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HLA-DQ-controlled T cell response to soluble egg antigen of *Schistosoma japonicum* in humans

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SUMMARY

We analysed regulatory mechanisms of the human T cell response to soluble egg antigen (SEA) of *Schistosoma japonicum in vitro*. SEA is a crude antigen mixture containing numerous epitopes. We obtained SEA-induced T cell lines from five patients with chronic schistosomiasis japonica, and tested their proliferative response to molecular weight fractions of SEA. Although all T cell lines showed strong responses to crude SEA, there was a heterogeneity in fraction-driven responsiveness. All but one T cell line tested failed to respond to SEA fraction I (mol. wt > 18 kD). One patient who was typed as HLA-DQwl/w4, did not show proliferation of $CD4^+$ T cells to fraction I; however, a fraction I-driven helper T cell response was observed when we added HU-11 monoclonal antibody specific for HLA-DQwl/w4. This indicated that the patient had helper T cells to the fraction even though their response was suppressed. Because HLA-DQ had an effect on functional expression of suppressor T cells, it was suggested that there was epitope-specific regulation of the T cell response to SEA, and HLA-DQ-controlled immune suppression might be involved in the regulatory system in human chronic schistosome infection.

Keywords human T cells *Schistosoma japonicum* HLA-DQ immune suppression

INTRODUCTION

It has been demonstrated that T cell-mediated immunity to schistosome egg antigen has an essential role in pathogenesis of hepatosplenic schistosomiasis (Byram *et al.*, 1979; Cheever, Byram & von Lichtenberg, 1985). In murine experimental studies there is evidence that egg antigen-specific T cells control intrahepatic granuloma formation around schistosome eggs (Ragheb, Mathew & Boros, 1987). During chronic infection, the egg antigen-specific T cell response is suppressed through various regulation systems (Colley, 1981; Olds *et al.*, 1982), and this process results in modulation of circumoval granuloma formation and lower portal pressure (Boros, Pelley & Warren, 1975). Those results in mice have suggested that the T cell response to the egg antigen also has important pathological roles in humans.

In our previous study we established T cell lines and clones specific for soluble egg antigen (SEA) of *Schistosoma japonicum* from patients with chronic schistosomiasis japonica (Ohta *et al.*, 1988). These T cells showed SEA-driven interleukin-2 (IL-2) production, and were involved in granulomatous hypersensitivity to *S. japonicum* eggs *in vitro*. All T cell clones tested responded to a relatively low molecular weight fraction of SEA (7–18 kD), while they did not respond to higher molecular weight ones on which many T cell recognition sites could be expressed. In the light of these observations, we intended to analyse why T cells of chronic patients responded only to a particular fraction, and to test whether there was epitope-specific regulation for T cell response in human chronic schistosomiasis japonica.

HLA-DQ is a genetic region which is somehow related to immune suppression system in humans (Hirayama *et al.*, 1987). In the present study, we tested effects of an anti-HLA-DQ monoclonal antibody (MoAb) on the SEA-driven human T cell response *in vitro*. We observed that the helper T cell response to higher molecular weight fractions of *S. japonicum* SEA was modulated in chronic patients, and their response was restored by adding an anti-DQ MoAb. We discuss possible HLA control of SEA-driven T cell response in human schistosome infections.

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MATERIALS AND METHODS

Patients

Five patients with chronic schistosomiasis japonica were studied. All five were born and raised in Yamanashi Prefecture where schistosomiasis japonica had been endemic. Their pre-

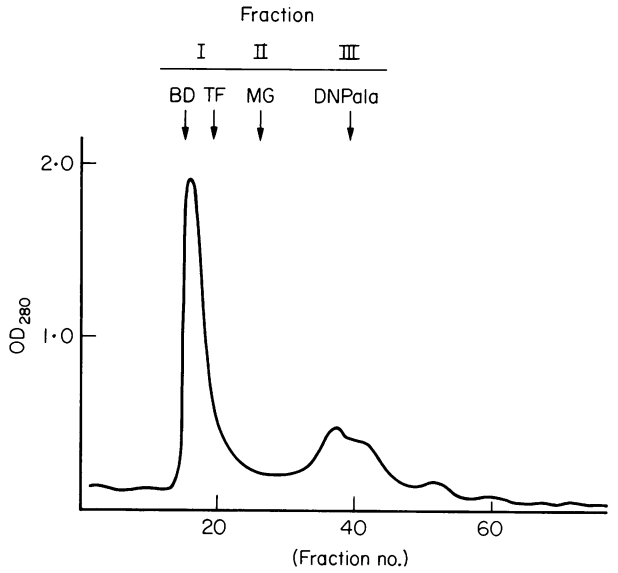

Fig. 1. Sephacryl S-200 gel filtration of crude soluble egg antigen of *S. japonicum*. Blue dextran (BD), transferrin (TF), myoglobin (MG) and DNP-alanine (DNP ala) were used as the molecular weight markers.

vious infection of *S. japonicum* was clearly demonstrated by former stool examination, rectal biopsy specimen and/or serodiagnosis with ELISA. They were diagnosed as having had schistosomiasis more than 20 years previously. All individuals had been treated, and we did not observe viable eggs in stool examination.

Lymphocyte preparation

Peripheral blood lymphocytes (PBL) were prepared from heparinized peripheral blood by Ficoll–Conray gradient solution method (specific gravity 1·077) (Sasazuki *et al.*, 1980).

Generation of T cell lines in vitro

PBL were suspended in RPMI 1640 culture medium (CM) supplemented with 15% heat-inactivated pooled human male sera, 100 μg/ml streptomycin, 100 U/ml penicillin and 20 mM L-glutamine. A PBL suspension of $1\text{–}2 \times 10^6$ cells/ml was dispensed into 6-well or 24-well plates (Nunc, Rockilde, Denmark), and was cultured with 5 μg/ml of crude or fractionated SEA for 7 days at 37°C in a 5% CO_2 humidified chamber. Blast cells were harvested and were resuspended in RPMI 1640 CM with 1 μg/ml of crude SEA and 10% of purified human IL-2 (Electro-Nucleonics, Silver Spring, USA), and were placed in 24-well plates. Irradiated autologous or HLA-compatible allogeneic PBL (3000 rad) were added as feeder cells. The culture medium was exchanged every 2–3 days, and feeder cells were added every 10 days. Those T cells were thought to be polyclonal.

SEA-driven proliferation of T cell lines

At least 10 days after the final addition of feeder cells, T cell lines were incubated overnight in IL-2-free medium to wash away the effects of exogenous IL-2. Ten thousand blastoid T cells were co-cultured with 5 μg/ml of crude SEA or 3 μg/ml of SEA fractions, and 5×10^4 irradiated autologous PBL were added as antigen-presenting cells (APC). Cells were incubated in 0·2 ml of RPMI 1640 CM in 96-well flat-bottomed microtitre plates (Corning, Corning, NY) for 72 h, and 1 μCI of ^{3}H-thymidine was added for the final 8 h. Incorporated ^{3}H-thymidine into proliferated T cells of triplicate cultures was assessed by liquid scintillation spectrometry.

Effects of anti-HLA MoAb on SEA-driven T cell response in vitro

Two MoAbs were tested for their effects on SEA-driven T cell response. HU-4 was anti-monomorphic HLA-DR (Koide *et al.*, 1982), and HU-11 was anti-HLA-DQwl/w4 (Kasahara *et al.*, 1983). Both MoAbs were in culture supernatant forms. MoAbs were added to the culture medium at the concentration of 2% or 20% (v/v), and the T cell proliferative response was compared between cultures with or without MoAbs.

Removal of CD8-positive cells from PBL

Three million PBL of patient KY were treated with 50 μl of an anti-OKT8 MoAb (IgG2a) (Ortho, Westwood, MA) for 30 min on ice, and were washed once with phosphate-buffered saline (PBS) (pH 7·2). Then cells were treated with 0·3 ml of rabbit complement (Cedarlane Laboratories, Ontario, Canada) 45 min at 37°C. We tested the treated PBL for SEA-driven proliferation.

T cell–macrophage co-culture experiment

T cells and macrophages were separated from PBL as described elsewhere (Ohta, Minai & Sasazuki, 1983). T cells were mixed with autologous macrophages at the ratio of 10:1 in RPMI 1640 CM, and were incubated with 5 μg/ml of crude SEA or 3 μg/ml of SEA fractions in 24-well plates for 7 days. Response was assessed by counting the number of proliferated T cell blasts.

Antigen

Antigens used in the present study were SEA (Ishii & Owhashi, 1982), *S. japonicum* adult worm antigen (AWA) (Sasazuki *et al.*, 1980), and purified protein derivative (PPD) (Nihon BCG Seizo Co., Tokyo, Japan). For each experiment, the same lot of each antigen was used. Protein concentrations were determined by the method of Lowry *et al.* (1951), and each antigen was preserved at −20°C before use.

Fractionation of crude SEA

Molecular weight fractions of SEA were separated by using a sephacryl S-200 superfine (Pharmacia, Uppsala, Sweden) gel column described elsewhere (Ohta *et al.*, 1988).

Phenotyping of T cell blasts

The surface markers of cell lines were tested by membrane immunofluorescence using anti-CD3, CD4, CD8, CD19, HLA-DR and IL-2 receptor (IL-2R) MoAbs (Becton Dickinson, Mountain View, CA) as described elsewhere (Yasukawa *et al.*, 1987). In brief, aliquots of blast cells were incubated in appropriate dilutions of MoAb for 30 min at 4°C. The cells were washed twice and further incubated with fluorescein-conjugated goat anti-mouse immunoglobulin (Coulter, Hialeah, FL) for 30 min at 4°C. The cells were then washed twice and the percentage of the fluorescent cells was determined using a fluorescent-activated cell sorter (FACS) (Epics C, Coulter Electronics).

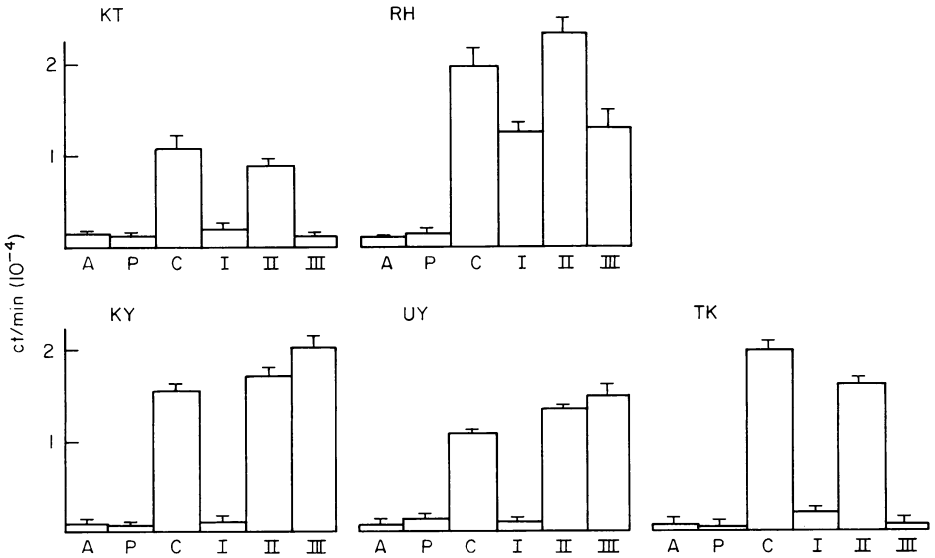

Fig. 2. Antigen-driven proliferation of T cell lines obtained from five patients with chronic schistosomiasis japonica. Mean ct/min of triplicate cultures. Antigens tested were as follows: A, *S. japonicum* adult worm antigen; P, purified protein derivative; C, crude soluble egg antigen (SEA); I, SEA fraction I; II, SEA fraction II; and III, SEA fraction III.

Table 1. Surface phenotypes of blast cells induced with soluble egg antigen (SEA) fractions in the presence or absence of monoclonal antibodies

		Positive for (%)					
Responder	Stimulation of	CD3	CD4	CD8	CD19	HLA-DR	IL-2R
KY PBL	Fraction II alone	96·0	92·0	0·3	0·2	90·1	60·0
KY PBL	Fraction I alone	28·2	28·3	5·3	0·2	84·6	36·4
KY PBL	Fraction I + HU-11 (× 5)	85·3	71·1	18·1	NT	76·5	62·2
KY PBL (CD8-depleted)	Fraction I alone	75·7	74·3	2·0	0·6	NT	NT

Peripheral blood lymphocytes were stimulated with SEA fractions for 7 days *in vitro*.
HU-11 monoclonal antibody was added at the concentration of 20% (v/v).
PBL, peripheral blood lymphocytes; IL-2R, interleukin-2 receptor; NT, not tested.

RESULTS

Molecular weight fractions of SEA

The crude SEA was applied to a sephacryl S-200 column, and we tentatively divided into this three fractions (I, II and III) as shown in Fig. 1. The molecular weight cut-off was set at 18 000 and 7000.

Response of SEA-reactive T cell lines to SEA fractions

We obtained crude SEA-induced blastoid cell lines from five patients with chronic schistosomiasis japonica. All these cells had helper T (Th) phenotyes of $CD3^+$, $CD4^+$, $CD8^-$ and $HLA\text{-}DR^+$ (data not shown). We observed fine specificities of these T cell lines to SEA, and there was no detectable proliferation to AWA and PPD (Fig. 2). However, there was a heterogeneity in responsiveness to SEA fractions among five T cell lines: T cells from two donors responded to both fractions II (7–18 kD) and III (< 7 kD), from two donors only to fraction II, and one T cell line responded to all three fractions (Fig. 2).

Phenotype analysis of fraction I-induced T cell response

Although we did not obtain T cell lines reactive to fraction I in four out of five patients, we observed vigorous PBL proliferation to the fraction in our previous study (Ohta *et al.*, 1988). Therefore, we tested what kind of cells in PBL responded to fraction I. We obtained blastoid cells by stimulating PBL with fraction I or II, and compared the surface phenotypes of these cells. Representative results are shown in Table 1. A crude SEA-induced T cell line of Patient KY responded to fraction II, but not to fraction I. Fraction II-induced blast cells were $CD3^+$, $CD4^+$, $CD8^-$; however, the majority of fraction I-driven blast cells were $CD3^-$ (Table 1). Those blast cells were HLA-DR-positive, but were negative for a B cell marker of CD19. In a repeated experiment, the similar phenotypes were observed for the fraction I-driven cells (data not shown).

Effects of anti-HLA MoAb on SEA-driven T cell response

To confirm the absence of fraction I-driven T cell proliferation, we stimulated purified T cells with SEA fractions in the presence of autologous macrophages. There was no detectable T cell proliferation to fraction I; however, a strong response was observed when HU-11 MoAb was added to the primary culture, and the effect was dose dependent (Fig. 3). Patient KY was typed as HLA-DQw1/w4 and Patient TK was negative for both specificities. HU-11 was effective only for Patient KY, but had

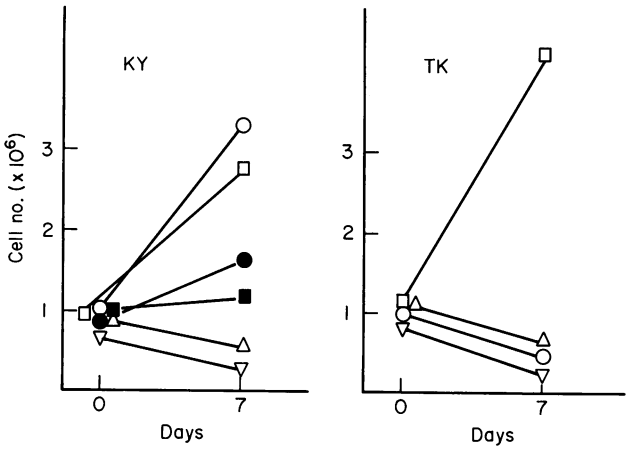

Fig. 3. Effects of anti-HLA monoclonal antibody (MoAb) on soluble egg antigen (SEA) driven T cell response were tested. HU-4 anti-HLA-DR MoAbs inhibited the crude SEA-driven T cell proliferation. Fraction I did not induce proliferation of T cell of patient KY (HLA-DQwl/w4); however, addition of HU-11 anti-HLA-DQwl/w4 MoAb restored the T cell response in a dose-dependent manner. HU-11 MoAb did not have any effect on T cells of patient TK (DQwl$^-$/w4$^-$). ○, Stimulation of fraction I with 20% HU-11; ●, fraction I with 2% HU-11; △, fraction I alone; □, crude SEA alone; ■, crude SEA with 20% HU-4; ▽, 20% HU-11 alone.

no effect on TK cells. HU-11 was not acting as a T cell mitogen because the MoAb alone did not induce T cell proliferation (Fig. 3). Phenotypes of fraction I-induced blast cells in this case were CD3$^+$, CD4$^+$ and CD8$^-$ (Table 1), and we confirmed that anti-HLA-DQ MoAb restored the suppressed response of CD4$^+$ T cells to fraction I. We prepared CD8$^+$ T cell-removed PBL of patient KY, and obtained fraction I-reactive blast cells. Those cells were also CD3$^+$, CD4$^+$ and CD8$^-$ (Table 1).

Specificity analysis of SEA-induced T cell lines

T cell lines induced with crude SEA or fraction I in the presence or absence of HU-11 MoAb were tested for antigen-driven proliferation. We confirmed that a crude SEA-induced T cell line of patient KY was reactive in HLA-DR restriction, and did not respond to fraction I. Addition of HU-11 MoAb to the established DR-restricted T cell line did not alter the responsiveness (Table 2). However, fraction I-induced CD4$^+$ T cells were proliferative to fraction I, and this response was inhibited by adding HU-4 anti-HLA-DR MoAb (Table 3). This suggested that patient KY had APC function capable of inducing a T cell response, and had HLA-DR-restricted Th cells reactive to fraction I; however, the response was suppressed through a regulation system involving the HLA-DQ molecule(s).

DISCUSSION

Modulation of SEA-driven T cell responsiveness during the chronic phase is one of the most characteristic immunological phenomena in schistosome infection. This results in diminished circumoval granulomas in the liver and improvement of clinical symptoms. It is likely that modulation of the T cell response determines in part the following pathological process or prognosis (Colley *et al.*, 1986; Tweardy *et al.*, 1987).

There is no direct evidence that T cells specific for SEA have similar pathological roles for developing hepatosplenic lesions in human schistosomiasis japonica. However, it was possible to demonstrate correlates of delayed hypersensitivity of human SEA-specific T cells such as IL-2 production or *in vitro* granulomatous hypersensitivity (Ohta *et al.*, 1988). In human subjects with previous *S. japonicum* infection, we observed SEA-induced vigorous proliferation of T cells *in vitro* (Ohta *et al.*, 1982, 1988). This might have suggested that there was no modulation of the T cell response to SEA, even in human chronic infection. However, because SEA is a crude antigen containing a lot of epitopes recognized by T and/or B cells (Auriault *et al.*, 1988; Harn *et al.*, 1989), we tested the T cell response to its molecular weight fractions. Although only a few individuals were tested, we observed that almost all patients failed to respond to fractions of >18 kD, while vigorous responses to other fractions were still observed. It could be that immunomodulation was functioning only against T cells reactive to a particular epitope(s) of SEA. We observed a fraction I-driven PBL response. However, in this case cellular characterization of those responding cells was not clarified. We tested only one B cell marker, CD19, and the cells were negative for this marker. Together with the observation that the blast cells were clearly positive for HLA-DR, we are not able to exclude a possibility of those cells being B cell lineage before testing other B cell markers.

Although we had thought that T cells of the chronic patients did not respond to fraction I, a patient tested had responsive T cells and sufficient APC functions because we were able to obtain a fraction I-reactive, HLA-DR-restricted Th cell line in the patient. The T cell response seemed to be suppressed in such chronic patients, and the suppression was relieved by adding a MoAb to HLA-DQ. It was likely that a gene product of HLA-DQ may have been involved in the suppression mechanism. In our previous study, immune suppression genes were mapped within the HLA-DQ region in humans, and antigen-specific CD8$^+$ suppressor T cells (Ts) were controlled by an HLA-DQ-linked immune suppression gene (Hirayama *et al.*, 1987). Although we did not get direct evidence for fraction I-specific Ts in this study, treatment of PBL with anti-CD8 MoAb and complement restored CD4$^+$ T cell response, and one of the most probable explanations for our present observation was the induction of fraction I-specific Ts in chronic patients. This means that T cells reactive to only limited epitopes were modulated. There was no effect of adding HU-11 MoAb on the response of an already established T cell line. The established T cell line was composed of homologous Th cells, and Ts that were controlled by HLA-DQ were already eliminated from the cell population.

In our previous study, we demonstrated the Ts system by adding or depleting putative suppressive T cells in the PBL culture (Ohta *et al.*, 1983). However, the Ts had no detectable effect on proliferation of the responsive T cell line (Hirayama *et al.*, 1987). Addition of purified autologous resting T cells to the T cell lines also did not inhibit their proliferation to fraction I during a 72-h culture period (Ohta, unpublished data). Our failure to detect Ts functions may be explained by the too short-term incubation, or by a possibility that activated Th cells were no longer under the control of the HLA-DQ-mediated suppression system. The T suppressor system is highly complicated consisting of a series of cellular interactions (Dorf & Benacerraf, 1984). Considering that HU-11 MoAb had an effect only on resting T cell response, it is possible to assume that the present

Table 2. No effect of anti-HLA-DQ monoclonal antibody on established HLA-DR-restricted helper T cell line

T cell line	MoAb added	Medium	SEA-C	SEA-Fraction I	SEA-Fraction II
KY anti-SEA-C	None	502±28	17707±1837	1682±819	15770±1272
KY anti-SEA-C	Anti-DR (×5)	773±171	2730±458	NT	NT
KY anti-SEA-C	Anti-DQ (×5)	560±205	17054±1274	2152±191	12946±861

Data are mean ct/min ± s.d.
MoAb, monoclonal antibody; SEA, soluble egg antigen; NT, not tested.

Table 3. Induction of HLA-DR-restricted, fraction-I-reactive T cells in patient KY

T cell source	Stimulation of	HU-4 added	Medium	Sj AWA	SEA-C	SEA-fraction I
KY PBL	SEA-C alone	−	1295±683	1072±612	31135±546	6037±742
KY PBL	SEA-C+HU-11	−	346±167	1272±573	33615±4776	19858±2050
KY PBL	SEA fraction I+HU-11	−	2302±291	4280±1143	58028±2684	62199±3385
KY PBL	SEA fraction I+HU-11	+	1773±485	NT**	5137±998	6281±2030

HU-4, anti-HLA-DR monomorphic; HU-11, anti-HLA-DQwl/w4.
Data are mean ct/min ± s.d.
AWA, adult worm antigen; SEA, soluble egg antigen; PBL, peripheral blood lymphocytes; NT, not tested.

putative Ts system might be directed to the induction phase of helper T cells. More detailed study is under way to analyse cellular and molecular basis of the suppression system.

It is not known which T cell epitope(s) of SEA mainly induce pathological lesions in human *S. japonicum* infection. Recent results have shown that a glycoprotein of 140 kD was responsible for egg-focused pulmonary granuloma formation in murine schistosomiasis japonica (Sidner, Carter & Colley, 1987). In our previous study we observed that epitopes on a relatively small molecular weight fraction (7–18 kD) seemed to induce pathological T cell response in humans (Ohta *et al.*, 1988). However, these results were obtained by testing only chronic patients. Considering murine experimental studies, it is probable that a pathologically important T cell response had been modulated in chronic patients. It is possible, therefore, that T cells reactive to a small molecular weight fraction, which persist even in the chronic phase, may be of less importance in immunopathogenesis. The present study suggests the existence of epitope-specific modulation of the T cell response in chronic patients. This means that only a particular T cell response is modulated. It might be possible that a pathological T cell response is induced mainly by epitopes expressed on fraction I. It will be interesting to investigate the T cell response in acute patients. Time-course kinetics of the T cell response to SEA fractions seem to give a clue for understanding the immunopathogenesis of hepatosplenic schistosomiasis japonica in humans.

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REFERENCES

AURIAULT, C., GRAS-MASSE, H., WOLOWCZUK, I., PIERCE, R.J., BALLOUL, J., NEYRINCK, J., DROBECQ, H., TARTAR, A. & CAPRON, A. (1988) Analysis of T and B cell epitopes of the *Schistosoma mansoni* P28 antigen in the rat model by using synthetic peptides. *J. Immunol.* **141,** 1687.

BOROS, D.L., PELLEY, R.P. & WARREN, K.S. (1975) Spontaneous modulation of granulomatous hypersensitivity in schistosomiasis mansoni. *J. Immunol.* **114,** 1437.

BYRAM, J.E., DOENHOFF, M.J., MUSALLAM, R., BRINK, L.H. & VON LICHTENBERG, F. (1979) *Schistosoma mansoni* infections in T-cell deprived mice, and the ameliorating effect of administering homologous chronic infection serum. II. Pathology. *Am. J. trop. Med. Hyg.* **28,** 274.

CHEEVER, A.W., BYRAM, J.E. & VON LICHTENBERG, F. (1985) Immunopathology of *Schistosoma japonicum* infection in athymic mice. *Parasite Immunol.* **7,** 387.

COLLEY, D.G. (1981) T lymphocytes that contribute to the immunoregulation of granuloma formation in chronic murine schistosomiasis. *J. Immunol.* **126,** 1465.

COLLEY, D.G., GARCIA, A.A., LAMBERTUCCI, J.R., PARRA, J.C., KATZ, N., ROCHA, R.S. & GAZZINELLI, G. (1986) Immune responses during human schistosomiasis mansoni. XII. Differential responsiveness in patients with hepatosplenic disease. *Am. J. trop. Med. Hyg.* **35,** 793.

DORF, M.E. & BENACERRAF, B. (1984) Suppressor cells and immunoregulation. *Annu. Rev. Immunol.* **2,** 127.

HARN, D.A., DANKO, K., QUINN, J.J. & STADECKER, M.J. (1989) *Schistosoma mansoni*: the host immune response to egg antigens. I. Partial characterization of cellular and humoral responses to pI fractions of soluble egg antigens. *J. Immunol.* **142,** 2061.

HIRAYAMA, K., MATSUSHITA, S., KIKUCHI, I., IUCHI, M., OHTA, N. & SASAZUKI, T. (1987) HLA-DQ is epistatic to HLA-DR in controlling the immune response to schistosomal antigen in humans. *Nature*, **327,** 426.

ISHII, A. & OWHASHI, M. (1982) *Schistosoma japonicum*: immediate hypersensitivity reactions to egg antigen in humans and rodents. *Exp. Parasitol.* **54,** 104.

KASAHARA, M., TAKENOUCHI, T., IKEDA, H., OGASAWARA, K., OKUYAMA, T., ISHIKAWA, N., WAKISAKA, A., KIKUCHI, Y. & AIZAWA, M. (1983) Serologic detection of HLA-D specificities by the use of monoclonal antibodies. II. Distinction between HLA-Dw2 and HLA-Dw12. *Immunogenetics*, **18,** 525.

KOIDE, Y., AWASHIMA, F., YOSHIDA, T.O., TAKENOUCHI, T., WAKISAKA, A., MORIUCHI, J. & AIZAWA, M. (1982) The role of three distinct Ia-like antigen molecules in human T cell proliferative response: effect of monoclonal Ia-like antibodies. *J. Immunol.* **129,** 1061.

LOWRY, O.H., ROSENBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193,** 265.

OHTA, N., MINAI, M. & SASAZUKI, T. (1983) Antigen specific suppressor T lymphocytes (Leu-2a$^+$3a$^-$) in human schistosomiasis japonica. *J. Immunol.* **131,** 2524.

OHTA, N., EDAHIRO, T., TOHGI, N., ISHII, A., MINAI, M. & HOSAKA, Y. (1988) Generation and functional characterization of T cell lines and clones specific for *Schistosoma japonicum* egg antigen in humans. *J. Immunol.* **141,** 2445.

OHTA, N., NISHIMURA, Y.K., IUCHI, M. & SASAZUKI, T. (1982) Immunogenetic analysis of post-schistosomal liver cirrhosis in man. *Clin. exp. Immunol.* **49,** 493.

OLDS, G.R., OLVEDA, R.D., TRACY, J.W. & MAHMOUD, A.A.F. (1982) Adoptive transfer of modulation of granuloma formation and hepatosplenic disease in murine schistosomiasis japonica by serum from chronically infected animals. *J. Immunol.* **128,** 1391.

RAGHEB, S., MATHEW, R.C. & BOROS, D.L. (1987) Establishment and characterization of an antigen-specific T-cell line from liver granulomas of *Schistosoma mansoni*-infected mice. *Infect. Immun.* **55,** 2625.

SASAZUKI, T., OHTA, N., KANEOKA, R. & KOJIMA, S. (1980) Association between an HLA haplotype and low responsiveness to schistosomal worm antigen in man. *J. exp. Med.* **152,** 314s.

SIDNER, R.A., CARTER, C.E. & COLLEY, D.G. (1987) Modulation of *Schistosoma japonicum* pulmonary egg granulomas with monoclonal antibodies. *Am. J. trop. Med. Hyg.* **36,** 361.

TWEARDY, D.J., OSMAN, G.S., EL KHOLY, A. & ELLNER, J.J. (1987) Failure of immunosuppressive mechanisms in human *Schistosoma mansoni* infection with hepatosplenomegaly. *J. clin. Microbiol.* **25,** 768.

YASUKAWA, M., IWAMASA, K., KAWAMURA, S., MURAKAMI, S., TAKADA, K., HATO, T., SHIOSAKA, T., TAMAI, T., FUKUOKA, T., FUJITA, S. & KOBAYASHI, Y. (1987) Phenotypic and genotypic analysis of chronic myelogenous leukemia with T lymphoblastic and megakaryoblastic mixed crisis. *Br. J. Haematol.* **66,** 331.