

THE ROLE OF EGG ANTIGENS, CYTOKINES IN GRANULOMA FORMATION IN MURINE SCHISTOSOMIASIS MANSONI

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The induction of granuloma formation by soluble egg antigens (SEA) of Schistosoma mansoni is accompanied by T cell-mediated lymphokine production that regulates the intensity of the response. In the present study we have examined the ability of SDS-PAGE fractionated SEA proteins to elicit granulomas and lymphokine production in infected and egg-immunized mice. At the acute stage of infection SEA fractions (<21, 25-30, 32-38, 60-66, 70-90, 93-125, and >200 kD) that elicited pulmonary granulomas also elicited IL-2, IL-4 lymphokine production. At the chronic stage a diminished number of fractions (60-66, 70-90, 93-125, and >200 kD) were able to elicit granulomas with an overall decrease in IL-2, IL-4 production. Granulomas were elicited by larval-egg crossreactive and egg-specific fractions at both the acute and chronic stage of the infection. Examination of lymphokine production from egg-immunized mice demonstrated that as early as 4 days IL-2 was produced by spleen cells stimulated with <21, 32-38, 40-46, 93-125, and >200 kD fractions. By 16 days, IL-2 production was evoked by 8 of 9 fractions. IL-4 production at 4 days in response to all fractions was minimal while at 16 days IL-4 was elicited with the <21, 25-30, 50-56, 93-125, and >200 kD fractions. The present study reveals differences in the range of SEA fractions able to elicit granulomas and IL-2, IL-4 production between acute and chronic stages of infection. Additionally, this study demonstrates sequential (IL-2 followed by IL-4) lymphokine production during the primary egg antigen response.

Key words: soluble egg antigens – cytokines – granuloma formation

Schistosoma mansoni eggs deposited in tissues of infected hosts induce the major pathological manifestations of the disease (Boros, 1989). In mice eggs lodged within the intestinal wall and presinusoidal capillaries of the liver induce a T cell mediated granulomatous response that causes subsequent fibrosis and organ pathology. In this model the peak inflammatory granuloma response occurs at the acute stage, 8-10 weeks post infection. The size of the granuloma response is subsequently downmodulated leading to the chronic stage of the disease around 18-20 weeks (Boros et al., 1975; Boros, 1986). Granuloma formation throughout the infection is induced and elicited by secreted soluble egg antigens (SEA) released by miracidia within deposited eggs (Boros & Warren, 1970).

Recent studies in our laboratory have delineated *in vitro* blastogenic splenic and granu-

loma T cell responses to fractionated antigens of soluble egg antigens (SEA) demonstrating a broader range of antigen responsiveness at the acute as compared to chronic stage of the infection (Lukacs & Boros, 1991a). In addition, we have also demonstrated that the granulomatous response which develops around the eggs is induced initially by larval-egg crossreactive and subsequently by egg-specific antigens. The larval antigens that share epitopes with SEA proteins presensitize the system and thus enhance the egg granuloma response (Lukacs & Boros, 1991b). These studies contribute to several others (Brown et al., 1977; Carter & Colley, 1979; Harn et al., 1989) that have attempted to unravel the complex granulomatous response to the immunogens of SEA.

T cell mediated granuloma formation has previously been shown to be dependent upon cytokine production in response to SEA (Mathew et al., 1990; Grzych et al., 1991). The importance of CD4⁺ T cells and IL-2 production in the granulomatous response was demonstrated by exogenous rIL-2 injections

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that restored augmented granuloma responses to chronically infected mice (Mathew et al., 1990). Other groups found a correlation between increases in SEA-specific IL-4 and IL-5 production and egg deposition (Grzych et al., 1991).

The present studies addressed the role of fractionated SEA proteins in the granulomatous response and correlated it with lymphokine production during the acute and chronic stages of *S. mansoni* infections.

MATERIALS AND METHODS

Mice – Female CBA/J mice used throughout the study were maintained under standard laboratory conditions.

Infection – Five-six week old mice were infected with 25 cercariae of the Puerto Rican strain of *S. mansoni*.

Egg isolation and SEA preparation – Eggs were isolated at 8 weeks from livers of 200 cercariae infected mice and used for immunization or SEA preparation.

Fractionation of SEA and sepharose bead coating – Crude SEA was fractionated as previously described (Lukacs & Boros, 1991a). The SDS-PAGE separated proteins were divided into nine fractions by molecular mass, < 21, 25-30, 32-38, 40-46, 50-56, 60-66, 70-90, 93-125, and > 200 kD fractions, and electroeluted from the gel slices. Pooled fractions were concentrated (30 µg/ml) and coated onto 50,000 CNBr-activated sepharose 4B beads (Pharmacia) or used *in vitro* (6-8 µg/ml) for lymphokine elicitation.

Granuloma elicitation and measurement – Two thousand SEA fraction-coated beads were injected iv into infected mice at the acute or chronic stage of infection. After 4 days mice were sacrificed, their lungs were inflated with buffered formalin and processed for histological examination. Granuloma areas (µm² x 10³) were measured in hematoxylin/eosin stained sections using computerized image analysis software.

Measurement of lymphokine production – T lymphocytes isolated from from liver granulomas of acutely and chronically infected mice were cultured for 24 h with each of the SEA fractions at a concentration of 3 x 10⁶ cells/ml. Supernatants from the cultured cells were

collected and the IL-2 and IL-4 levels were measured by CTLL and CT4S lymphokine-dependent cell lines, respectively. Specificity was confirmed by S4B6 anti-IL-2 or anti-IL-4 (11B11) mAbs. Lymphokine levels were determined by comparisons with rIL-2 and rIL-4 standards, respectively.

Egg immunizations – Normal mice were immunized ip with 2000 live, mature eggs. Splenic lymphokine (IL-2 and IL-4) response to crude and fractionated SEA was measured at day 4 and 16 post egg immunization. Lymphokines were elicited by SEA fractions cultured with 3 x 10⁶ cells/ml. Lymphokines were measured as described above.

RESULTS

Correlation between lymphokine production and granuloma elicitation – In order to identify granulomagenic antigens of SEA we separated the crude preparation by SDS-PAGE and correlated granuloma elicitation with lymphokine production for the various fractions. As shown in Table I the acute infection granuloma T lymphocytes produced IL-2 when stimulated with 7 (< 21, 25-30, 32-38, 50-56, 60-66, 70-90, and 93-125 kD) of the 9 SEA fractions. With the exception of 70-90 kD fraction eight of the 9 SEA fractions elicited IL-4 production. Granuloma elicitation in the lungs of acutely infected mice showed correlation primarily with those fractions, 32-38, 60-66, 93-125, that elicited both IL-2 and IL-4 but also with fractions that elicited either IL-2 (70-90 kD) or IL-4 (> 200 kD).

TABLE I

Granuloma T cell-mediated lymphokine production and pulmonary granuloma formation elicited by SEA fractions in acutely infected mice

SEA fraction (KD)	Lymphokine Production IL-2	IL-4	Granuloma ^a elicitation
< 21	+	++	+ ^b
25-30	+	++	+
32-38	++	++	++ ^c
40-46	–	++	–
50-56	++	++	–
60-66	++	++	++
70-90	++	–	++
93-125	++	++	++
> 200	–	++	++

a: mean values of 5-6 mice per group.

b: significant at P < 0.05.

c: significant at P < 0.001.

TABLE II

Granuloma T cell-mediated lymphokine production and pulmonary granuloma formation elicited by SEA fractions in chronically infected mice

SEA fraction (KD)	Lymphokine Production		Granuloma ^a elicitation
	IL-2	IL-4	
< 21	-	-	-
25-30	-	-	-
32-38	-	-	-
40-46	-	++	-
50-56	-	++	-
60-66	++	+	++
70-90	++	-	++ ^c
93-125	-	++	+ ^b
> 200	+	-	+

a: mean values of 5-6 mice per group.

b: significant at P < 0.05.

c: significant at P < 0.001.

Examination of lymphokine production with chronic infection granuloma T cells, showed greatly decreased responsiveness. Only the 60-66 and 70-90 kD fractions elicited significant IL-2 while the 40-46, 50-56, and 93-125 kD fractions elicited significant IL-4 production. In chronically infected mice the 60-66 and 70-90 kD fractions evoked good sized granulomas, while the 93-125 and > 200 kD elicited significantly smaller granulomas. Therefore, a correlation between IL-2 but not IL-4 production and granuloma formation was observed during the chronic infection (Table II).

Lymphokine production by splenic lymphocytes in egg immunized mice – To examine lymphokine responses induced by only egg specific and not by larval-egg crossreactive antigens (Lukacs & Boros, 1991b) we immunized normal mice with 2000 mature eggs. At 4 days after immunization the splenic T cells in response to crude SEA produced primarily IL-2 (75 pM/ml) and very little IL-4 (3 pM/ml). The individual SEA fractions demonstrated similar results at 4 days with the highest levels of IL-2 elicited by the < 21, 32-38, 40-46, 93-125, and > 200 kD fractions (8.9, 8.9, 7.5, 5.8, and 9.3 pM, respectively) (Fig. 1). At 16 days post immunization IL-2 production (> 5 pM/ml) was elicited with all SEA fractions except the 70-90 kD group (1.3 pM/ml) (Fig. 1). At 4 days post immunization splenic lymphocytes produced very low levels of IL-4 (< 1 pM/ml) upon stimulation with the SEA fractions (Fig. 2). At 16 days with the exception of the 70-90 kD fraction, IL-4 production increased substantially in response to several SEA fractions

(< 21, 25-30, 50-56, 93-125, and > 200; 3-5 pM/ml) (Fig. 2).

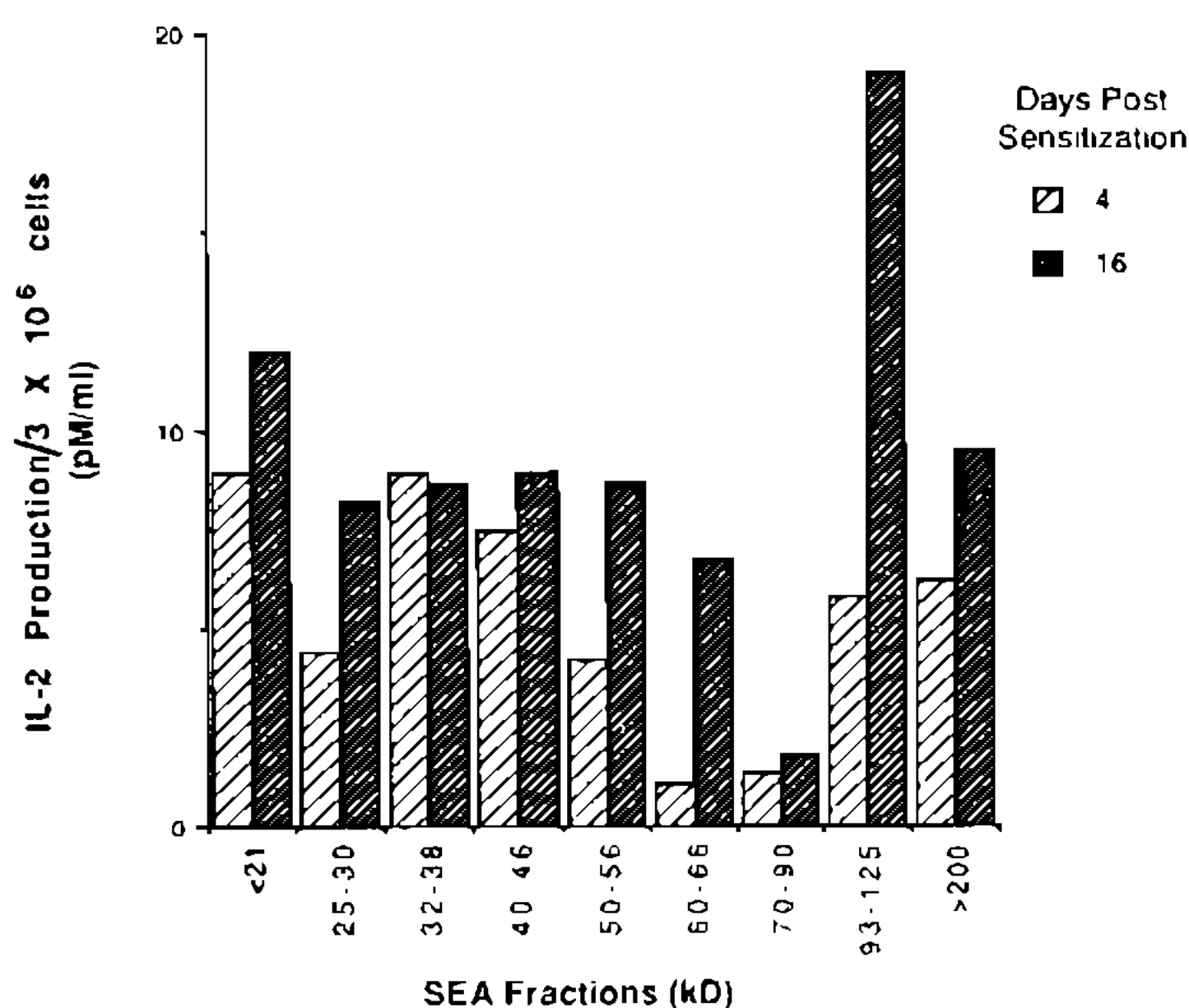


Fig. 1: SEA fraction-elicited production of IL-2 by splenic T cells from egg-immunized mice. Culture supernatants were collected after 24h of incubation and measured by CTLL cell assay. Data depict a representative experiment with each fraction measured in triplicate. A repeat experiment showed similar results. In each experiment spleens pooled from at least three mice were used.

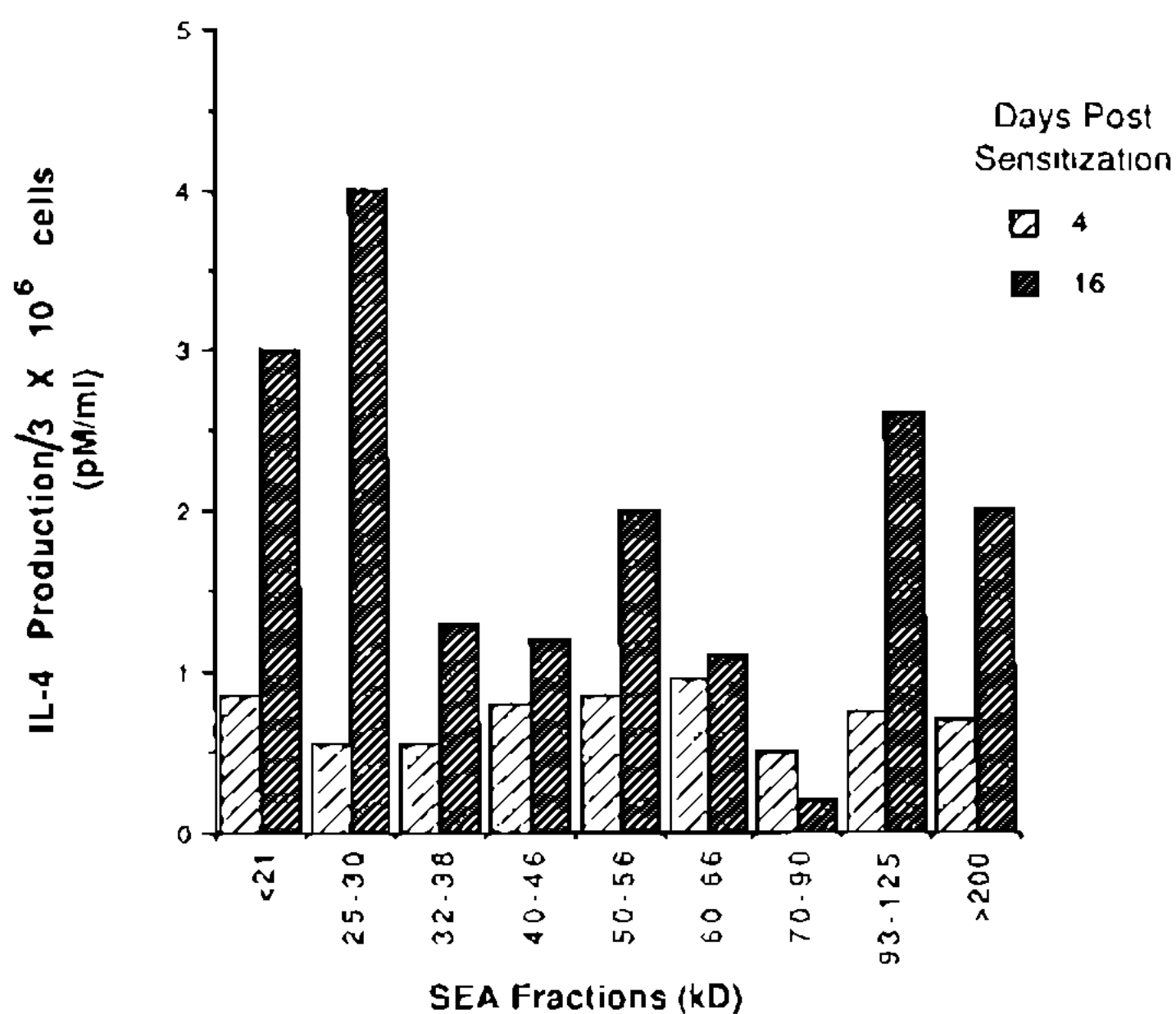


Fig. 2: SEA fraction-elicited production of IL-4 by splenic T cells from egg-immunized mice. Culture supernatants were collected after 24h of incubation and measured by CT4S cell assay. Data depict a representative experiment with each fraction measured in triplicate. A repeat experiment showed similar results. In each experiment spleens pooled from at least three mice were used.

DISCUSSION

In the present study we continued our ongoing effort to outline and define the various SEA protein fractions associated with granuloma formation and correlate the ability of

specific SEA fractions to elicit lymphokine production with the capability to initiate and maintain the granulomatous response. Additionally, we addressed the question of sequential lymphokine production during immunization by egg antigens.

Previous studies have demonstrated that during the chronic stage of the infection the downmodulated granulomatous response is accompanied by enhanced T suppressor cell activity (Boros, 1986) and diminished CD4⁺ T cell responsiveness expressed by low levels of lymphokine production and lymphoproliferation (Boros et al., 1975; Boros, 1986). Recent studies in our laboratory identified larval-egg cross reactive (60-66, 93-125, and > 200 kD) and egg-specific fractions (25-30, 32-38, and 70-90 kD) within crude SEA (Lukacs & Boros, 1991a).

In the present study we demonstrate that the acute stage granuloma response (8-10 w post infection) results from the combined stimuli of these 2 groups of egg antigens (Table I). Moreover, a correlation was seen between immunogenicity and lymphokine production because those antigenic fractions (32-38, 60-66, and > 200 kD) that elicit both IL-2 and IL-4 production in granuloma T cells were also good elicitors of pulmonary granuloma formation. However, antigens that evoked either IL-2 or IL-4 production also proved to be granulomagenic implicating the participation of both lymphokines in granuloma formation. Examination of the responsiveness of granuloma T lymphocytes of chronically infected mice demonstrated diminished reactivity to 5 of the SEA fractions (< 21, 25-30, 32-38, 93-125, > 200 kD) with an overall decrease in IL-2 and IL-4 production to all SEA fractions. It is noteworthy that 3 of the 4 granulomagenic fractions (60-66, 93-125, and > 200) that remained active and evoked responsiveness in chronically infected mice are larval-egg crossreactive while only one fraction (70-90) is egg-specific (Lukacs & Boros, 1991b). Thus a novel facet of the downmodulation of the granulomatous response is described; the selective unresponsiveness of granuloma T cells to certain antigens of SEA. The significance of the ability of the crossreactive fractions to persevere in granuloma elicitation as opposed to the egg-specific fractions needs further elucidation.

To clarify the sequence of lymphokine release we have studied splenic cell responsive-

ness during the primary egg-induced immune response. Previous studies have established that the primary granuloma response in the lungs appears at 4 and peaks at 16 days after the iv injection of eggs (Boros, 1986). The present data show that at 4 days significant levels of IL-2 are produced by spleen cells stimulated with the majority of SEA fractions. This is indicative of the potent immunogenicity of soluble egg antigens that unaided by adjuvant generate IL-2 production and initiate the granulomatous responses. Because IL-4 production is still minimal at that time one may argue that IL-2 is the primary lymphokine that is produced after the sensitization process. By 16 days IL-2 production is further increased and IL-4 levels increase substantially in response to 5 of the 9 fractions (Fig. 2). These results concur with experiments conducted with infected mice (Table I) that show peak granuloma formation only when granuloma lymphocytes produce significant amounts of both IL-2 and IL-4 lymphokine. It is noteworthy that comparison of granuloma T cell lymphokine responses with those of spleen cells from immunized mice indicates different patterns of antigen recognition.

In conclusion, the present studies identified groups of larval crossreactive and egg-specific antigens that participate in the granulomatous response. During the transition from the acute to the chronic stage of the infection responsiveness to certain egg antigens disappears, whereas responses to larval antigens persist. Production of IL-2, IL-4 lymphokines is strongly associated with granuloma formation and is diminished concurrently with the downmodulation of the granulomatous response. Initial studies in egg-immunized mice establish that IL-2 is the primary lymphokine released in response to the SEA protein-induced stimuli with subsequent release of IL-4. Future studies should examine the mechanism(s) that cause diminished antigen responsiveness and lymphokine production during the chronic stage of infection.

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