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Immunological Parameters Affected by Larval *Ascaris suum* Infection

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Abstract

High levels of immunoglobulins were produced in mice infected with larval *A. suum*, whereas antibody responses to an unrelated antigen administered 4 days before the infection were suppressed. In the spleen, B cells remarkably increased, while T cells decreased during the first two weeks after the infection. The thymic atrophy and the elimination of immature thymocytes indicated the operation of systemic feedback-regulation. Lipopolysaccharide (LPS) carried over from the intestine was not concerned with the induction of the regulation. Activities of proinflammatory cytokines such as IL-6 and IFN were elevated in the infected mice, but the IL-1α activity was rather diminished. The elevation of TNF-α activity was negligible, but it was enhanced by a challenge with a low dose of LPS. The IL-4 activity was undetectable either in sera or culture supernatants of splenocytes from infected mice. Results suggest that the perturbation of immune system may be caused in the process of a sequence of physiological reactions.

Key words: larval *Ascaris suum* infection; immunosuppression; immunoglobulin production; cellular kinetics; thymic atrophy; cytokines.

Introduction

Helminth infection affects murine immune systems in a variety of ways. The most typical example for helminth-induced immunomodulation is the polyclonal enhancement of IgE synthesis by *Nippostrongylus brasiliensis* (Jarrett and Stewart, 1972). This is due to the induction of IL-4 production by this parasite (Finkelman *et al.*, 1986; Coffman *et al.*, 1989). However, the effect of parasite infection is never uniformly understandable and variable depending on the kind of parasite and infectious conditions. For example, the *N. brasiliensis* infection suppresses IgE antibody productions in some cases (Watanabe *et al.*, 1976; Haig *et al.*, 1980). It is one of the reason for the variety that parasites give hosts systemic stimuli beside a stimulus that is recognized in local, immunological compartments.

The immune system is bi-directionally linked to the nervous and endocrine systems (Blalock, 1989; Goetzl *et al.*, 1990). Events that stimulate physiological systems are integrated as stress. Stresses modulate immune responses in various ways depending on the nature and intensity of the stimuli (Stein *et al.*, 1985). The immunomodulation is considered one of terminal results mediated by neurotransmitters, hormones, autacoids and cytokines that resulted from complex physiological reactions to stress (Blalock, 1989; Goetzl *et al.*, 1990). However, the intense stress such as tissue damage generally induces immunosuppression through the release of glucocorticoids (Freire-Garabal *et al.*, 1991; Munck *et al.*, 1984; Stein *et al.*, 1985).

The helminth infection is considered as a stressful event probably with both cognitive and noncognitive stimuli. The cognitive stimuli may be generated by dynamic movement. Although it is difficult to assess the sole effect of the cognitive

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stimuli, some products resulting from tissue damages or subsequent inflammatory reactions can stimulate the central nervous system. Noncognitive stimuli such as antigens are trapped by immune systems. The latter stimuli may be also transmitted to the neuroendocrine system through the production of cytokines and peptide hormones when the production is serious (Blalock, 1989; Goetzl *et al.*, 1990).

The larvae of *Ascaris suum* migrate through the liver and lung in mice, resembling that in its definitive host, the swine and being synchronous (Sprent, 1952; Mitchell *et al.*, 1976). These organs supposedly become damaged by the larvae, resulting in the induction of a variety of physiological reactions as a homeostatic mechanism. The larvae are naturally expelled from mice by two weeks after the infection. The enhanced formation of immunoglobulins, including IgE is observed thereafter (Crandall and Crandall, 1971; Crandall, 1976). However, antibody responses against unrelated antigens that are administered in the period of larval migration are suppressed (Crandall and Crandall, 1976). This contradictory phenomenon is not explained solely by the cytokine network in local immunocompartment and the mechanism remains obscure. We approached the problem in terms of systemic, physiological reactions. We have shown in this report how immunological parameters are varied along with larval migration of *A. suum*, and we have also presented evidences of the induction of physiological reactions in the separate manuscript (Komatsu *et al.*, 1996).

Materials and Methods

Animals

Female BDF1 mice at 7 weeks of age were obtained from Shizuoka Laboratory Animal Center (Hamamatsu, Japan). Female C3H/HeN and C3H/HeJ mice at 7 weeks of age were obtained from Nihon Clea Co. Ltd. (Tokyo, Japan).

Infection with Ascaris suum *larvae*

The coats of fertilized eggs obtained from the uterus of adult *Ascaris suum* were removed with 10% NaOCl and incubated at 28°C for 4 weeks. Mice were infected with 1×10^4 embryonated eggs each by stomach intubation.

Immunization

Hen egg white lysozyme (HL) that was recrystallized 6 times (Seikagakukogyo Company Ltd., Osaka, Japan) was used as an antigen. Experimental and control groups were composed of 6 BDF1 mice. Both groups were immunized with an intraperitoneal injection of the antigen (10 μg) emulsified in a total volume of 0.2 ml Freund's incomplete adjuvant and boosted with a repeat injection 9 weeks later. The experimental group was infected with embryonated eggs of *A. suum* 4 days after the first immunization.

Sera

For the antibody assay, blood was taken from the orbital vein, diluted simultaneously five times with saline and kept overnight at 4°C. The serum was separated by centrifugation. For the cytokine assay, the serum was separated from the blood taken by heart puncture and frozen until use.

Antibody assay

Total IgE, IgG and IgM concentrations in the sera from infected mice were estimated by means of sandwich enzyme-linked immunosorbent assays (ELISA) using multiplates with 96 wells (MS-3596F/H, Sumitomo Bakelite Co. Ltd., Tokyo, Japan). Monoclonal rat anti-mouse IgE (LO-ME-3, Serotec Ltd., Kidlington, England), goat anti-mouse IgG (affinity-isolated antibody specific to gamma chain, Caltag Laboratories Inc., So. San Francisco, CA) and monoclonal rat anti-mouse IgM (LO-MM-9, Zymed Laboratories, Inc., So. San Francisco, CA) were used as the first antibodies. The peroxidase conjugate of affinity-isolated goat IgG specific to the epsilon chain of mouse IgE (Nordic Immunological Laboratories, Capistrano Beach, CA), the peroxidase conjugate of affinity-isolated goat antibody specific to gamma chain of mouse IgG (Caltag Laboratories Inc.) and the peroxidase conjugate of goat IgG fraction monospecific to mouse IgM (Organon Teknika Corporation, West Chester, PA) were used as second antibodies. The standard ligands were monoclonal mouse IgE, IgG and IgM. The optical density at 492 nm (OD492) was read using a microplate reader (MTP-32, Corona Electric Co., Ltd., Ibaragi, Japan). HL-specific antibodies were titrated also by ELISA. The standard for anti-HL

IgE titration was pooled serum taken from mice immunized with HL. That for anti-HL IgG assay was a monoclonal mouse anti-HL IgG, which was provided by Dr. H. Fujio of the Research Institute for Microbial Diseases, Osaka University.

Cell analyses

Fluorescein (FITC) conjugated rat anti-mouse Thy1.2 monoclonal antibody (30-H12), FITC conjugated rat anti-mouse Lyt-2 monoclonal antibody (53-6.7) and phycoerythrin (PE) conjugated rat anti-mouse L3T4 monoclonal antibody (GK1.5) were purchased from Becton Dickinson Immunocytometry Systems (Mountain View, CA). PE conjugated rat anti-mouse Thy1.2 monoclonal antibody (30-H12) was obtained from Pharmingen (San Diego, CA). FITC conjugated goat anti-mouse immunoglobulin antibody was obtained from Caltag Laboratories (So. San Francisco, CA). Tris-ammonium chloride-treated whole spleen cells or thymocytes (1×10^6/ml PBS-7 containing 0.5% BSA and 0.1% NaN_3) were stained with the respective antibodies at 4°C for 30 min. In some experiments, cells were stained doubly with FITC and PE conjugates. The cells were analyzed flow cytometrically using a FACScan (Becton Dickinson Immunocytometry Systems, San Jose, CA).

Supernatant of the cell cultures

RPMI 1640 (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) containing heat inactivated FCS (10%), L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml) and 2-ME (5×10^{-5} M) was used as the culture medium. Tris-ammonium chloride-treated whole spleen cells (4×10^6/ml) prepared from infected mice were cultured for 48 h, then the supernatants were separated by centrifugation.

Assay of cytokine activities

IL-6 activities in sera taken from BDF1, C3H/HeN or C3H/HeJ mice infected with *A. suum* larvae were determined by measuring the growth of IL-6 dependent murine hybridoma MH60.BSF-2 as described (Matsuda *et al.*, 1988; Yoshimoto *et al.*, 1992). Briefly, test samples (100 μl/well) were serially diluted in duplicate in 96-well microtiter plates with culture medium and incubated for 48 h with IL-6-dependent cells (10^4/well). Human recombinant IL-6 (rIL-6) was used as the standard. The viability was measured in a colorimetric assay with a tetrazolium salt (Sigma Chemical Co., St. Louis, MO). IL-4 activities in sera and culture supernatants of spleen cells taken from infected mice were determined by means of a bioassay using an IL-4 dependent murine T cell clone (CT.4S) and a standard murine rIL-4 as described (Hu-Li *et al.*, 1989). IFN activities in sera were estimated by measuring the capacity to inhibit the cytopathic effect of vesicular stomatitis virus on mouse L-929 cells (Nakamura *et al.*, 1993). TNF-α activities in sera were estimated by a cell lytic assay using L-929 cells (Aggarwal *et al.*, 1985; Carswell *et al.*, 1975). The cell viability was measured in a colorimetric assay (at 550 nm) using crystal violet (Sigma Chemical Co.). The standard was a murine r-TNF-α. IL-1α activities in sera were measured using a mouse IL-1α ELISA kit (InterTest-1αX™, Genzyme Immunobiologicals, Cambridge, MA). This is a solid-phase enzyme-immunoassay based on the multiple antibody sandwich principle. The first antibody was a monoclonal anti-mouse IL-1α and the second was a biotinylated polyclonal antibody. The enzyme was peroxidase conjugated with avidin and the standard was mouse rIL-1α.

Statistical analyses

Data are expressed as the means and standard errors. Data from ELISA were computed by regression analyses. Anti-HL IgE titers are expressed as reciprocals of dilution of test samples that showed an OD492 of 0.050 by ELISA. The significance of the data was evaluated by Student's t-test.

Results

Antibody responses in infected mice

The experimental group was composed of 6 mice. Two groups were immunized with an intraperitoneal injection of HL (10 μg) emulsified in Freund's incomplete adjuvant and boosted with a repeat injection 9 weeks later. One of these groups was infected with a dose of 1×10^4 embryonated *A. suum* eggs 4 days after the first immunization. Anti-HL antibodies and total immunoglobulins in both groups of mice were assayed by ELISA. Anti-HL

IgE antibodies were expressed as endtiters estimated by linear regression analysis (Fig. 1A). Others were expressed quantitatively (Fig. 1). In the primary response of infected mice, the anti-HL IgE production was maximally suppressed by 85.6% ($p<0.05$). The suppression (89.8% at $0.05<p<0.1$) persisted into the secondary response. The production of anti-HL IgG was also maximally suppressed by 90.4% ($p<0.02$). In contrast to the production of antibodies specific to HL, total IgE, IgG and IgM immunoglobulins markedly increased in infected mice (Fig. 1B). The maximal concentrations were 9.189±1.455 μg, 3.848±0.356 mg and 2.661±0.271 mg per ml serum, respectively. The normal levels in

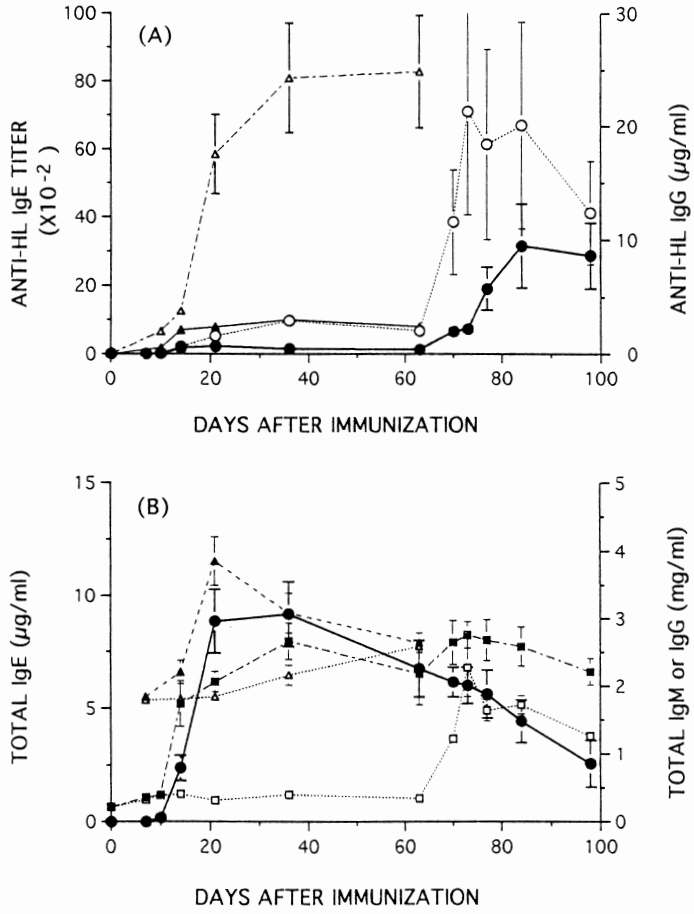

Fig. 1 Production of antibodies and total immunoglobulins in BDF1 mice infected with 1×10^4 embryonated eggs of *Ascaris suum*. (A) Mice were immunized with an intraperitoneal injection of 10 μg of HL emulsified in a total volume of 0.2 ml incomplete Freund's adjuvant. The immunization was boosted with a repeat injection 9 weeks later. One group of mice was infected with *A. suum* 4 days after the first immunization. Anti-HL IgE titers are expressed as reciprocals of dilution of sera showing 0.05 of OD492 by ELISA. Anti-HL IgG antibodies were quantified using a monoclonal anti-HL IgG as the standard for the ELISA. ●, anti-HL IgE in infected mice; ○, anti-HL IgE in uninfected mice; ▲, anti-HL IgG in infected mice; △, anti-HL IgG in uninfected mice. (B) Total immunoglobulins quantified by ELISA in the sera of these mice. ●, IgE in infected mice; ▲, IgG in infected mice; △, IgG in uninfected mice; ■, IgM in infected mice; □, IgM in uninfected mice.

uninfected mice were 0.191±0.026 μg, 1.834±0.061 mg and 0.400±0.095 mg per ml serum, respectively.

Analysis of spleen cells in infected mice

In BDF1 mice infected with 1×10^4 embryonated eggs of *A. suum*, about 800 larvae were focused in the liver on the 5th day, then about 600 larvae migrated into lung two days later. Most larvae were expelled 2 weeks after infection (Fig. 2A). In C3H/HeJ mice, the larval migration resembled that in BDF1 mice. During the migration period in both strains of mice, counts of whole splenocytes increased while thymus weights decreased (Fig. 2B). These phenomena were induced by larval infection itself, but not by lipopolysaccharide (LPS) carried over from intestinal bacteria flora, because C3H/HeJ mice are unresponsive to LPS. The increase in count of splenocytes was irregular and usually accompanied by descendant phases on the 5th and 7th day (BDF1). This timing corresponded with that of larval migration into the liver and lung. The splenocyte population of both strains varied irregularly

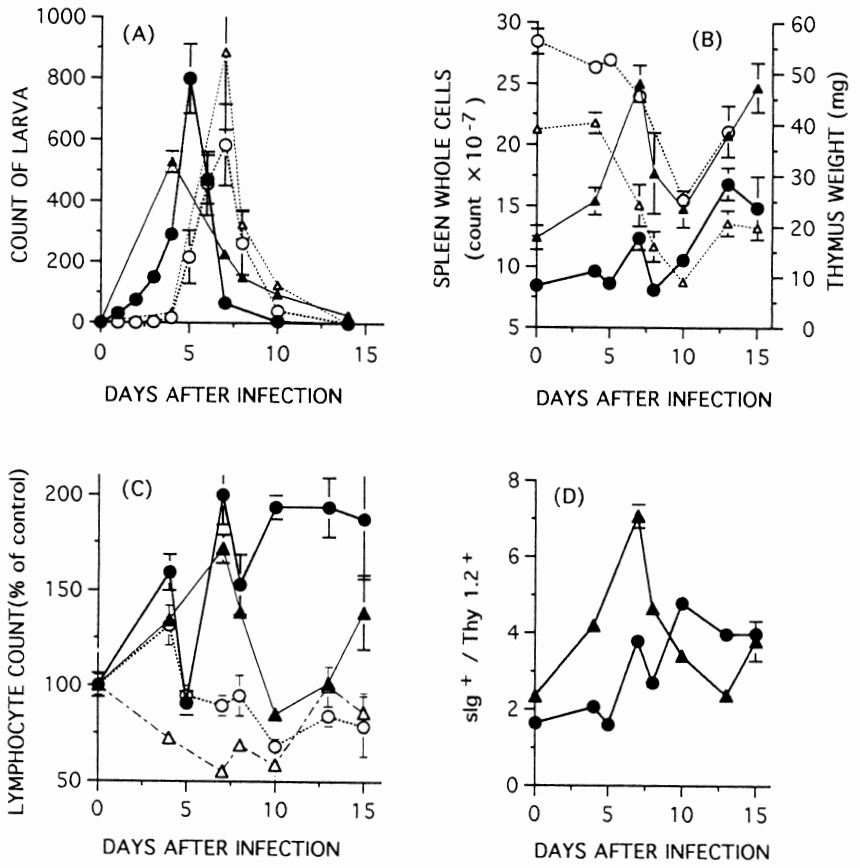

Fig. 2 Cellular events in BDF1 and C3H/HeJ mice infected with 1×10^4 embryonated eggs of *Ascaris suum*. (A) Larval migration in these mice. Counts of larvae in the liver (●) and lungs (○) of BDF1 mice; those in liver (▲) and lung (△) of C3H/HeJ mice. (B) Kinetics of whole splenocytes and thymus weight. Counts of whole splenocytes in BDF1 (●) and C3H/HeJ (▲); Thymus weights of BDF1 (○) and C3H/HeJ (△). (C) Counts of splenic lymphocytes. Data are expressed as percentages of preinfection levels. (●), slg^+ (surface immunoglobulin-positive) cells in BDF1 and (▲), those in C3/HeJ; (○), $Thy1.2^+$ cells in BDF1 and (△), those in C3H/HeJ. (D) Ratios of slg+ cells to $Thy1.2^+$ cells in BDF1 (●) and C3H/HeJ (▲).

along the process of larval migration. The count of slg^+ cells (B cells) was maximally elevated about 2-fold on the 10 day, whereas that of $Thy1.2^+$ cells (T cells) was diminished to 68% of the preinfection level (BDF1 in Fig. 2C). Therefore, the ratio of slg^+ cells to $Thy1.2^+$ cells changed as shown in Fig. 2D.

Analysis of T lymphocyte subpopulations in the spleen

T cell subpopulations in spleen of BDF1 mice infected with the same dose of *A. suum* eggs were analyzed by doubly staining cells with monoclonal antibodies conjugated with fluorescein or phycoerythrin. Data obtained from triplicate experiments are expressed as averages and standard errors. The data on minor cell populations that were not directly counted were computed from the data. The decrease in percentages of both $Thy1.2^+CD4^+CD8^-$ and $Thy1.2^+CD4^-CD8^+$ cells was significant ($p<0.05$) from the 5th day (Fig. 3B). There was no parallelism in the decrease between both cells. Thus, the ratio of total $CD4^+$ to total $CD8^+$ cells changed as shown in Fig. 3B (5th and 10th day, $p<0.01$). Immature cells bearing both CD4 and CD8 phenotypes were not found in the spleen, but small percentages (1.4% at maximum) of cells bearing only Thy1.2 phenotype were detected from the 5th day. On the contrary, cells bearing only CD4 or CD8 that were detected in the normal spleen (1.5% and 0.9%, respectively) disappeared from the 10th day.

Analysis of T lymphocyte subpopulations in the thymus

Fig. 3A shows FACScan windows of two-dimensional scatter plots of 1×10^4 thymocytes from normal mice (Left) or mice infected 10 days before (Right) and the gated area for the analysis. The forward light scatter reflects the relative cell size and the side light scatter are generated by the distortion of cellular microorgans. Normal thymocytes were scattered as a belt along the horizontal axis (Left). This indicated that the normal thymocytes are composed of cells with a variety of sizes. Most of these cells were Thy1.2-positive (98.4%). The cells from infected mice were scattered rather as a spot than a belt, indicating the reduction of large cells (Right). In the normal thymus, detectable subpopulations were $Thy1.2^+CD4^+CD8^+$ (86.9±1.0%), $Thy1.2^+CD4^+CD8^-$ (7.0±0.8%), $Thy1.2^+CD4^-CD8^+$ (1.5±0.2%), $Thy1.2^+CD4^-CD8^-$ (2.1±0.8%) and unstained cells (1.6±0.7%). After infection, the count of total thymocytes decreased from the 5th day (by 5.4±0.1%) and 98.7±0.5% of them were eliminated on the 10th day. The count returned thereafter, but it was still 24.7±19.5% of normal level on the 14th day. As seen in Fig. 3C, a significant decrease in percentage of $Thy1.2^+CD4^+CD8^+$ was evident on the 10th day (by 94.9±3.5%, $p<0.01$). The percentage was still abnormal on the 14th day (50.6±16.9%). On the contrary, percentages of $Thy1.2^+CD4^+CD8^-$, $Thy1.2^+CD4^-CD8^+$, $Thy1.2^+CD4^-CD8^-$ and unstained cells increased. The ratio of total $CD4^+CD8^-$ cells to total $CD4^-CD8^+$ cells decreased to 56.2±14.2% of the normal level ($p<0.05$) on the 14th day.

Cytokine activities in infected mice

Mice were infected with 1×10^4 embryonated eggs of *A. suum*. IL-6 activities in pooled sera from three mice were bioassayed. As shown in Fig. 4, two peaks of activity were observed in BDF1 mice. The first (276 pg/ml) was on the 4th day and the second (360 pg/ml) was on the 7th day of infection. Peaks of activity on the 7th day were also apparent in other strains (C3H/HeJ, 500 pg/ml; C3H/HeN, 750 pg/ml). TNF-α and IFN (not classified) activities in pooled BDF1 sera were estimated by means of a bioassay. A slight activity of TNF-α (14.8 units/ml) was detected in the pooled serum taken 10 days after infection (Table 1). In order to confirm the inductive effect of infection on this cytokine production, five mice were injected intravenously, on the 10th day of infection, with 20 μg of LPS of *Escherichia coli* as described (Mannel *et al.*, 1980), bled 90 minutes later and their sera were pooled. The activity of the cytokine was more evident in the serum of infected mice (Table 1). A single injection of this dose of LPS had no effect on uninfected mice. IFN activities were detected at least between the 5th and 10th day of infection (Table 1). In contrast to these cytokines, IL-1α activity in serum decreased from the 10th day of infection (Table 1). The activities were estimated by means of a sensitive ELISA method. On the other hand, IL-4 activities that were measured by means of a bioassay (sensitivity, 0.05 units/ml) were not found in any sera. Spleen cells taken from the

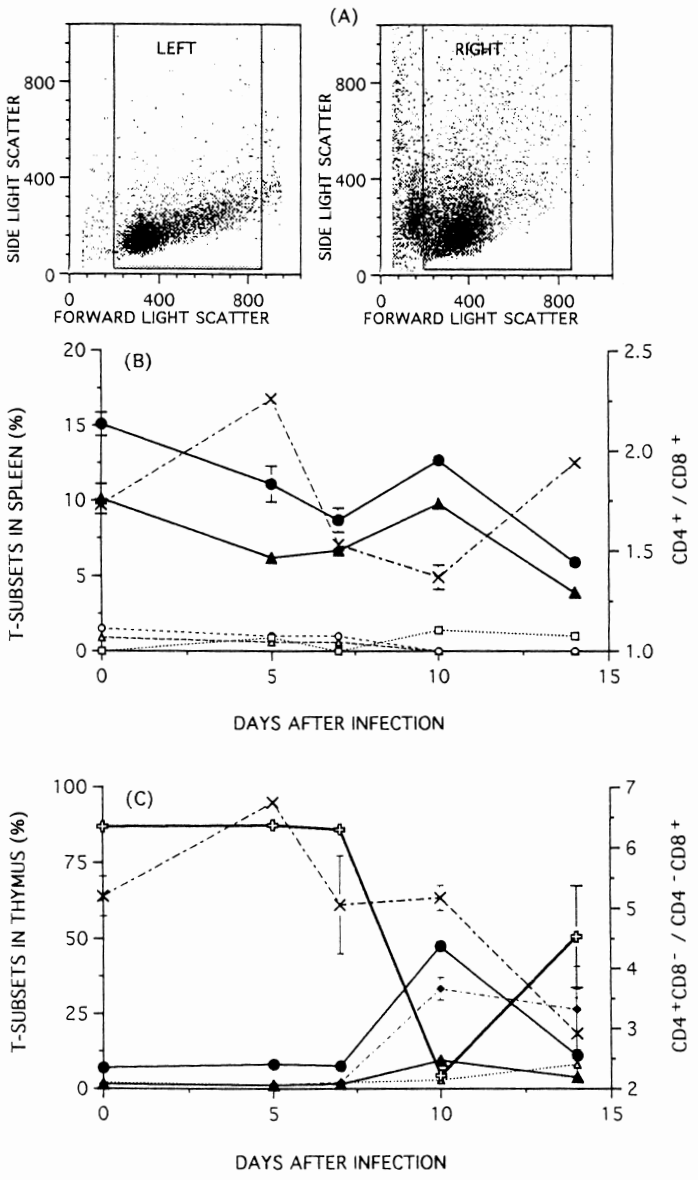

Fig. 3 Lymphocyte subpopulations in BDF1 mice infected with 1×10^4 embryonated eggs of *Ascaris suum*. (A) Two dimensional scatter plots of thymocytes in uninfected (Left) and infected (Right) mice, and the gated area for analyses. Horizontal and vertical axes indicate 0 and 90 degrees of the scattering angles, respectively. (B) Percentages of lymphocyte subpopulations in spleen. (●), $Thy1.2^+CD4^+CD8^-$ cells; (▲), $Thy1.2^+CD4^-CD8^+$ cells; (○), $Thy1.2^-CD4^+CD8^-$ cells; (△), $Thy1.2^-CD4^-CD8^+$ cells; (□), $Thy1.2^+CD4^-CD8^-$ cells; (×), Ratios of total $CD4^+$ cells to total $CD8^+$ cells. (C) Percentages of lymphocyte subpopulations in thymus. (+), $Thy1.2^+CD4^+CD8^+$ cells; (●), $Thy1.2^+CD4^+CD8^-$ cells; (▲), $Thy1.2^+CD4^-CD8^+$ cells; (△), $Thy1.2^+CD4^-CD8^-$ cells; (◆), Unstained cells; (×), Ratios of $CD4^+CD8^-$ cells to $CD4^-CD8^+$ cells.

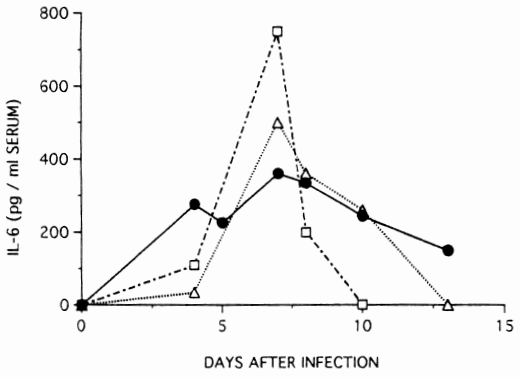

Fig. 4 IL-6 activities in pooled sera of three mice infected with 1×10^4 embryonated eggs of *Ascaris suum*. (●), BDF1; (△), C3H/HeJ; (□), C3H/HeN.

infected mice on the indicated days in Table 1 were cultured *in vitro* for 2 days using RPMI 1640 medium supplemented with standard culture components. No activities of IL-4 were detected in the medium.

Discussion

It has been reported that the larval *A. suum* infection does not enhance the reaginic antibody response to an unrelated antigen (Crandall, 1976). Our preliminary studies using PCA reaction showed that IgE antibody responses against HL or human γ-globulin were usually suppressed by the infection with larval *A. suum* when embryonated eggs (1×10^3 to 1×10^4) were given 4 days before the antigen administration. The reinfection with the same dose again failed to elevate IgE productions to preimmunized antigens while it enhanced IgE antibody formation against the *A. suum* antigen (Data not shown).

The ability of the used antigen-adjuvant system to induce three classes of antibody has been well established (Komatsu *et al.*, 1979; Komatsu *et al.*, 1988). The weak reaction should be sensitive to regulatory signals. In this study, HL was administered 4 days before the infection to generate some preferences to HL-specific antibody responses. However, the suppression of both IgE and IgG responses was again evident. The formation of IgE-memory cells was partially suppressed besides (Fig. 1A). On the other hand, the abnormal increase in total immunoglobulin production in these mice was confirmed (Fig. 1B).

The kinetics of splenocytes during larval migration seemed to represent these contradictory phenomena (Fig. 2C). The number of B cells increased about 2-fold, whereas that of T cells decreased significantly. The ratios of B to T cells ($slg^+/Thy1.2^+$) in the spleen are varied as seen in Fig. 2D. Moreover, the composition of T cell subpopulations ($CD4^+/CD8^+$) changed along with the decrease in their counts (Fig. 3B). These suggest that antagonistic

Table 1 Cytokine activities in mice infected with larval *Ascaris suum*

Days after Infection	Challenge* With LPS	TNF-α (U./ml Serum)	IL-1α† (pg/ml Serum)	IFN (U./ml Serum)	IL-4‡ (U./ml)
0	–	<3.7	69.1±21.0	<62.5	<0.05
	+	<3.7			
5	–	<3.7	70.9±5.8	250	<0.05
7	–		55.2±15.9	125	<0.05
10	–	14.8	14.8±7.0 (P<0.05)	125	<0.05
	+	622.5			
13	–	<3.7	22.8±2.8 (P<0.1)		

*Lipopolysaccharide of *Escherichia coli* (20 μg) was injected intravenously 10 days after the infection with *A. suum*, then blood was taken 90 min later.

†IL-1 activity was measured by ELISA and others were determined by bioassays on pooled sera (Data are expressed as units/ml).

‡IL-4 activities in culture mediums of whole splenocytes taken from infected mice on indicated days. The culture was performed for 2 days.

signals may be brought into the spleen by the infection.

We observed under the microscope that plasmacytes appeared in the spleen in the later phase of larval migration (Data not shown). The proliferation and differentiation of B cells indicate that the immune system was not artificially disrupted by over infection and reactions were observed within physiological levels. The most conceivable cause to proliferate and differentiate B cells is the antigenic stimulus. However, it seems unreasonable that stimuli of conventional antigens can accomplish it under splenic circumstances where $CD4^+$ cells are diminished and the balance of subpopulations is irregularly changed. In fact, antibody responses against a protein antigen that should have been proceeding in this period were suppressed (Fig. 1A). The B cell proliferation rather appears to be brought about by the T-independent stimulus. The relevance of LPS was completely eliminated by the experiment using C3H/HeJ mice. Alternatively, it is noted that *A. suum* larvae release materials like a T-independent antigen (Brown *et al.*, 1977).

The increase in IgE in mice infected with *N. brasiliensis* is due to the stimulation of a subset of helper T lymphocytes (T_{H2}) which finally leads to the polyclonal synthesis of IgE through IL-4 production (Finkelman *et al.*, 1986; Coffman *et al.*, 1989). This cytokine is almost undetectable in culture supernatants of spleen cells from mice primed with conventional antigens, whereas it is detected in the culture supernatant of lymphoid cells of animals infected with *N. brasiliensis* (Ishizaka, 1989). As we examined during 10 days after the infection, the cytokine activity was, however, undetectable in the sera or the culture supernatants of splenocytes from mice infected with larval *A. suum*. The decrease in $CD4^+$ subsets during this period may result in less production of this cytokine.

IL-6 acts on activated B cells and makes them terminally differentiate into antibody forming cells (Muraguchi *et al.*, 1988). IFNs make B cells mature directly or in combination with IL-2 and stimulate immunoglobulin production (Bich-Thuy and Fauci, 1986; Defrance *et al.*, 1986; Morikawa *et al.*, 1987; Neubauer *et al.*, 1985; Sidman *et al.*, 1984). These cytokine activities were augmented in the circulation by the larval *A. suum* infection (Table 1). They may act on the B cell proliferation and differentiation during the larval migration.

The levels of all three classes of immunoglobulin were vigorously elevated from 2 weeks after the infection (Fig. 1B). It is unclear whether B cells that produce these immunoglobulins are activated in the same manner as seen during larval migration or by the conventional manner under normally returned splenic conditions.

The specificities of the immunoglobulins were not completely determined, but it is sure that they contain antibodies against *A. suum* antigens. IgE showed high binding capacities to the crude extract from adult worms as examined by ELISA. The maximal titer ($2.16\pm0.22\times10^3$ at $OD_{492}=100$) was found 5 weeks after the infection (Data not shown in text). The binding was specific, because the capacity was completely eliminated by the preincubation with the extract. The IgE well exhibited the biological capacity to induce PCA reaction against the *A. suum*-derived antigen. The previous study also showed the production of *A. suum*-specific IgE (Crandall and Crandall, 1976). Therefore, materials derived from *A. suum* larvae considerably contain selective stimuli.

The decrease in T cells in the spleen and the concomitant atrophy of the thymus imply the operation of systemic down-regulation (Figs. 2 and 3). The effect of the regulation was also reflected on the irregularity of B cell increase. As the effect on proliferating B cells was transient, the timing of down-regulation was critically defined. It was associated with the timing of larval migration to the liver and lung.

Similar changes in T lymphocyte subpopulations in spleen and thymus are induced in mice by acute infection with *Trypanosoma cruzi*. In the mice, levels of circulating glucocorticoids are elevated (Leite-de-Moraes *et al.*, 1991). Alterations of the cellular composition and functions in the spleen and thymus are also induced by physiological causes such as surgeries (Freire-Garabal *et al.*, 1991; Stein *et al.*, 1985) or a binge drinking (Han *et al.*, 1993). All of these stress factors induce glucocorticoid secretion.

Corticosterone is a conventional product of the neuroendocrine response to stressful events in rodents. It is a key factor of systemic feedback-regu-

lation that results in the impairment of immune system (Andreoli *et al.*, 1989; Claman, 1988; Munck *et al.*, 1984). The cellular aspect in the atrophic thymus (Fig. 3C) suggested the influence of corticosterone. In the separate manuscript, we have presented the evidence that the infected mice secrete corticosterone in accordance with the larval migration (Komatsu *et al.*, 1996).

The cytokines detected in the infected mice are proinflammatory cytokines as they participate in the development and maintenance of the inflammatory reaction. These cytokines are produced by a variety of cell types in the inflammatory site. The IL-6 activity was elevated in the circulation in accordance with the larval migration to the liver and lung (Fig. 4). The IL-6 production in the infected mice was never induced by LPS carried over from the intestine, because it was also observed in C3H/HeJ mice (Fig. 4). The induction seems due to the tissue damage of target organs.

Although multisystem networks of cytokines and neuromediators are extremely complex (Goetzl *et al.*, 1990), IL-6 is one factor related to the release of glucocorticoids (Lyson *et al.*, 1991; Naitoh *et al.*, 1988; Salas *et al.*, 1990). The kinetics of IL-6 production shown in this paper almost paralleled that of corticosterone of which data are presented in the separate manuscript (Komatsu *et al.*, 1996). The correlation coefficient (R^2) between the circulating levels of IL-6 and corticosterone was 0.827 in BDF1 mice.

On the other hand, IFN-α rather inhibits hypothalamo-pituitary-adrenal secretion following peripheral and central administration (Saphier *et al.*, 1994). Although we did not determine the type of IFN in the circulation of infected mice, it may not be relevant to the secretion of corticosterone.

The elevation of TNF-α activity was negligible in the infected mice, but the level of the cytokine was well augmented by the challenge with a low dose of LPS (Table 1). This indicates that the infection can prime mice to produce the cytokine.

The relevance of IL-1α to the secretion of corticosterone may be excluded because of the significant decrease below the normal level. The production of prostaglandin E is involved in inflammatory responses (Kurland *et al.*, 1979). The decrease in the IL-1α level in infected mice may be due to the production of prostaglandin E, because this can suppress the production of IL-1α, while enhancing that of IL-6 by mouse macrophages (Haynes *et al.*, 1992).

In summary, the infection with larval *A. suum* perturbs the cellular composition of lymphoid organs during larval migration. During this period, IL-6 and IFN activities are elevated in the circulation while the IL-1α activity is decreased. The increase in TNF-α activity in the circulation is negligible, but the production is primed by the infection. The IL-4 activity can neither be detected in the sera nor in the culture supernatant of splenocytes from infected mice. It is suggested that the increase in IL-6 production that are induced by the tissue damage of target organs may stimulate the corticosterone secretion. The secretion may cause the immunoperturbation.

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