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# Studies on the immunological disturbance in murine *Schistosomiasis japonica* from the viewpoint of the interleukin cascade reaction

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## SUMMARY

We examined the mechanisms of disturbed T-lymphocyte function that occurred during *Schistosoma japonicum* infection of BALB/c mice from the viewpoint of the interleukin cascade reaction. Each point of the interleukin cascade reaction was examined. First, IL-1 production by adherent peritoneal or spleen cells, as a source of macrophages, was normal or rather enhanced during the infection, the values being 100–180% of the control. Secondly, proliferative response to exogenous IL-1 of thymocytes from *S. japonicum*-infected mice progressively decreased during 3–7 weeks of infection. Thirdly, IL-2 production of *S. japonicum*-infected mice was significantly inhibited, even at 2 weeks of infection, and the activity was 10–20% of the control at 5–8 weeks of infection. Diminished IL-2 production was not caused by suppressive factors, such as $PGE_2$ or suppressor macrophages, or a decrease in the number of IL-2-producing T cells. Finally, the response to exogenous IL-2 in *S. japonicum*-infected mice was suppressed markedly by 4 weeks of infection, and the responsiveness was reduced to 20% of the control at 8 weeks of infection. The mechanisms of disturbances in T-cell functions in *S. japonicum*-infected mice are discussed.

## INTRODUCTION

The adult *Schistosoma japonicum* resides in the host's mesenteric veins, thereby escaping from immunological attack by the host and causing severe infection. Immune responses of the host are disturbed by the infection. Many approaches have been made to elucidate the mechanisms of immunological disturbance induced by *S. japonicum* infection, and some interesting results have been obtained. As to disturbances in T-cell lineage, depression of mitogenic response (Lewert, Yogore & Blas, 1979; Garb, Stavitsky & Mahmoud, 1981) and the presence of antigen-specific suppressor T cells have been demonstrated (Ohta, Minai & Sasazuki, 1983). An impairment in B-cell lineage was described by polyclonal B-cell activation and the presence of autoantibodies (Kawabata *et al.*, 1981). On the other hand, the suppressor macrophage was reported as another candidate for immunosuppression in *Schistosomiasis japonica* (Amano & Oshima, 1984). However, relationships among these phenomena and the precise mechanisms of the immunological disturbance have not been fully clarified. Recently, two important cytokines with effects on immune regulation, IL-1 and IL-2 have been biochemically characterized. We thought that it might be possible to analyse immunological disturbances in *Schistosomiasis japonica* in a more sophisticated manner if we studied the immunological disturbance in schistosomiasis from the viewpoint of cascade reactions mediated by these interleukins. In the present paper, we report a disturbed interleukin cascade reaction in mice infected with *S. japonicum*.

Abbreviations: Con A, concanavalin A; FCS, fetal calf serum; [$^3$H]TdR, [$^3$H]thymidine; IL-1, interleukin-1; IL-2, interleukin-2; IMDM, Iscove's modified Dulbecco's medium; α-MM, α-methyl-D-mannoside; MEM, Eagle's minimum essential medium; P A, phytohaemagglutinin; PEC, peritoneal exudate cells; $PGE_2$, prostaglandin $E_2$; PNA, peanut agglutinin; SE, standard error.

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## MATERIALS AND METHODS

### *Mice*

Male BALB/c mice aged 5 weeks were purchased from the Shizuoka Laboratory Animal Center (Shizuoka, Japan) and maintained in our animal facility during the experiments. For IL-1 assay, C3H/HeN mice were bred and maintained in our animal facility from breeding pairs originally obtained from the Charles River Animal Co. (Atsugi, Japan).

### *Infection*

Six-week-old BALB/c mice were infected intraperitoneally by the injection of approximately 20 cercariae of *S. japonicum* (Yamanashi, Japan strain) which had been maintained in our laboratory by passage in *Oncomelania hupensis nosophora* and BALB/c mice. Mice were killed at various points of infection, and their immunological activities were examined. Age-matched mice were used as a control.

*Media*

All cell washing was carried out with Eagle's minimum essential medium (MEM) (Nissui, Tokyo, Japan) containing 1% heat-inactivated fetal calf serum (FCS; Filtron, Altona, Australia). For culture medium, Iscove's modified Dulbecco's medium (IMDM; Boehringer Mannheim, Mannheim, FRG) supplemented with 10% FCS, penicillin and streptomycin (100 U/ml and 100 $\mu$g/ml) was used in all experiments.

*Cell preparation*

Whole spleen cells were prepared by teasing the spleen with a glass homogenizer. Erythrocytes were lysed with 0·15 M Tris–0·75% $NH_4Cl$, and then the whole spleen cells were washed three times with the washing medium and passed through a sterilized nylon mesh to exclude tissue debris.

Peritoneal exudate cells (PEC) were obtained by lavage of the peritoneal cavity with 10 ml of MEM containing heparin (5 U/ml) on Day 3 after intraperitoneal injection with 0·5 ml of 2·5% glycogen. For preparation of adherent cells, PEC or whole spleen cells were pooled from five mice for each group. Adherent PEC or adherent spleen cells were obtained from PEC or whole spleen cells seeded in a plastic dish (Falcon 3003; Falcon, Cockeysville, MD) that had been coated with FCS at 4° for 24 hr. After incubation in a $CO_2$ incubator (a humidified atmosphere of 7% $CO_2$ in air at 37°) for 1 hr, the dish was washed vigorously four times with MEM to remove non-adherent cells. Adherent PEC or adherent spleen cells were harvested with a rubber policeman and then each cell suspension was centrifuged at 200 *g* for 5 min and resuspended in the culture medium.

T-enriched cell suspensions were obtained by the method described by Julius, Simpson & Herzenberg (1973). The nylon-column effluent cells contained 70–80% T cells determined by a cytotoxicity test using anti-Thy-1.2 and rabbit complement (Cedarlane, Ontario, Canada).

Peanut agglutinin-negative thymocytes ($PNA^-$ thymocytes) were prepared from the thymocytes of C3H/HeN mice using the method of Reisner, Linker-Israeli & Sharon (1976). Briefly, the thymus was removed, dissociated into single cell suspensions, and then washed and suspended in MEM at a concentration of $1 \times 10^9$ cells per ml. Peanut agglutinin (0·5 mg/ml) (PNA) (E. Y. Laboratories, San Mateo, CA) was added to the cell suspension, and incubated for 10 min at room temperature. After incubation, a five-fold volume of MEM was added to this cell suspension, and then 1 ml of the cell suspension was layered gently on 5 ml IMDM plus 20% FCS. The top layer consisting of $PNA^-$ cell-enriched thymocytes was harvested and washed twice with a culture medium containing 0·2 M galactose (Sigma Chemicals, St Louis, MO) to remove any residual PNA, and then it was suspended in the culture medium at a concentration of $5 \times 10^6$/ml.

*IL-1 production*

We used silica particles to stimulate IL-1 production in this experiment. Two ml (per well) of each cell suspension of adherent PEC or adherent spleen cells ($1 \times 10^6$/ml) were stimulated with 200 $\mu$g (per well) of silica particles (Sigma, S505) in six-well Falcon Tissue trays (Falcon 3046) in a $CO_2$ incubator for 24 hr. The supernatants were centrifuged at 1500 *g* for 10 min, passed through 0·45-$\mu$m filters and then stored at −70° until IL-1 assay. The silica-induced supernatant of adherent PEC obtained from 6-week-old normal mice was used as the standard preparation of IL-1.

*IL-1 assay*

IL-1 activity of *S. japonicum*-infected mice was assayed by proliferation of $PNA^-$ thymocytes prepared from C3H/HeN mice (Conlon, Henny & Gillis, 1982). Briefly, 0·1 ml of $PNA^-$ thymocyte suspension ($5 \times 10^6$/ml) was added to each well of a 96-well microculture plate containing 0·1 ml of two-fold diluted samples. Then, the plates were incubated for 72 hr in a $CO_2$ incubator. The proliferation of $PNA^-$ thymocytes was determined by [$^3$H]thymidine ([$^3$H]TdR) (2 mCi/ml; Amersham Corp., Amersham, Bucks, U.K. (uptake during the last 24 hr of the culture. The sample dilution giving a c.p.m. value equal to 50% of the maximum c.p.m. obtained with the standard preparation was determined from the regression line. The IL-1 activity of the sample was determined by the following equation:

$$\frac{\text{reciprocal dilution of samples corresponding to 50\% of the maximum c.p.m. of standard preparation}}{\text{reciprocal dilution giving 50\% of the maximum c.p.m. of standard preparation}} \times 100 = \text{unit/ml.}$$

*Responses to exogenous IL-1*

The response to exogenous IL-1 was examined by the proliferation of whole thymocytes to IL-1 preparation derived from the P388D1 cell line. Briefly, whole thymocytes ($5 \times 10^5$ cells/0·1 ml/well) from normal or *S. japonicum*-infected mice were cultured with the IL-1 preparation in a volume of 0·2 ml/well for 72 hr in a $CO_2$ incubator, and [$^3$H]TdR (0·5 $\mu$Ci/well) was added to each well at 24 hr before the end of the culture. The cells were harvested and the incorporation of [$^3$H]TdR was determined. The IL-1 preparation used in these experiments was obtained from a mouse macrophage-like cell line, P388D1, which spontaneously secretes IL-1 into the culture medium during incubation (a gift from Drs M. Izawa and J. Hamuro, Ajinomoto Co. Inc., Yokohama, Japan). This supernatant was concentrated approximately 10-fold by ultrafiltration and dialysed against RPMI-1640 and then filter-sterilized and stored at −70° until use. IL-1 assays usually measure the proliferation of whole thymocytes in the presence of mitogen. However, $PNA^-$ thymocytes proliferate with IL-1 alone (Conlon *et al.*, 1982). The IL-1 preparation used in the present experiment was of high titre, so it stimulated a small population of $PNA^-$ thymocytes among the whole thymocytes without the aid of mitogen.

*IL-2 production*

Whole spleen cells were cultured at a density of $25 \times 10^5$/ml with various concentrations of Con A (0·5–25 $\mu$g/ml) for 24–72 hr in a $CO_2$ incubator. Furthermore, T-enriched cells ($25 \times 10^5$/ml) were also cultured with Con A (5 $\mu$g/ml) in the presence of adherent spleen cells ($5 \times 10^4$/ml) from normal mice for 24 hr. In some experiments, whole spleen cells or T-enriched cells were stimulated with Con A (5 $\mu$g/ml) in the presence of indomethacin (Sigma) or adherent spleen cells ($5–500 \times 10^2$/ml) from normal or infected mice. Indomethacin was used according to Rappaport & Dodge (1982). After incubation, each culture supernatant was harvested and passed through 0·45-$\mu$m filters and stored at −70° until IL-2 assay.

**Table 1.** IL-1 production by adherent PEC or adherent spleen cells of BALB/c mice infected with *S. japonicum*

| Weeks after infection | IL-1 production (U/ml) Adherent PEC Normal | Infected | % of normal | IL-1 production (U/ml) Adherent spleen cells Normal | Infected | % of normal |
|---|---|---|---|---|---|---|
| 1 | 100±15 | 185±21* | 185 | 30±6 | 46±8* | 153 |
| 2 | 90±9 | 84±8 | 93 | 50±11 | 51±9 | 100 |
| 4 | 91±11 | 85±13 | 93 | 54±9 | 54±12 | 100 |
| 5 | 94±13 | 126±18 | 134 | 58±15 | 56±6 | 96 |
| 7 | 107±16 | 114±11 | 107 | 36±9 | 68±14* | 189 |
| 8 | 80±21 | 98±15 | 122 | 65±14 | 80±12 | 123 |

Adherent PEC or adherent spleen cells were obtained from five normal or five infected BALB/c mice, and the cells were pooled for each group. For IL-1 production, the cells ($1 \times 10^6$/ml) were stimulated with 100 μg of silica particles for 24 hr in a $CO_2$ incubator. The cultures were performed in duplicate for each group. The same experiment was done twice for each week after infection. The results were expressed as mean U/ml±SE from four individual assays per each group.

*$P < 0.02$, with respect to the value of normal.

**Table 2.** Response to IL-1 of thymocytes from *S. japonicum*-infected BALB/mice

| Weeks after infection | [$^3$H]TdR uptake (c.p.m.) Normal | Infected | % of normal |
|---|---|---|---|
| 3 | 4521±1234* | 4296±1228* | 82 |
| 5 | 3263±295 | 1590±92† | 50 |
| 7 | 4750±108 | 950±86† | 20 |

Whole thymocytes ($5 \times 10^6$/well) were cultured with IL-1 preparation for 72 hr in a $CO_2$ incubator. The cultures were performed in triplicate per mouse. [$^3$H]TdR was pulsed for 24 hr of culture.

* Each value represents the mean c.p.m. ±SE of five mice per each group.

† $P < 0.05$, with respect to the value of normal.

*IL-2 assay*

IL-2 activity was detected by [$^3$H]TdR incorporation of an IL-2-dependent cytotoxic lymphocyte line (CTLL). Briefly, $4 \times 10^3$ CTLL cells were cultured in 96-well, flat-bottomed microtitre plates in the presence of the serially diluted test supernatant for 24 hr in a $CO_2$ incubator, and 0·5 μCi [$^3$H]TdR was added during the last 4 hr of culture. The uptakes of [$^3$H]TdR by CTLL-2 cells in response to the sample were compared to the response of a standard rat IL-2 preparation by probit analysis (Gillis *et al.*, 1978).

*Response to exogenous IL-2*

The response to IL-2 was examined by the proliferation assay of Con A-stimulated spleen cells to exogenous IL-2 (Wofsy *et al.*, 1981). Briefly, whole spleen cells ($5 \times 10^6$/ml) were cultured for 48 hr with Con A (5 μg/ml) and subsequently washed three times with α-methyl-D-mannoside (10 mg/ml) (α-MM) (Sigma) to remove the remaining Con A. These Con A-stimulated cells ($5 \times 10^5$/ml) were then incubated in a 96-well microculture plate with lectin-free IL-2 for 48 hr in a $CO_2$ incubator. [$^3$H]TdR (0·5 μCi/well) was added during the last 24 hr of the 48-hr culture. The cells were harvested and the incorporation of [$^3$H]TdR was determined in triplicate. The lectin-free IL-2 was kindly supplied by Dr J. Hamuro (Ajinomoto).

*Statistics*

The *P* values were determined by using a non-parametric Mann–Whitney *U*-test. A *P* value of 0·05 or less was considered to be significant.

## RESULTS

### IL-1 production in *S. japonicum*-infected mice

As the first step in the interleukin cascade reaction, we examined the level of IL-1 production in *S. japonicum*-infected mice. Adherent spleen cells or adherent PEC were stimulated with silica particles for 24 hr in a $CO_2$ incubator, and the IL-1 activity of the supernatants was assayed. The level of IL-1 production by adherent spleen cells or adherent PEC was equal or rather enhanced during 1–8 weeks after the infection as compared to its production in age-matched normal mice (Table 1). Furthermore, the number of recovered spleen cells and peritoneal exudate cells began to increase at 4 weeks of infection (data not shown).

### Responses to exogenous IL-1 of *S. japonicum*-infected mice

As the next step in the interleukin cascade reaction in *S. japonicum*-infected mice, we observed the responses to exogenous IL-1. When thymocytes were supplemented with culture supernatants of P388D1 cells, the level of [$^3$H]TdR incorpora-

**Table 3.** IL-2 production by whole spleen cells from *S. japonicum*-infected BALB/c mice

| Weeks of infection | IL-2 production (U/ml) Whole spleen cells* Normal | Infected | % of normal | IL-2 production (U/ml) T-enriched cells† Normal | Infected | % of normal |
|---|---|---|---|---|---|---|
| 1 | 140±17 | 115±12‡ | 82 | ND | ND§ | |
| 2 | 148±5 | 81±9‡ | 55 | ND | ND | |
| 3 | 231±6 | 122± 6‡ | 53 | 166±16‡ | 42±8 | 25 |
| 4 | 130±6 | 59±14‡ | 46 | ND | ND | |
| 5 | 158±8 | 13±0‡ | 11 | 155± 7‡ | 23±6 | 15 |
| 6 | 131±5 | 16±10‡ | 12 | ND | ND | |
| 7 | 155±5 | 25±0‡ | 16 | 165±12‡ | 20±5 | 12 |
| 8 | 194±2 | 29±13‡ | 15 | ND | ND | |

* Whole spleen cells ($25 \times 10^5$/ml) were cultured for 24 hr with Con A (5 μg/ml) in triplicate per mice.

† T-enriched cells were prepared by nylon-column fractionation of whole spleen cells, which were pooled from five mice per group. T-enriched cells were cultured with Con A (5 μg/ml) in the presence of adherent cells ($5 \times 10^4$/ml) from normal mice for 24 hr in duplicate for each group. The same experiment was done three times. The results using whole spleen cells are expressed as the mean ± SE of five individual mice per each group. The results using T-enriched cells are expressed as the mean ± SE from three experiments for each group.

‡ $P < 0.05$, with respect to the value of normal.

§ ND, not done.

tion (5000–6000 c.p.m.) was greater than the [$^3$H]TdR incorporation (50–100 c.p.m.) in cultures incubated with the medium alone. Using these results, in the following experiment we used the supernatant of P388D1 cells as the source of IL-1. Table 2 shows the proliferative response to IL-1 of thymocytes from *S. japonicum*-infected mice killed at the third, fifth and seventh week of infection. The values gradually decreased after the infection, and reached 20% of control at 7 weeks after infection. From this result, it can be said that the response to IL-1 was impaired in the *S. japonicum*-infected mice.

**IL-2 production during the course of the infection**

As the third step in the interleukin cascade reaction, IL-2 production in *S. japonicum*-infected mice was examined. Spleen cells from the mice at 1–8 weeks of infection were stimulated with Con A. IL-2 production was significantly lower than in the control, even after 2 weeks of infection. Thereafter, IL-2 production further decreased, and it reached 10–16% of normal at 5–8 weeks of infection (Table 3).

**Table 4.** Effect of Con A dose and culture time on IL-2 production by normal and *S. japonicum*-infected mice

| Concentration of Con A (ug/ml) | IL-2 production (U/ml) 24 hr Normal* | Infected† | 48 hr Normal* | Infected† | 72 hr Normal* | Infected† |
|---|---|---|---|---|---|---|
| 0·5 | 33± 3 | 10±4 | 2±1 | 1±2 | 0±0 | 0±0 |
| 2·0 | 82± 6 | 21±2‡ | 12±2 | 1±1‡ | 0±0 | 0±0 |
| 5·0 | 122±11 | 24±2‡ | 23±4 | 4±3‡ | 0±0 | 0±0 |
| 20·0 | 44± 4 | 19±1‡ | 50±3 | 8±1‡ | 47±8 | 0±0 |

Whole spleen cells ($25 \times 10^5$/ml) from either normal or the infected BALB/c mice were cultured with varying concentrations of Con A for 24 hr, 48 hr or 72 hr in a $CO_2$ incubator. IL-2 activity was expressed as mean U/ml ± SE of five individual mice for each group.

* Eleven-week-old normal BALB/c mice.

† Eleven-week-old BLAB/c mice infected with *S. japonicum* for 5 weeks.

‡ $P < 0.05$, with respect to the value of normal.

**Table 5.** IL-2 production by whole spleen cells from *S. japonicum*-infected BALB/c mice in the presence of indomethacin

| Weeks after infection | IL-2 production (U/ml) | | % of normal |
|---|---|---|---|
| | Normal | Infected | |
| 1 | 178±9* | 183±14 * | 91* |
| | (140±17)† | (115±1)† | (82)† |
| 2 | 272±2 | 176±5‡ | 65 |
| | (148±5) | (81±9) | (55) |
| 3 | 204±8 | 123±5‡ | 60 |
| | (231±6) | (122±6) | (53) |
| 4 | 225±1 | 101±15‡ | 45 |
| | (130±6) | (59±14) | (46) |
| 5 | 133±18 | 66±9‡ | 49 |
| | (121±8) | (13±0) | (11) |
| 6 | 174±3 | 41±9‡ | 23 |
| | (131±5) | (16±10) | (12) |
| 7 | 220±4 | 24±6‡ | 11 |
| | (155±5) | (25±0) | (16) |
| 8 | 268±0 | 58±1‡ | 22 |
| | (194±2) | (29±13) | (15) |

Whole spleen cells ($25 \times 10^5$/ml) were cultured for 24 hr with Con A (5 μg/ml) or in the presence of indomethacin (2 μg/ml). The results represent the mean ± SE of five individual mice for each group.

* Stimulation with Con A in the presence of indomethacin.

† Stimulation with Con A alone.

‡ $P < 0{\cdot}05$, with respect to the value of normal.

**Table 6.** Effect of adherent spleen cells from *S. japonicum*-infected mice on IL-2 production by normal mice

| Animal group | T-enriched cells (/ml)* | | Source of adherent spleen cells (/ml)† | IL-2 production (U/ml) |
|---|---|---|---|---|
| Normal | $25 \times 10^5$ | + | Medium alone | 122±6 |
| Normal | $25 \times 10^5$ | + | Normal ($5 \times 10^2$) | 139±5 |
| Normal | $25 \times 10^5$ | + | Infected ($5 \times 10^2$) | 133±3‡ |
| Normal | $25 \times 10^5$ | + | Normal ($5 \times 10^4$) | 141±6 |
| Normal | $25 \times 10^5$ | + | Infected ($5 \times 10^4$) | 145±8‡ |
| | Medium | + | Normal ($5 \times 10^2$) | <4±0 |
| | Medium | + | Infected ($5 \times 10^2$) | <4±0 |
| | Medium | + | Normal ($5 \times 10^4$) | <4±0 |
| | Medium | + | Infected ($5 \times 10^4$) | <4±0 |

* T-enriched cells were fractionated with nylon-wool column from whole spleen cells that were pooled from five normal mice.

† Adherent spleen cells were obtained from spleen cells that were pooled from either five normal mice or five mice infected for 5 weeks. T-enriched cells ($25 \times 10^5$/ml) were cultured in the presence of adherent spleen cells and Con A (5 μg/ml) for 24 hr in duplicate for each group. The same experiment was done three times. The results represent the mean ± SE of three experiments for each group.

‡ $P > 0{\cdot}1$, with respect to the value of normal.

One of the possible reasons for the decrease in IL-2 production may be the relative reduction in the number of T cells resulting from splenomegaly accompanied by polyclonal B-cell activation and eosinophilia during the infection. In order to investigate whether this was the case, spleen cells were fractionated by nylon-wool column, and column non-adherent T-enriched cells were stimulated with Con A in the presence of normal splenic adherent cells. The IL-2 production of T-enriched cells was 15–25% of the normal level during 3–7 weeks after infection (Table 3). A second possible reason for the reduction of IL-2 production in *S. japonicum*-infected mice may be concerned with experimental conditions such as the dose of Con A or the culture time. To exclude the possibility, spleen cells from mice infected with *S. japonicum* 5 weeks earlier were incubated with varying doses of Con A for varioues periods of time, and IL-2 production was examined. As shown in Table 4, IL-2 production of *S. japonicum*-infected mice was decreased at all Con A doses and culture times compared with that of normal mice. From these results, we ascertained that the reduction in IL-2 production by *S. japonicum*-infected mice was independent of the dose of Con A or the duration of culture. A third possible reason for the depressed IL-2 production in *S. japonicum*-infected mice has been deduced from the experiments of others (Rappaport & Dodge, 1982; Walker *et al.*, 1983), namely that prostaglandin $E_2$ ($PGE_2$ secreted by adherent cell population) suppresses IL-2 production, and that the suppressive effect of $PGE_2$ can be reversed by the addition of indomethacin. To examine whether the decrease in IL-2 production of *S. japonicum*-infected mice was caused by $PGE_2$ produced by a certain cell population, indomethacin (2 μg/ml) was added to the system of IL-2 production by whole spleen cells. The addition of indomethacin enhanced the IL-2 production of normal and *S. japonicum*-infected mice at many points of measurement, but at other points it did not. Furthermore, even when IL-2 production of *S. japonicum*-infected mice was enhanced by indomethacin treatment, their IL-2 titre did not reach the level of control normal spleen cells that received indomethacin treatment (Table 5). This result indicates that $PGE_2$ is not a main factor in the decrease in IL-2 production of *S. japonicum*-infected mice.

Macrophages are also known to inhibit the lymphocyte proliferative response by mitogen in *S. japonicum or S. mansoni* infections (Amano & Oshima, 1984; Ottesen, 1979). Therefore, to examine the effect of macrophages on IL-2 production in *S. japonicum*-infected mice, splenic adherent spleen cells from *S. japonicum*-infected mice as a source of macrophages were added directly to the system of IL-2 production by normal T-enriched spleen cells. These splenic adherent cells were obtained from mice in the fifth week of infection, whose IL-2 production was strongly suppressed. As shown in Table 6, splenic adherent cells from *S. japonicum*-infected mice did not inhibit IL-2 production by T-enriched cells from normal mice.

### Response to exogenous IL-2 of *S. japonicum*-infected mice

As the final part of the interleukin pathway, we examined the IL-2 response of *S. japonicum*-infected mice. First, to determine the IL-2 dose to be used in these IL-2 responses, Con A-activated spleen cells from normal mice were cultured in the presence of various concentrations of lectin-free IL-2, and the [$^3$H]TdR

**Table 7.** Responses to exogenous IL-2 of *S. japonicum*-infected BALB/c mice

| Weeks of infection | [$^3$H]TdR uptake (c.p.m.) Normal | Infected | % of normal |
|---|---|---|---|
| 1 | 55,758±1751* | 53,122±2465* | 95 |
| 2 | 42,542±4356 | 48,693±4440 | 114 |
| 3 | 66,902±1537 | 46,831±5800† | 70 |
| 4 | 42,665±1447 | 27,578±4607† | 65 |
| 5 | 56,674±1162 | 34,004±2442† | 60 |
| 6 | 63,759±2236 | 22,315±3821† | 35 |
| 7 | 47,756±664 | 14,326±360† | 30 |
| 8 | 47,892±5633 | 8620±1638† | 18 |

Whole spleen cells ($5 \times 10^6$/ml) were stimulated with Con A (5 μg/ml) for 48 hr in a $CO_2$ incubator. After stimulation, the Con A-activated cells were washed with a medium containing α-MM and adjusted to $5 \times 10^5$/ml. Then, the cells were cultured with IL-2 (100 U/ml) in triplicate per mouse. [$^3$H]TdR (0·5 μCi/well) was added during the last 24 hr of the 48-hr culture.

* The results represent the mean c.p.m. ± SE of five individual mice for each group.

† $P < 0{\cdot}02$, with respect to the value of normal.

uptake was examined. IL-2 was used in a final concentration of 100 U/ml in examining the proliferative response to IL-2 in *S. japonicum*-infected mice, in as much as IL-2 at this dose produced a significant proliferative response. As shown in Table 7, the proliferative response to IL-2 gradually decreased as compared with age- and sex-matched normal mice during the first to the eighth week of the infection. These results clearly indicated that, in *S. japonicum*-infected mice, the response to IL-2 was impaired.

## DISCUSSION

In the present study, we demonstrated that, compared with normal mice, the ability to produce IL-1 by *Schistosoma japonicum*-infected mice was similar or enhanced during a period of infection, whereas proliferative responses to IL-1 and IL-2 and IL-2 production progressively decreased during the period of the infection. Although the precise mechanisms of the disturbed immune response in *S. japonicum*-infected mice from the viewpoint of the interleukin cascade reaction are far from clear, several possibilities are conceivable concerning the disturbance at each point of the cascade reaction. Firstly, concerning the present observation that the IL-1 production of *S. japonicum*-infected mice was rather enhanced at some points during the infection, a possible explanation is that this phenomenon may be related to granuloma formation in schistosomiasis. IL-1 was first reported as a factor released from macrophages in connection with their accessory function in antigen recognition. Thereafter, it has been reported that IL-1 has multiple biological functions—that is, it not only induces IL-2 production by T cells, but also stimulates fibroblast proliferation (Schmidt *et al.*, 1982). Although Wyler *et al.* (1984) reported that fibroblast-stimulating factors *in vivo* secreted by egg granuloma macrophages in *Schistosomiasis mansoni* are functionally distinct from IL-1, they reported that IL-1 also has fibroblast proliferation-stimulating activity. In *Schistosomiasis mansoni* and *japonica*, macrophages and fibroblasts have been reported to be involved in granuloma formation (Phillips & Fox, 1984). Taken together, an enhanced ability of IL-1 production in *S. japonicum*-infected mice may be associated with granulomatous inflammation in *S. japonicum* infection.

Secondly, as to the decreased response to exogenous IL-1 of thymocytes of *S. japonicum*-infected mice, one possibility is that the number of thymocytes responsive to IL-1 is reduced. It has been reported that atrophy of the thymus occurs during *S. japonicum* infection (Amano & Oshima, 1983), and in our present study the recovery of thymocytes from *S. japonicum*-infected mice progressively decreased after the infection (unpublished result). However, it is a well-known fact that thymic atrophy induced by the infection occurs especially in the thymus cortex, whereas thymocytes responding to IL-1 are cortisone resistant and reside in the medulla of the thymus. Furthermore, thymocytes might be killed by a thymocytotoxic antibody, which has been reported to increase in *S. japonicum*-infected mice (Kawabata *et al.*, 1981). Another possibility is that, although the number of IL-1-responsive thymocytes was not reduced, the function of the thymocytes was impaired in terms of responsiveness to IL-1. This might be caused by the reduced expression of IL-1 receptors on cell surfaces. Anyway, further studies are required with the use of labelled IL-1 and other tools.

Thirdly, several possibilities are conceivable concerning the decreased production of IL-2 in *S. japonicum*-infected mice. (i) A relative decrease in the number of IL-2 producer cells by polyclonal B-cell activation or eosinphilia may not be the cause of the result observed, since the decrease in IL-2 production in *S. japonicum*-infected mice was also observed even when T-enriched cell suspensions were used as a source of IL-2 producer (Table 3). (ii) The result may not be ascribed to an artifact in *in vitro* experimental conditions such as the dose of Con A or the culture time, in as much as the decreased IL-2 production was irrelevant to these experimental conditions, as shown in Table 4. (iii) $PGE_2$ which has been reported to inhibit IL-2 production (Walker *et al.*, 1983), and $PGE_2$-induced suppressor T cells (Chouaib *et al.*, 1984) may not be closely involved in the decreased IL-2 production in *S. japonicum*-infected mice because the restoration of the IL-2 production by the addition of indomethacin was only partial (Table 5). Suppressor macrophages, which have been reported to be inhibitors of mitogenic response in *S. japonicum*-infected mice (Amano & Oshima, 1984), also might not be a cause of depressed IL-2 production (Table 6). (iv) It has been reported that a certain serum factor in *S. japonicum*-infected mice inhibits T-cell mitogenesis (Garb *et al.*, 1982). In as much as this factor binds to thymocytes, it seems interesting to examine whether this factor inhibits IL-2 production. Recently it has been revealed that not only Lyt-$1^+$ $2^-$ cells, but also Lyt-$2^+$ cells produce IL-2 (Andrus, Prowse & Lafferty, 1981), so it may be necessary to investigate IL-2 production in each T-cell subset for understanding the more exact mechanisms of depressed IL-2 production in *S. japonicum*-infected mice. As to the substantial intra-group variation of IL-2 production in the control normal spleen cells (Table 5), it may be ascribed to the difference in the day of spleen cell culture for IL-2 production. As shown in the Materials and Methods, mice were infected with *S. japonicum* at 6 weeks after birth, so mice were killed at various different days of infection.

Resting T cells are activated to express IL-2 receptors

following Con A stimulation (Larsson, 1979), and subsequently these activated T cells respond to IL-2 by proliferation (Wagner *et al.*, 1980). Therefore, as the last step in the interleukin cascade reaction, we examined the responses to IL-2 of *S. japonicum*-infected mice by the proliferation rate of Con A blasts in the presence of exogenous IL-2. Our results demonstrated that responses to exogenous IL-2 in *S. japonicum*-infected mice were gradually suppressed as compared with normal mice. Two possible explanations may account for the depression of responses to IL-2 in *S. japonicum*-infected mice. First, the number of T cells in the spleen cells of *S. japonicum*-infected mice was decreased by the appearance of polyclonal B-cell activation. However, this possibility can be excluded by the result that the response to IL-2 of nylon-column effluent spleen cells from mice in the sixth week of infection was smaller than that of whole spleen cells from normal mice (data not shown). A second possibility may involve a down-regulation of the IL-2 receptor expression or impairment of IL-2 receptors by unknown factors in *S. japonicum*-infected mice.

It may be very difficult to explain the disturbed interleukin cascade reaction observed in *S. japonicum*-infected mice by a single central defect in the interleukin cascade reaction, in as much as the complicated host–parasite relationships may be established for the reason that the *S. japonicum* worm actively works on the host to escape from the host defence. It is conceivable that the most important central defect might be the decreased response to IL-1, in as much as this disturbance might consequently induce decreased IL-2 production. The low responsiveness to IL-2 might be explained by insufficient expression of IL-2 receptors on T cells because of disturbed differentiation in IL-2-reactive T cells or by the decreased number of IL-2-reactive T cells, both of which might be induced by low titre of IL-2 in the host.

Studies to elucidate the mechanisms of disturbed interleukin cascade reactions observed in the present experiments are under way.

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