IL-4 mRNA was found to be expressed in 5 and 8 wk infected mice, and an appreciable enhanced expression of IL-4 mRNA was observed in SEA stimulated spleen cells at 8 wk infection than at 5 wk. However, neither ConA nor SEA could induce IL-4 mRNA transcription in 10 and 12 wk infected mice indicating the modulated expression of IL-4 mRNA.

Key words: IL-4 mRNA; *Schistosoma japonicum*; mice.

Introduction

Because of the importance of egg granulomas in the pathogenesis of schistosomiasis (Warren, 1982), the study on the mechanisms of granuloma development and regulation is of great significance both theoretically and practically. Experiments conducted in mice have demonstrated that schistosome granuloma formation is a schistosome egg antigen (SEA) induced T cell-dependent granulomatous inflammatory response (Boros, 1989). Several lines of evidence have also indicated that the spontaneous modulation from large granulomas in the acute stage to small granulomas in the chronic stage in schistosome infection is accompanied by systemically regulated immune responses to SEA (Chensue *et al.*, 1980; Kresina *et al.*, 1986).

It has been suggested that Th2 and its product IL-4 are involved in granuloma modulation in schistosomiasis mansoni. By Northern blot quantification of IL-4 mRNA in the spleens, mesenteric lymph nodes, and granulomatous livers, and *in vitro* comparison of SEA stimulated IL-4 mRNA in mice infected with *S. mansoni* for various lengths of time, Henderson *et al.* (1992) demonstrated that both peak level of tissue IL-4 mRNA and IL-4 mRNA produced by SEA stimulated spleen cells were seen in acutely infected mice. Lukacs and Boros (1993) further examined the *in vivo* effect of IL-4 in granuloma formation during the acute stage of *S. mansoni* infection in mice. They found that repeated injections of anti-IL-4 mAb significantly diminished pulmonary granuloma formation in infected mice and concluded that IL-4 was important in influencing the intensity of granuloma formation.

Several pathologic and immunological differences have been found between murine schistosomiasis mansoni and schistosomiasis japonica (Stavitsky, 1987). However, compared with the study of schistosomiasis mansoni, modulation mechanisms in schistosomiasis japonica is less clear. Therefore, the present study attempts to investigate SEA-stimulated capacity for IL-4 mRNA expression in different stages of murine *S. japonicum* infection in order to gain insight into the possible role of IL-4 in the process of the regulation of SEA related systemic immune responses including the modulation of granulomatous responses in schistosomiasis japonica.

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Materials and Methods

Animal and infection

Male BALB/c (7–8 wk old) mice were obtained from the Animal Center of Chinese Medical Academy and were percutaneously infected with 30 cercariae of *S. japonicum*. Infected *Oncomelania hupensis* were provided by Hunan Province Parasitosis Research Institute. Uninfected BALB/c mice were used as control.

Histological examination

Liver samples were obtained at necropsy and fixed in 10% buffered formalin. The tissues were processed for histologic study by embedding, sectioning, and hematoxylin-eosin staining. The slides were examined under a microscope at 100 × magnification. Diameters were determined by the means of two measurements made across perpendicular axes. Only newly-formed granulomas were measured. Confluent granulomas and multi-egg granulomas were not included.

Spleen cells preparation and culture

Spleen cell suspensions were prepared individually from 3, 5, 8, 10 and 12 wks infected and uninfected mice (4 mice per group). Viability was determined by trypan blue exclusion test and viability of all preparations was >95%. Spleen cells were cultured in 25 cm^2 flask (Costar, USA) at 10^6 cells/ml in 7 ml of RPMI 1640 (Sigma Chemical Co., USA) supplemented with penicillin 100 U/ml, streptomycin 100 U/ml, 2 mM L-glutamine and 10% heat-inactivated FCS (Sigma Chemical Co.) in the presence or absence of 5 μg/ml ConA or 7 μg/ml SEA for 4 hrs, in a humidified atmosphere of 5% CO_2 at 37°C.

Various concentrations of SEA and ConA ranged from 1–10 μg/ml and various time of stimulation from 0 (unstimulated) to 24 hr were investigated in the preliminary study and then the appropriate concentration of SEA and ConA, and time of stimulation were determined for the induction of IL-4 gene expression.

Total RNA extraction

Equal number of pooled cells from each experimental condition were harvested. After washing, the total RNA was isolated using a single-step acid guanidinium thiocyanate-phenol-chloroform extraction method (Chomczynski and Sacchi, 1987). The concentration and purity of the isolated RNA was monitored by the measurements of light absorbance in DU-70 Spectrophotometer (Beckman, USA). Only those RNA preparations with 260/280 ratio >1.7 and the 260/230 ratio >2.0, the isolated RNA were used in this study. The RNA pellet was dissolved in diethyl pyrocarbonate (DEPC)-treated water prior to use for reverse transcription.

Reverse transcription

Reverse transcription was performed using a reverse transcription system (Promega, USA), containing 1 μl of extracted RNA, 4 μl 25 mM $MgCl_2$, 2 μl 10 × buffer, 2 μl 10 mM dNTPs, 0.5 μl recombinant RNasin ribonuclease inhibitor, 15U AMV reverse transcriptase, 0.5 μg oligo (dT) and DEPC-treated water. The reaction was carried out in a DNA thermal cycler (Perkin Elmer Co., USA). The mixture was incubated at 42°C for 60 min to allow first strand cDNA synthesis. Enzyme activity was terminated by following incubating at 99°C for 5 min.

Polymerase chain reaction

Five μl of diluted cDNA sample were amplified 35 cycles in a 50 μl reaction mixture containing 5 μl 10 × PCR buffer, 4 μl 25 mM $MgCl_2$, 4 μl 10 mM dNTPs, 2.5U Taq DNA polymerase (Promega), 1 μl each of 40 pM sense and antisense primers and sterile water. The cycling condtions were denaturing at 94°C for 1 min, annealing at 50°C for 1 min, and extending at 72°C for 1 min. After the final cycle, the temperature was maintained at 72°C for 5 min. β-actin cDNA was used as a positive control and its RT-PCR product for normalization of IL-4 RT-PCR product. Primers used were as follows: β-actin, sense: ATGGATGACGATATCGCT, antisense: ATGAGGTAGTCTGTCAGGT (Chen *et al.*, 1993). IL-4, sense: ACAAAAATCACTTGAGAGAGATCAT, antisense: AGTAATCCATTTGCATGATGCTCTT (Ehlers *et al.*, 1992). The amplified fragments for β-actin and IL-4 were 569 bp and 351 bp respectively. The PCR reaction products were analysed by agarose gel electrophoresis in 2% agarose stained with 0.5 μg/ml ethidium bromide.

The pBR322/Msp I was also electrophoresed as size marker.

Results

IL-4 mRNA expression by spleen cells of *S. japonicum* infected mice in response to stimulation with ConA or SEA was investigated at various time post-infection. Correspondently, liver granuloma measurements were performed, so as IL-4 mRNA expressions were analysed covering the time points when up-regulation and down-regulation of granuloma formation occurred.

Fig. 1 illustrates the mean diameters of the newly-formed liver granulomas from 5 to 12 wk post-infection. It was found that granuloma formation in *S. japonicum* infected BALB/c mice is a regulated phenomenon, with no granuloma formation at 3 wk, granuloma appearing between wk 4 and 5, peaking at 8 wk, and declining at 10 wk after infection. The significant decrease of granuloma size at 10 wk indicated that the beginning of the modulation of granuloma formation at this time.

To find out how relevant of the *in vitro* SEA or ConA stimulated IL-4 mRNA expression to the *in vivo* SEA elicited granulomatous responses, ConA and SEA induced IL-4 gene transcript was analysed by RT-PCR. After normalization to β-actin RT-PCR product, the data were presented. As shown in Fig. 2 both ConA and SEA failed to stimulate IL-4 mRNA expression in 3 wk infected mice. However, immediately after egg deposition and granuloma formation, which occurred at 4–5 wk of infection, obvious expression of IL-4 gene transcript was detected (Fig. 3). It suggested that IL-4 mRNA expression is closely associated with the event of

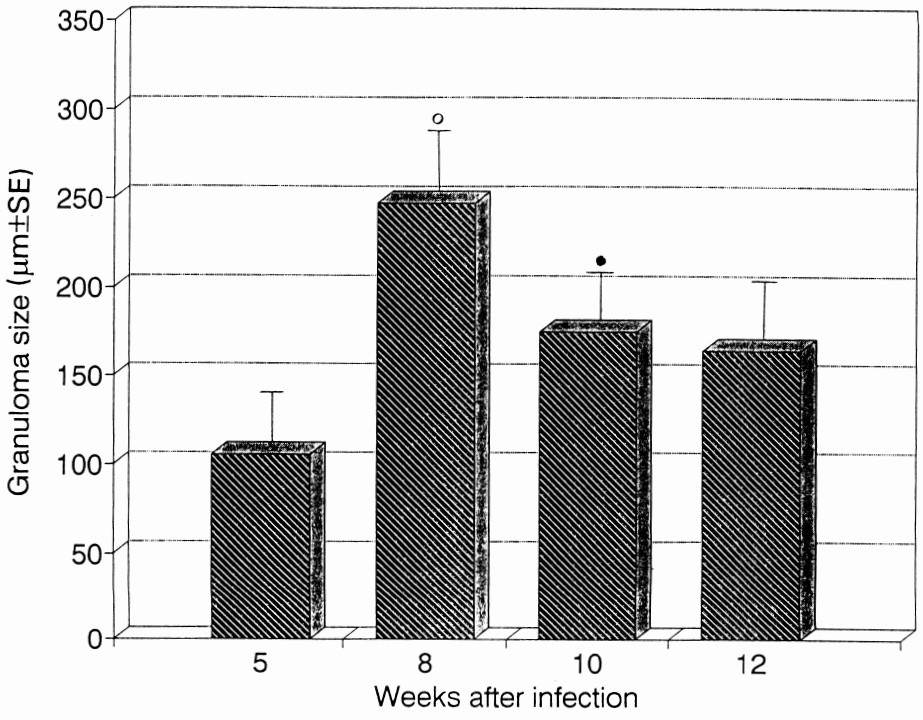

Fig. 1 Liver granuloma size are expressed as group mean diameters±SE (four mice per group). Tissue processing for hematoxylin-eosin staining and the measurement of granulomas are described in materials and methods.

o Group mean is significantly different from group mean at 5 wk ($p<0.01$)

● Group mean is significantly different from group mean at 8 wk ($p<0.01$)

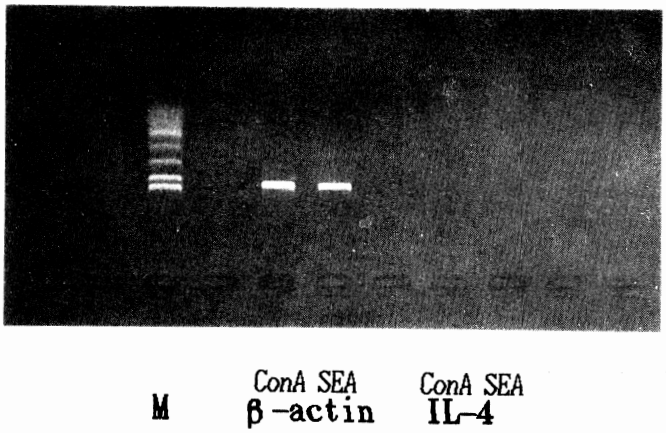

Fig. 2 RT-PCR analysis of the transcription of IL-4 mRNA in spleen cells of 3 wk *S. japonicum* infected mice. Total RNA was isolated after 4 hr incubation with SEA or ConA. DNA size marker fragments (M) are from a pBR322/Msp I digest (622, 527, 404, 309, 242, 238 and 217 bp respectively).

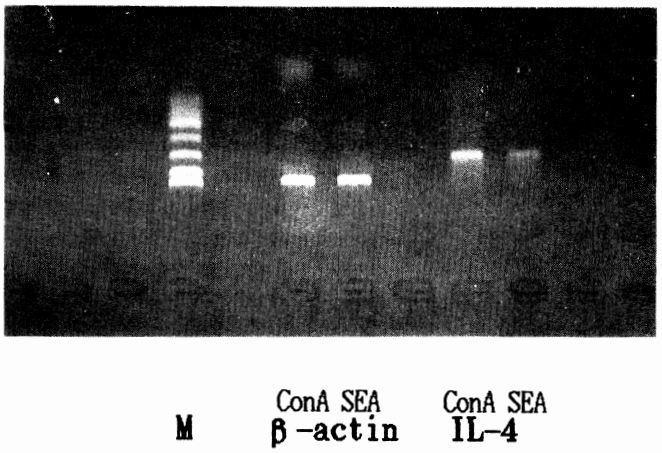

Fig. 3 RT-PCR analysis of the transcription of IL-4 mRNA in spleen cells of 5 wk *S. japonicum* infected mice. Total RNA was isolated after 4 hr incubation with SEA or ConA. DNA size marker fragments (M) are from a pBR322/Msp I digest (622, 527, 404, 309, 242, 238 and 217 bp respectively).

granuloma formation.

The kinetic analysis of IL-4 RT-PCR products have yielded interesting results. It was found that IL-4 gene transcript was found to be expressed in both 5 and 8 wk infected mice. It should be remarked that an appreciable enhanced IL-4 mRNA expression was only observed in SEA (Fig. 4), but not in ConA (data not shown) stimulated spleen cells of 8 wk infection when the peak granulomas appeared ($p<0.01$). However, accompanying the dramatic decrease of granuloma size at 10 and 12 wk ($p<0.01$), neither ConA nor SEA could induce IL-4 mRNA expression in 10 and 12 wk infected mice.

No IL-4 gene transcript was detected in both

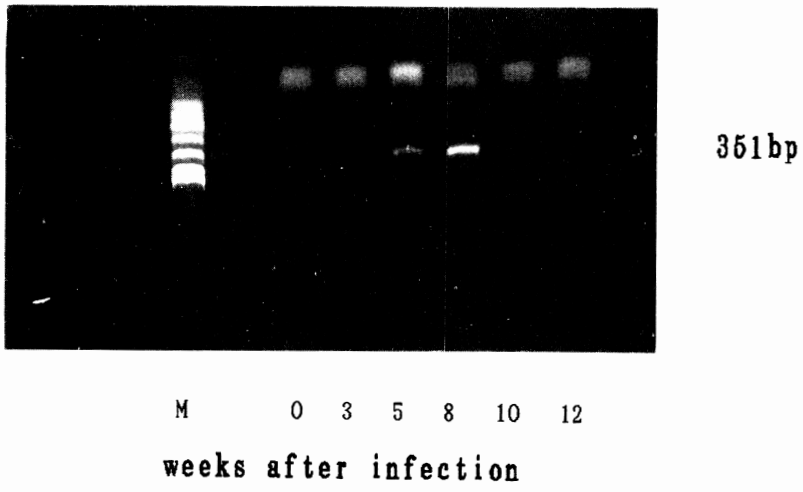

Fig. 4 RT-PCR analysis of the transcription of IL-4 mRNA in spleen cells of *S. japonicum* infected mice.
Spleen samples were prepared from noninfected (normal) and 3, 5, 8, 10, and 12 wk infected mice respectively. Total RNA was isolated after 4 hr incubation with SEA.
DNA size marker fragments (M) are from a pBR322/Msp I digest (622, 527, 404, 309, 242, 238 and 217 bp respectively).

unstimulated and ConA and SEA stimulated control mice.

Discussion

The current study revealed that significant changes of IL-4 mRNA expression in SEA stimulated spleen cells during *S. japonicum* infection, and this change is coincident with SEA elicited granuloma formation and modulation *in vivo*. It is possible that regulation at the population level of Th2 cells and/or IL-4 gene transcription level of the Th2 could occur and be responsible for the overall regulation of IL-4 mRNA expression. In addition, it is reasonable to speculate that the changes in the SEA induced IL-4 mRNA expression capacity may be part of the active regulation responsible for the up and down regulation of SEA related immune responses *in vivo*.

IL-4 is a lymphokine produced predominantly by activated Th2 subset. It has been demonstrated that IL-4 has immunoregulatory actions on T cells, B cells, mast cells and macrophages. In addition, IL-4 may induce the expression of class II MHC antigen and enhance antigen presenting ability on monocyte/macrophage. Furthermore, it is suggested that IL-4 has the potential to induce selective eosinophil recruitment which occurs not only in allergic diseases, but also in schistosome egg granulomatous response (Paul, 1991). Although the molecular mechanisms involved in the modulation of granuloma is unclear, a number of studies have suggested that egg deposition is the major stimulus for the production of Th2 cytokines and IL-4 is of major importance to the regulation of granulomatous responses in schistosomiasis mansoni (Grzych *et al.*, 1991). IL-4 production throughout *S. mansoni* infection in response to SEA was examined with ELISA by Yamashita and Boros (1992). The results showed that peak production of IL-4 was consistent with maximal granulomas of 8 wk infection. Moreover, exogenous rIL-4 treatment significantly enhanced granulomatous response of chronically infected compared with untreated mice. Chensue *et al.* (1994) investigated the relationship of some Th1 and Th2 cytokines with regard to granulomas, they found that depletion of IFN-γ with mAb resulted in augmentation of granulomas. In contrast, depletion

of IL-4 abrogated granuloma area by 40% in anti-murine IL-4 treated mice, along with a reduction in local granuloma IL-4 production. Experiments conducted by Lukacs and Boros (1993) also shown similar results. Studies on the mechanisms of IL-4 on the granuloma formation and regulation in schistosomiasis mansoni have suggested that IL-4 may synergize with GM-CSF and M-CSF, and promote cellular recruitment during the early phase of granuloma formation (Chensue *et al.*, 1992). Our results support these published data in *S. mansoni* and suggest that IL-4 may play an important role in the regulation of granulomatous responses in schistosomiasis japonica.

It is interesting to notice that some differences in the dynamic of SEA induced IL-4 expression exist between our results in murine schistosomiasis japonica and the studies in murine schistosomiasis mansoni mentioned above. First, obvious SEA induced IL-4 gene expression was detected at 5 wk of infection in this study, whereas SEA stimulated IL-4 production were reported still minimal at 6 wk post-infection by Grzych *et al.* (1991), and Yamashita and Boros (1994). Second, although in both systems, IL-4 level peaked at 8 wk of infection, the profile of modulation of IL-4 expression or production was also different. An dramatic decrease of IL-4 gene expression appear at 10 wk of infection in murine *S. japonicum* infection, however, gradually diminished IL-4 production was observed, and an apparent decreased level of IL-4 was found until 12 wk or later stage post-infection in murine *S. mansoni* infection. Considering it has been demonstrated that both oviposition and granuloma modulation in murine *S. japonicum* infection occurred earlier than those in *S. mansoni* infection, the results taken together further indicate that there is a clear association between the formation and modulation of granuloma, and the ability of SEA stimulated spleen cells IL-4 induction and regulation.

ConA has been regarded as the polyclonal T cell activator. The facts that ConA could not induce IL-4 mRNA expression at 10 and 12 wk, as well as the significant increased granuloma size did not accompany dramatic enhancement of IL-4 mRNA expression at 8 wk post-infection suggest ConA stimulated IL-4 mRNA expression may only reflect the non-specific IL-4 producing capacity of the spleen cells; further, the depressed IL-4 mRNA expression may also indicate the non-specific immune suppression occurred at 10 wk post-infection.

The present study investigated SEA elicited IL-4 mRNA expression in *S. japonicum* infected mice. However, granuloma formation and modulation are complicated events, involving the interaction of different cell subpopulations, lymphokines, and local regulatory factors. In addition, the regulation of receptor expression may also play important roles. Further studies to explore their relative contributions should provide valuable information for the understanding of the mechanism of granuloma formation and regulation.

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