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## Abstract

Progenitor cells in the bone marrow and the spleen of mice, whether infected with *Schistosoma japonicum* or not, formed cell clusters and colonies when incubated with culture supernatant fluid of spleen cells incubated with soluble egg antigen (SEA). The egg extract, up to a concentration of 250 micrograms/ml protein, did not directly stimulate progenitor cell proliferation in the bone marrow. Eosinophilia in mice infected with *S. japonicum* may be mediated indirectly by egg antigen-stimulated immune lymphocytes and not directly by the egg antigen.

**KEYWORDS:** *Schistosoma japonicum*, soluble egg antigen, colony stimulating factor, bone marrow, spleen cell

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## Bone Marrow and Spleen Cell Colony Formation in Mice Infected with *Schistosoma japonicum*

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Progenitor cells in the bone marrow and the spleen of mice, whether infected with *Schistosoma japonicum* or not, formed cell clusters and colonies when incubated with culture supernatant fluid of spleen cells incubated with soluble egg antigen (SEA). The egg extract, up to a concentration of 250  $\mu\text{g}/\text{ml}$  protein, did not directly stimulate progenitor cell proliferation in the bone marrow. Eosinophilia in mice infected with *S. japonicum* may be mediated indirectly by egg antigen-stimulated immune lymphocytes and not directly by the egg antigen.

**Key words :** *Schistosoma japonicum*, soluble egg antigen, colony stimulating factor, bone marrow, spleen cell

A high level of eosinophilia occurs in schistosomiasis japonica, especially in the acute stage of infection. Local accumulation of the eosinophils around eggs is clearly recognized in egg granulomas in the intestine and the liver. These eosinophils originate in the bone marrow and spleen from granulocytic progenitor cells. In *Schistosoma mansoni* infected mice, Miller *et al.* (1) described a diffusible factor capable of stimulating eosinophilopoiesis within bone marrow. In *Trichinella spiralis* infection, studies by Basten and Beeson (2) and Ruscetti *et al.* (3) indicated eosinophilia in infected mice is related to lymphocytes involved in immune responses to the parasites.

Balb/c mice, 4-6 weeks old, were infected with 30 *Schistosoma japonicum* cercariae. They

were sacrificed after 6-16 weeks of infection for bone marrow cell cultures and after 9-16 weeks for spleen cell cultures. Normal mice were used at 8-16 weeks of age. Bone marrow cells were flushed out of excised femurs by syringe with alpha-modified Eagle's medium with Earle's salts ( $\alpha$ -medium, Flow Lab. USA). Cells were cultured according to the methods described by Niho (4). Final composition of the medium was as follows: one part of cell suspension ( $5 \times 10^5$ ) in  $\alpha$ -medium, two parts of  $\alpha$ -medium with 2.2 % methylcellulose (Nakarai Chem. Co., Japan), one part of heat inactivated horse serum and one part of a liquid substance to be examined for colony stimulation in  $\alpha$ -medium. L-cells were used as the positive control of colony stimulating factor (CSF). L-cells ( $6 \times 10^5/\text{ml}$ ) were cultured in  $\alpha$ -medium with 20 % calf serum and antibiotics at 37°C in 5 % CO<sub>2</sub> for 7 days to yield a super-

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natant. The supernatant was membrane filtered ( $0.2\mu\text{m}$  pore size, Sartorius SM 16529). Cell counts were made in a Neubauer chamber plate with Turk solution. Normal saline or  $\alpha$ -medium was used for negative controls.

Soluble egg antigen (SEA) of *S. japonicum* was prepared by the methods of Ishii *et al.* (6). The house dust mite extract of *Dermatophagoides pteronyssinus* (Dp2) was a semi-purified allergenic fraction as described by Kabasawa and Ishii (7).

In each experiment, four petri dishes (#3040 Falcon, USA) for every sample were incubated for 7 days at  $37^\circ\text{C}$  in 7.5%  $\text{CO}_2$  and examined for colonies under an inverted microscope to give average numbers. To maintain enough humidity, petri dishes were placed in larger petri dishes with water. Cell aggregates of more than 20 cells were counted as a colony, and less than 20 as a cluster. Cells from colonies or clusters were collected by aspiration with a capillary pipette in  $\alpha$ -medium and centrifuged on glass slides with a cell-centrifuge apparatus (SC-2, Tominaga, Tokyo). They were stained with Giemsa or in some case with Dominici or Litt stain.

Spleen cells were cultured in  $\alpha$ -medium to yield supernatant and examined for their ability to produce CSF. Spleen cells were cultured in  $\alpha$ -medium with methylcellulose to examine their

remaining hematopoietic activity. For colony formation, spleen cells were adjusted to  $1.5 \times 10^7/\text{ml}$  cells and cultured according to Saito and Tadakuma (6). Spleen cells from infected mice were cultured with SEA of *S. japonicum* at a concentration of  $40\mu\text{g}/\text{ml}$  to give reactant supernatant fluids. Culture fluids were membrane filtered and stored at  $-20^\circ\text{C}$ .

Bone marrow cells collected from normal mice were composed mainly of erythroblasts and early neutrophils whereas cells from *S. japonicum* infected mice were composed mostly of early granulocytic cells with ring shaped nuclei. There were some eosinophil myemocytes with eosinophilic granules. It was concluded that the infection stimulated the bone marrow to increase granulocyte production.

L-cell conditioned medium showed colony formation stimulating activity which positively correlated with concentration up to a 1:3 dilution (Fig. 1). SEA of *S. japonicum* did not show any effect on colony or cluster formation of bone marrow cells either from normal mice or from infected mice. The house dust mite extract did not have a direct activity either. As some inhibitory activity was seen at higher concentrations of the L-cell conditioned medium on colony formation, lesser concentrations were also examined

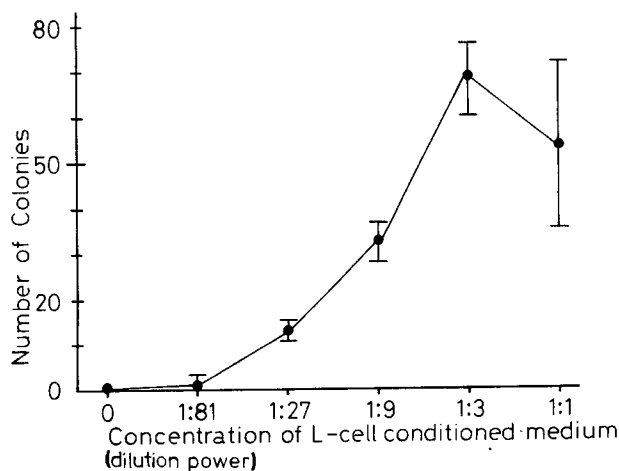


Fig. 1 Dose-response curve of L-cell conditioned medium to colony formation of bone marrow cells. L-cell conditioned medium was diluted with  $\alpha$ -medium. The average number of colonies in four dishes of 1.5 cm radius is shown with the standard deviation.

with egg extract and dust mite extract, but still no stimulating activity was found. When spleen cells from normal mice were incubated with SEA, bone marrow cells formed only a few clusters with the supernatant. However, culture supernatant fluid of spleen cells from infected mice incubated with SEA induced cluster and colony formation of bone marrow cells either from normal or infected mice. This result was confirmed using culture supernatant fluids without calf serum, which excluded the possibility of the effect of nutrient serum. The culture fluids from cells incubated with the house dust mite extract did not show activity which would confirm the specificity of the production.

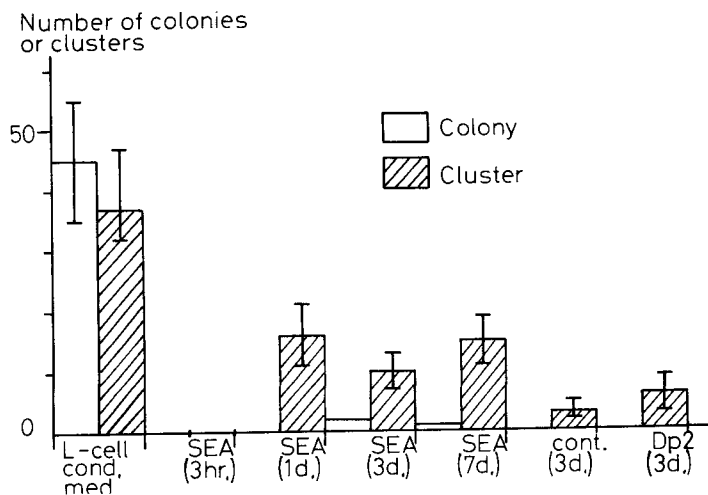
The time course of cluster and colony formation activity is shown in Fig. 2. The activity was first detected 24 h after the initiation of incubation. Fig. 3 shows the same activity of the infected mouse bone marrow cells. The same time course was seen. Morphological characters of the colonies, clusters or dispersed-granulocytic colonies were examined for cellular differentiation, but we could not demonstrate any different staining characteristics.

L-cell conditioned medium showed colony or

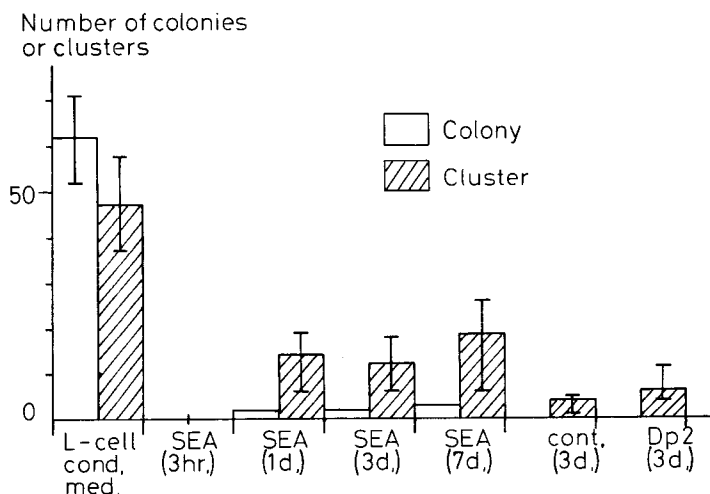
cluster formation activity on the spleen cells from normal mice (Fig. 4). Neither the egg extract nor the house dust mite extract showed any direct spleen cell stimulation activity. The supernatant fluid of the cultures incubated with SEA stimulated proliferation of spleen cells of normal mice. On the other hand, stimulation of spleen cells from infected mice was not seen.

Peripheral blood eosinophilia is a distinctive characteristic of many parasitic infections, especially of tissue invading parasites. It is mainly caused by recruitment of eosinophils from local tissue and increased production of eosinophil precursor cells. In schistosomiasis, marked eosinophilia is known to occur at the acute egg laying stage of the infection. The exact mechanism has not been elucidated to date. However, the importance of eosinophils in killing the schistosomules has been established. Because of the importance of the egg in the pathogenicity of schistosome infections, egg extract would be expected to interact with the immune system and/or hematopoietic systems which cause peripheral blood eosinophilia and local eosinophil accumulation around the egg.

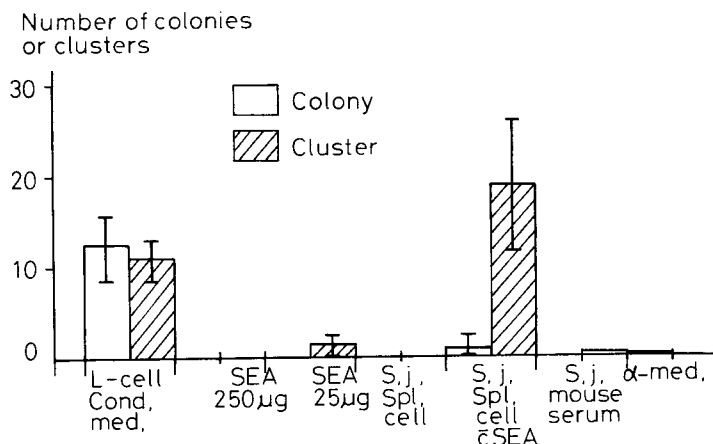
The results in this study showed that egg



**Fig. 2** Colony forming units of normal mouse bone marrow cells exposed to culture fluid from *Schistosoma japonicum*-infected mouse spleen cells with or without antigens. The average number per dish is shown. L-cell cond. med. is L-cell conditioned medium, SEA is soluble egg antigen of *Schistosoma japonicum*, and Dp2 is a dust mite antigen. Figures in parentheses show the incubation period for the preparation of culture supernatant fluid (hr.=hour; d.=day)



**Fig. 3** Colony forming units of *Schistosoma japonicum*-infected mouse bone marrow cells stimulated with culture fluid of *S. japonicum*-infected mouse spleen cells with or without antigens. Abbreviations and figures in parentheses are the same as in Fig. 2.



**Fig. 4** Colony forming units of normal mouse spleen cells stimulated with culture fluid of *Schistosoma japonicum* infected mouse spleen cells with or without antigens. S. j. spl. cell means culture supernatant fluid after incubation with or without antigen (SEA). ε denotes "incubated with". Other abbreviations are the same as in Fig. 2.

extract did not have a direct action on eosinophilopoiesis. This lack of a direct action is in contrast to the presence of an eosinophil chemotactic activity in the egg extract (8). On the other hand, immune system seems responsible for granulocyte (and perhaps eosinophil) production in schistosome infections. Basten *et al.* (9) reported that *Trichinella* antigen itself did not show any stimulating activity on peripheral

eosinophilia or bone marrow cell colony formation. McGarry *et al.* (10) showed that colony stimulating factor was produced *in vitro* by spleen cells from *S. mansoni* infected CBA/J mice starting from 3h after incubation with SEA. Robinson *et al.* (11) examined the ability of *Schistosoma mansoni* adult worm antigen to produce eosinophil colony stimulating factor to the human bone marrow cells when incubated with peripheral

blood mononuclear cells. Our results confirmed these previous results in *S. japonicum* infected Balb/c mice with relatively late onset of one day. Clusters counted in this study were expected to grow to colonies after two weeks as documented by Dresch *et al.* (12).

Granulocyte colony stimulating factor was also detected in the sera of *S. japonicum* infected mice (13) and human urine of *S. haematobium* and *S. mansoni* patients (14), suggesting that the factor has a role in the pathogenesis of schistosomiasis. Stimulating factors need to be further characterized biochemically in order to determine whether they are an eosinophilopoietin, which Mahmoud *et al.* (15, 16) detected in mice with *S. mansoni*, an eosinophil stimulation promotor (ESP) described by Colley (17), or Eo-CSF described by Metcalf *et al.* (18) from mitogen-stimulated spleen cells.

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