Schistosoma japonicum egg antigen-specific T cell lines in man. Induction of helper and suppressor T cell lines and clones in vitro in a patient with chronic schistosomiasis japonica.

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Research Article

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Schistosoma japonicum Egg Antigen-specific T Cell Lines in Man

Induction of Helper and Suppressor T Cell Lines and Clones In Vitro in a Patient with Chronic Schistosomiasis Japonica

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Abstract

T cell lines (TCLs) specific for *Schistosoma japonicum* egg antigen were established from a patient with chronic schistosomiasis japonica to investigate the regulatory mechanism of *S. japonicum* egg antigen-driven T cell responses in man. All five TCLs tested were $CD2^+$,$CD4^+$,$CD8^-$, and were strongly proliferative only to *S. japonicum* egg antigen in the absence of exogenous IL-2. All but one TCL produced IL-2–like lymphokines in vitro, indicating their helper T cell functions. One TCL, SjE-3, failed to produce IL-2–like lymphokines. Moreover, this TCL suppressed the specific proliferation of autologous peripheral blood lymphocytes to *S. japonicum* egg antigen. This TCL produced a soluble suppressor factor(s). These functional diversities among established TCLs were also confirmed by cloned T cells. Our observations might suggest that the regulatory system through helper and suppressor T-T interactions somehow involved in T cell responses to the egg antigen in human chronic schistosomiasis japonica.

Introduction

It is well known that immune responsiveness to schistosomal antigens is modulated through various immune suppression mechanisms in chronically infected mice in a manner of antigen specific as well as nonspecific (1–6). These phenomena also have been observed in human hosts (7–13). In our previous studies we demonstrated that Leu-$2a^+3a^-$ suppressor T cells are involved in immune modulation specific for *Schistosoma japonicum* adult worm antigen in patients with chronic schistosomiasis japonica (14, 15). However, most clinical attention has been focused on the regulatory mechanisms of schistosome egg-specific T cell responses in chronically infected hosts because those responses are critically involved in the formation of intrahepatic granulomas in *Schistosoma mansoni* (16–18) as well as in *S. japonicum* infections (19, 20). This egg granuloma formation is closely related to the development of typical hepatosplenic lesions in schistosomiasis, and the modulation of granulomatous responses seems to directly reflect the clinical fluctuation in a chronic infection phase.

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Immunoregulation of T cell responses has been thoroughly investigated in murine *S. mansoni* infection. Analysis in *S. japonicum* infection is still not enough due to the difficulties of assay systems for their T cell responses to this parasite (21, 22). In spite of the phenomenological similarities in modulation of granulomatous responses in these two schistosome infections, the current concept indicates that the regulatory mechanisms are apparently different between these two schistosome infections in mice: in *S. mansoni* infection, specific suppressor T cells but not serum factors are dominantly involved (23–25), whereas suppressor serum factors but not suppressor T cells are essentially involved in *S. japonicum* infection (26, 27). These unique profiles of immune regulation systems for *S. mansoni* and *S. japonicum* in mice raised many questions about the situations in human hosts.

In the present study we intended to analyze the induction and regulation of T cell responses to *S. japonicum* egg antigen in humans by using T cell lines (TCLs)[1] and clones specific for *S. japonicum* egg antigen in vitro. We obtained specific helper T cells as well as suppressor T cells directed at *S. japonicum* egg antigen in vitro in a patient with chronic schistosomiasis japonica, and we discuss the regulatory system for T cell responses to this antigen in humans, comparing the data obtained from studies in mice.

Methods

Patient. A 45-yr-old male patient with chronic schistosomiasis japonica was studied. This patient was born and raised in Nirasaki City, Yamanashi Prefecture, Japan, which had been an endemic area of schistosomiasis japonica in Japan. His previous infection with *S. japonicum* was clearly documented by rectal biopsy specimen. This patient had no past history of the treatment for schistosomiasis till our present study, and no particular clinical symptom was observed except for slight hyperbilirubinemia. This patient has strong cellular responses to *S. japonicum* egg, adult worm antigens, and tuberclin purified protein derivative (PPD) in vitro.

Lymphocyte preparation. PBL were prepared by Ficoll-Conray gradient solution method (specific gravity, 1.077) (11).

Antigen-driven proliferative response of PBL in vitro. PBL were suspended in RPMI 1640 (Irvine Scientific, Santa Ana, CA) supplemented with 20% heat-inactivated human sera, 100 μg/ml streptomycin, 100 U/ml penicillin, and 20 mM L-glutamine, and were cultured with antigens in 96-well flat-bottomed microtiter plates (Nunc, Copenhagen, Denmark). In our previous studies, the optimal culture condition for PBL responses to these antigens was determined (11, 28). In brief, 1×10^5 of PBL were cultured for 7 d. Antigen concentrations were 5 μg/ml for *S. japonicum* egg and adult worm antigens, and 2 μg/ml for PPD. For the last 16 h 1 μCi of [^{3}H]thymidine was pulsed,

1. *Abbreviations used in this paper:* APC, antigen presenting cell; PPD, pure protein derivative; TCL, T cell line.

and the incorporated [^{3}H]thymidine was assessed by liquid scintillation spectrometry.

Generation of TCLs in vitro. PBL were suspended in RPMI 1640 conditioned medium in 6-well plates (Nunc), and were incubated with 5 μg/ml of *S. japonicum* egg antigen for 7 d in 5% CO_2/95% humid air at 37°C. Activated blast cells were harvested and suspended in RPMI 1640 conditioned medium together with autologous irradiated PBL (3,000 rad) as feeder cells, 1 μg/ml of the egg antigen, and 10% of MLA-144 culture supernatants as exogenous IL-2 (29) in 24-well plates. The culture medium was exchanged every 2 d and the feeder cells were added to the culture every 2 wk. All five established cell lines were shown to be T cells by observation of their surface phenotypes of $CD2^+$.

Antigen-specific proliferation of TCLs. At least 14 d after the last addition of the feeder cells, those TCLs were incubated overnight in IL-2-free medium to wash away the remaining exogenous IL-2, and then 1×10^4 of TCLs and 5×10^4 irradiated autologous PBL (3,000 rad) as antigen presenting cells (APC) were mixed, and were cultured with antigens in 96-well flat-bottomed microtiter plates for 72 h. For the last 8 h of the incubation, [^{3}H]thymidine was pulsed. Specificities of these TCLs were determined by their positive proliferation to particular antigen(s) in the absence of exogenous IL-2.

Assay of IL-2 production by TCLs in vitro. In vitro production of IL-2-like lymphokines was evaluated by the method described by Wee et al. (30). In brief, 1×10^5 of TCLs were cultured with IL-2-free medium overnight, and then mixed with APC and 5 μg/ml of *S. japonicum* egg antigen in 24-well plates for 24 h. The culture supernatants were collected and added to murine IL-2-dependent CTLL-2 cells (31) at various concentrations (1:2 to 1:32 dilution) for 24 h in 96-well round-bottomed plates. For the last 8 h of the second 24-h-incubation, 1 μCi of [^{3}H]thymidine was pulsed, and the incorporated [^{3}H]thymidine into CTLL-2 cells was assessed.

Inhibition of autologous PBL proliferation to S. japonicum egg antigen by TCLs. 1×10^5 of PBL were suspended in RPMI 1640 conditioned medium containing *S. japonicum* egg antigen or PPD in 96-well flat-bottomed plates. TCLs were then added to PBL at various cell ratios, and were co-cultured for 7 d in CO_2 incubator. For the last 16 h, 1 μCi of [^{3}H]thymidine was pulsed and the incorporated [^{3}H]-thymidine was compared between the presence and absence of added TCLs. Effects of added TCLs were analyzed by percent suppression calculated as follows: % suppression [1 − (PBL response in the presence of added TCLs)/(PBL response in the absence of added TCLs)] × 100.

Suppressor factors produced by a TCL in vitro. 1×10^5 of TCLs were suspended in RPMI 1640 conditioned medium and 20 μg/ml of *S. japonicum* egg antigen was added in the absence of APC. After 24 h incubation in 24-well plates, the supernatants were collected, and were passed through filter membrane of diameter = 0.45 μm. The supernatants were immediately added to PBL at a final concentration of 50%. Effects by adding the supernatants on antigen-driven PBL responses were analyzed by the method described above except for the raised egg antigen dose (10 μg/ml). As controls, culture supernatants of the egg antigen alone as well as TCL culture alone were tested. Data analyses were done by percent suppression calculated as follows: % suppression = [1 − (PBL response of factor-added culture)/(PBL response of factor-free culture)] × 100.

S. japonicum egg antigen-specific T cell clones. After 10 d culture in RPMI 1640 conditioned medium containing IL-2 and APC, T cell blasts were cloned by single cell deposition by the micromanipulation method described elsewhere (32). Cells were sorted into individual wells of 96-well round-bottomed microtiter plates, and were cultured in RPMI 1640 conditioned medium with 1 μg/ml of the egg antigen and 10% MLA-144 culture supernatants. Irradiated autologous PBL were also added as APC every 2 wk. Of 260 single cell depositions, nine clones were obtained.

Antigens. Three antigens were used in this study: PPD (Nihon BCG Seizo Co., Tokyo, Japan), *S. japonicum* egg, and adult worm antigens, as described elsewhere (11, 28, 33). All the schistosome antigens were crude ones, and for the present study, a single lot of each antigen was used throughout the experiments.

Results

PBL responses to schistosomal antigens in vitro. The patient was tested for in vitro PBL proliferation to three antigens. This patient showed vigorous PBL responses to both *S. japonicum* egg and adult worm antigens, whereas a negative control PBL did not respond at all to these schistosomal antigens. Both the patient and a negative control subject showed strong proliferation to PPD, the positive control antigen (Table I).

Generation of S. japonicum egg antigen-specific TCLs in vitro. We established five TCLs (SjE-1 to SjE-5) from different series of experiments, all of which were $CD4^+CD8^-$ T cells by fluorescence staining technique (Table II). These TCLs were not proliferative to any antigen stimulation in the absence of exogenous APC. However, by adding APC strong proliferation was observed only against *S. japonicum* egg antigen without exogenous IL-2 (Table II). A kinetic study showed that the peak response was observed at 72–96 h incubation (data not shown).

In vitro production of IL-2-like lymphokines by TCLs. The established TCLs were tested for their in vitro production of IL-2 by the stimulation of *S. japonicum* egg antigen. All but one TCLs produced IL-2-like lymphokines in the culture supernatants. One TCL, SjE-3, failed to produce the lymphokines in our assay system. A typical result by SjE-2 and SjE-3 cells was shown in Fig. 1. The ability of IL-2-like lymphokines production suggested that all these TCLs but SjE-3 were helper ones.

Specific inhibition of S. japonicum egg antigen-driven proliferation of PBL by adding SjE-3 TCL. To analyze the functional profile of SjE-3 TCL, we tested the effect of the TCL on antigen-driven PBL responses in vitro. SjE-3 TCL as well as SjE-2 helper TCL were added to 1×10^5 of PBL at the cell ratio of 1:20 to 1:200. After 7 d culture, responses of TCLs themselves could no longer be detected. By adding SjE-2 TCL, we did not observe any effect on antigen-driven PBL proliferation at any TCL/PBL ratio tested. On the other hand, SjE-3 TCL

Table I. Antigen-driven PBL Proliferation of S. japonicum-infected or -noninfected Individuals

Donor	Age	Sex	*S. japonicum* infection	Antigen(−)	PPD (2 μg/ml)	Sj egg (5 μg/ml)	Sj adult worm (5 μg/ml)
						cpm	
K.Y.	44	M	+	256±11	87,489±3,429	248,763±16,084	76,663±2,925
N.O.	35	M	−	429±104	201,649±17,528	349±17	915±7

Table II. S. japonicum Egg Antigen-specific TCLs Derived from a Patient with Chronic Schistosomiasis Japonica

Cell line	APC	Proliferative responsiveness to: Antigen(−)	Sj egg (5 μg/ml)	Sj adult worm (5 μg/ml)	PPD (2 μg/ml)	Phenotypes CD2+	CD4+	CD8+
							%	
SjE-1	−	1.3±0.1*	1.8±0.1	NT‡	NT	97.9	90.4	5.3
	+	1.0±0.1	36.3±1.5	1.3±0.2	1.5±0.1			
SjE-2	−	0.2±0.1	0.2±0.1	NT	NT	100.0	92.2	2.7
	+	0.2±0.1	42.3±1.7	1.5±0.2	1.0±0.1			
SjE-3	−	0.2±0.1	0.3±0.1	NT	NT	95.8	88.9	3.5
	+	0.6±0.1	6.2±0.7	0.7±0.1	0.3±0.1			
SjE-4	−	0.4±0.1	0.4±0.1	NT	NT	87.2	88.0	7.2
	+	1.1±0.2	26.3±1.0	1.4±0.3	1.1±0.2			
SjE-5	−	0.4±0.1	0.7±0.1	NT	NT	92.2	84.3	4.0
	+	0.8±0.1	41.1±1.8	0.5±0.1	1.5±0.2			
−	+	0.2±0.1	0.3±0.1	0.2±0.1	0.2±0.1			

* cpm±SE × 10^{-3}. ‡ NT, Not tested.

showed significant suppressive effects on *S. japonicum* egg antigen-driven PBL proliferation in a dose-dependent manner. SjE-3 TCL had no effect on PPD-driven PBL response even at a TCL/PBL ratio of 1:20 (Table III). This suggests that SjE-3 is an *S. japonicum* egg antigen-specific suppressor TCL. The specific suppression by this TCL became more apparent when stimulating antigen doses were elevated (60.3% suppression for 5 μg/ml antigen vs. 95.2% suppression for 80 μg/ml antigen) (Fig. 2). By irradiation of SjE-3 cells, the suppressive effect was diminished (Table III). When SjE-3 TCL were co-cultured with SjE-2 cells, additive incorporation of [^{3}H]thymidine was observed, suggesting no direct suppression of SjE-3 on SjE-2 TCL response.

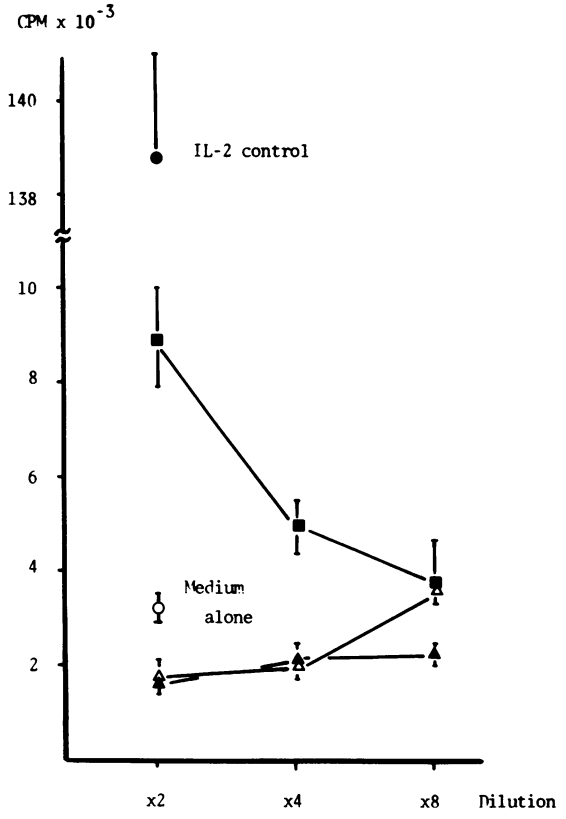

Figure 1. In vitro production of IL-2-like lymphokines by TCL. (■) Culture supernatant of SjE-2 TCL with *S. japonicum* egg antigen. (▲) Culture supernatant of SjE-3 TCL with the egg antigen. Only the supernatant from SjE-2 culture induced proliferation of CTLL-2 cells, indicating IL-2-like activity. (△) Negative control to exclude the possibility that the observed IL-2-like activity is APC origin.

Suppressor factor production by SjE-3 TCL. To test the possibility of suppressor factor production by SjE-3 TCL, these cells were preincubated with or without the antigen, and the culture supernatants were added to proliferative responses of autologous PBL. After the preincubation, no apparent effect was observed for the stimulatory activity of the egg antigen against PBL (76,871 cpm by fresh antigen vs. 61,543 cpm by preincubated antigen, or 75,131 cpm by fresh vs. 92,202 cpm by preincubated) (Table IV). The supernatant from preincubation of SjE-3 with the egg antigen clearly suppressed the egg antigen-driven PBL proliferation (98.7% suppression). In case of SjE-2 helper TCL, no inhibitory effect was observed in the supernatant (1.2% suppression). When the supernatant from SjE-3 culture alone was tested, the suppression was less effective (Table IV).

S. japonicum egg antigen-specific helper and suppressor T cell clones. Since these TCLs were still polyclonal, we intended to confirm these functional diversities in TCLs at the clonal level. We obtained only nine clones from 260 single cell depositions (cloning efficiency, 0.035); of these nine clones, seven were from SjE-2 helper TCL and two from SjE-3 suppressor TCL. Two out of seven clones from SjE-2 showed strong proliferation to *S. japonicum* egg antigen, and one of the two clones was confirmed to produce IL-2-like lymphokines (Table V). On the other hand, one of the two clones from SjE-3 showed significant proliferation to the egg antigen, and also had a suppressive effect on *S. japonicum* egg antigen-driven PBL responses, but not on PPD-driven responses (Table VI). This suppressive clone, No. 7/8/1, was also $CD4^+8^-$ (90.3 and 6.6% by fluorescence staining, respectively). Thus, distinct T cell functions observed in TCLs were confirmed at the cloned T cells.

Table III. Specific Suppression of SjE-3 TCL against S. japonicum Egg Antigen-driven PBL Proliferation

Responder	Experiment	TCL added	Antigen(−)	Sj egg (5 μg/ml)	PPD (2 μg/ml)
				cpm	
PBL (1×10^5)	1	—	447±142	82,641±228	43,588±5,333
		SjE-2 (1:20)	1,619±235	85,614±2,688 (−3.6)‡	52,573±4,664
		SjE-3 (1:20)	1,649±651	33,247±3,499 (59.8)	54,068±2,369
		(1:200)	421±82	81,717±4,121 (1.1)	38,597±4,072
		SjE-3^{R}* (1:20)	915±337	60,270±1,407 (27.1)	50,496±5,022
PBL (1×10^5)	2	—	256±11	52,609±609	14,749±655
		SjE-2 (1:20)	635±178	59,192±1,483 (−12.5)	17,100±3,294
		SjE-3 (1:20)	2,700±734	27,480±3,914 (47.8)	33,634±6,929
		SjE-3^{R} (1:20)	1,566±289	35,262±2,333 (33.0)	NT§
SjE-2 (1×10^4)		—	151±18	4,366±133	NT
+APC		SjE-3 (1:20)	185±8	7,251±71 (−67.2)	NT

PBL proliferation was assayed by 7-d culture, and SjE-2 proliferation by 3-d culture. * SjE-3^{R}, Irradiated SjE-3 T cell line (3,000 rad). ‡ Percent suppression. § NT, Not tested.

Discussion

In murine experimental schistosome infections, modulation of the granulomatous responses during chronic infection results in substantial clinical improvement in the hepatic lesions. In *S. mansoni* infection, it has been shown that the granulomatous responses are induced by Ly1$^+$, Ia$^-$, Qa-1$^+$ T cells. Ly1$^+$, Ia$^+$, Qa-1$^+$, and Ly1$^-$2$^+$ I-J$^+$ T cells were then involved in the regulation through T-T interactions (23–25). On the other hand, granulomatous responses to eggs of *S. japonicum* have not been well characterized. Recent studies by Cheever and colleagues demonstrated that T cells were also critically involved in the granuloma formation in schistosomiasis japonica in mice (19, 20). However, detailed analysis of the regulatory mechanisms of the T cell responses still remained unclear. In human *S. mansoni* infection, T cell responses involved in the granuloma formation have been studied by T cell blastogenic responses or by egg granuloma formation experiments in vitro (34, 35). Although those studies have revealed the involvement of CD4$^+$ as well as CD8$^+$ T cells in granulomatous responses around eggs, the regulatory system for the T cell responses has not yet been addressed.

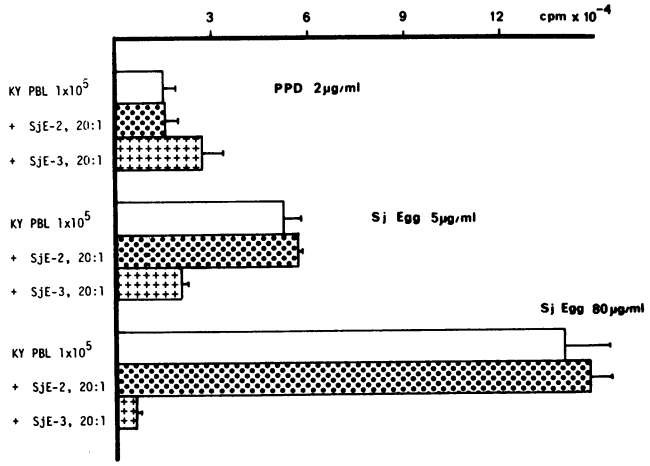

Figure 2. Effects on antigen-driven proliferation of PBL by adding SjE-2 helper TCL or SjE-3 suppressor TCL in vitro. Although no effect was observed for PPD-driven PBL proliferation, *S. japonicum* egg antigen-driven proliferation was specifically suppressed by adding SjE-3, but not by SjE-2 cells. Moreover, the suppression became more apparent when the stimulating antigen dose was elevated.

In our present study, we obtained TCL and clones specific for *S. japonicum* egg antigen. In our TCLs established, we observed at least two functionally distinct T cell subsets, both of which are CD2$^+$,CD4$^+$,CD8$^-$. Helper TCLs were strongly proliferative to the egg antigen in the absence of exogenous IL-2, and produced IL-2–like lymphokines by stimulation of the specific antigen. On the other hand, SjE-3, a CD4$^+$ TCL, showed less vigorous proliferation compared with helper TCLs, and this TCL did not produce IL-2–like lymphokines. Since IL-2 production is not a sufficient criterion for helper T cells (36), we could not exclude other possible helper functions in this TCL. However, this TCL clearly suppressed the *S. japonicum* egg antigen-driven proliferation of autologous PBL. The suppression was antigen specific, and PPD-driven proliferation was never affected. Because added TCLs were proliferative during the 7-d culture, other factors such as antigen consumption or altered culture condition should be considered. However, SjE-2 TCL did not affect the PBL responses, or suppression became apparent when the antigen dose was elevated. These results suggest that the suppression was a specific function of SjE-3 TCL. Moreover, this TCL seemed to produce a soluble suppressor factor(s) by the stimulation of the egg antigen, and it was indicated that SjE-3 TCL was a suppressor T cell lineage. Together with the phenotype of the TCL being CD4$^+$, it is possible that SjE-3 was a suppressor inducer TCL. Suppressor inducer itself is not a direct effector in immune suppression, but it initiates the series of suppressor T cell cascade (37, 38). Our inference might be supported by the results that SjE-3 TCL was effective only when these cells were added to PBL, and that it was not directly suppressive against CD4$^+$ helper TCLs.

It is possible that SjE-3 TCL might produce a factor(s) as a mode of suppression. Suppressor factors have been reported in *S. mansoni* infections, some of which are products of T sup-

Table IV. Induction of a Soluble Suppressor Factor by an Antigen-specific Suppressor TCL, but not by a Helper TCL

Responder	Factor added	Medium	Sj egg 10μg/ml
		cpm	*cpm*
PBL (1×10^5 cells)	—	220±85	75,131±2,680
	Sup* (Sj egg antigen alone)	92,202±3,153	NT§
	Sup (SjE-2 + Sj egg antigen)	74,262±7,144 (1.2)‡	NT
PBL (1×10^5 cells)	—	349±17	76,871±904
	Sup (Sj egg antigen alone)	61,543±5,956	NT
	Sup (SjE-3 + Sj egg antigen)	1,010±94 (98.7)‡	NT
PBL (1×10^5 cells)	—	287±74	247,660±10,395
	Sup (SjE-3 + Sj egg antigen)	38,423±3,354 (84.5)‡	NT
	Sup (SjE-3 alone)	2,644±658	114,643±5,958 (53.7)‡

* Sup, Supernatants used were from preculture for 24 h in 5% CO_2, 37°C, humid air, and were tested at the final concentration of 50%. ‡ Percent suppression. § NT, Not tested.

pressor cells. Chensue and colleagues reported an I-C^+ suppressor T cell factor in mice (6), but it seems not to be comparable with the factor(s) in our study because that factor is produced by $Ly1^-2^+3^+$ cells, or is directed against $Ly1^+$ T cells. It is not clear whether the factor in our study is HLA restricted. To date there is no report on factors in schistosomiasis japonica that seem to be equivalent or related to our present observation. Future attempts planned at uncovering the physicochemical basis of the factor will be important.

It is likely that a T suppressor inducer needs APC for functional activation; however, the requirement of APC for suppressor T cells is still controversial (39, 40). The factor(s) seemed to be produced by activated SjE-3 TCL because it was observed only when both SjE-3 and the egg antigen were cocultured, or because irradiation diminished the effect of the TCL. Our results thus suggested that functional activation of SjE-3 was independent of exogenous APC. This interpretation raised the question of how SjE-3 was activated even in the absence of APC. There is accumulation of a lot of data showing diversities in pathways for activating specific T cells. Moldwin and colleagues demonstrated that direct stimulation of T cell receptors with high doses of stimulating antigens induce IL-2–independent activation in some T cell clones (41). Although it is the current concept that T cells recognize the processed antigen in the context of the self major histocompatibility determinant, Olds and Kresina suggested the possibility that *S. japonicum* egg–specific T cells might have the antigen receptors that are somehow homologous to those of B cells (27). If it were the case, it might have been possible to stimulate the egg-specific T cell receptors by high doses of intact egg antigen under certain conditions. One of the speculations we would make is that SjE-3 TCL might have a unique activating pathway which does not necessarily require exogenous APC. And the dose of the antigen might be important since SjE-3 was APC dependent in proliferative response at a low antigen dose (5 μg/ml). The detailed analyses of the mechanisms of the antigen-driven activation of SjE-3 TCL are now under investigation.

Table V. S. japonicum Egg Antigen-induced IL-2–like Lymphokines Production by the Helper T Cell Clone No. 1/6 In Vitro

Factor	CTLL proliferation by addition of factors of:		
	× 2 dilution	× 4 dilution	× 8 dilution
IL-2–free medium	0.3±0.1*	NT‡	NT
IL-2 control (2 U/ml)	37.0±3.0	27.1±1.4	13.4±1.2
No. 1/6 alone culture	0.6±0.1	0.5±0.1	0.7±0.2
No. 1/6 + Sj egg antigen culture	3.4±0.3	2.3±0.2	1.1±0.1

Clone No. 1/6 was derived from SjE-2 helper TCL.
* cpm±SE × 10^{-3}.
‡ NT, Not tested.

In murine *S. mansoni* infection, the regulation of granulomatous response is modulated through interactions between functionally distinct T cell subsets. Doughty et al. clearly showed that the modulation mechanism includes two distinct T cell subsets; of these, $Ly1^-2^+$ T cells act directly, and $Ly1^+2^-$,Qa-1^+ T cells induce feedback suppression (42). In our study, it was suggested that both T helper and suppressor cells are clonally induced in human infection of *S. japonicum,* and that the T-T interaction between these T cell subsets might be somehow functioning in the regulatory mechanism as seen in *S. mansoni* infection. Note that the patient studied was a high responder to the egg antigen at PBL level (Table I). Our previous study on human T cell responses to *S. japonicum* adult worm antigen demonstrated that the $CD8^+$ suppressor T cells controlled low responsiveness at PBL level (14). We observed diversities in responsiveness to *S. japonicum* egg antigen in human population (28). It is thus interesting to know how these high and low responses to the egg antigen are determined. In the patient tested in this study, suppressor T cell functions are somehow blocked at PBL level although T cells of suppressor lineage are clonally present. This might be a unique profile of anti-*S. japonicum* egg T cell responses in humans. Alternatively, it is possible that these functionally distinct $CD4^+$ T cells might be generally observed in highly sensitized individuals such as the present case, and that these T cells might be less important for the immunomodulation in schistosomiasis japonica. Analysis of the biological significance of SjE-3 TCL in the immunoregulation is still underway.

Table VI. Specific Suppression of S. japonicum Egg Antigen-driven PBL Proliferation by the Suppressor Clone No. 7/8/1 In Vitro

Responder	Experiment	Clone added (No.)	Antigen(−)	Sj egg (5 μg/ml)	PPD (2 μg/ml)
PBL (1×10^5)	1	—	0.6±0.1*	154.9±18.7	69.8±2.6
		7/8/1 (1:20)	1.3±0.2	84.5±8.0 (45.8)‖	79.9±8.2
		(1:200)	1.3±0.1	138.1±8.6 (10.8)	NT‡
		7/8/1^{R}§ (1:20)	1.0±0.1	133.0±6.1 (14.2)	NT
PBL (1×10^5)	2	—	0.2±0.1	75.1±2.3	46.8±7.3
		7/8/1 (1:20)	5.0±1.5	35.0±1.3 (53.4)	40.8±2.4
		(1:200)	5.0±1.7	98.4±10.7 (−30.9)	NT
		7/8/1^{R} (1:20)	6.4±1.4	58.5±2.6 (22.2)	NT

Clone No. 7/8/1 was derived from SjE-3 TCL. * cpm±SE × 10^{-3}. ‡ NT, Not tested. § No. 7/8/1^{R}, Irradiated No. 7/8/1 T cell clone (3,000 rad). ‖ Percent suppression.

In murine schistosomiasis japonica, a suppressor serum factor is one of the most important regulatory mechanisms in anti-egg T cell responses. Olds and Kresina have clearly shown that an IgG_1 fraction of chronic infection serum was highly suppressive. This strong suppressive effect was mainly mediated by anti-egg antibody-idiotypic antibodies through direct interactions with $L3T4^+$,$Ly2^-$ T helper cells (27). In our preliminary experiments, the autologous serum of this patient did not affect the helper T cell proliferation, and even the IgG_1 fraction did not inhibit SjE-2 response to the egg antigen. Our egg antigen used is a crude one containing many antigenic determinants. The antigen was used at the low concentration (5 μg/ml), where only a few determinants could be stimulatory, but the determinant(s) recognized by mice might be different from that by humans. Alternatively, we also speculate that the T cell regulatory system of responses to *S. japonicum* eggs in humans is not necessarily analogous to that in mice.

Finally, the studies using antigen-specific TCL and clones could provide us new methods for studies of immunoparasitology and clinics in tropical diseases (43, 44). It might be possible to observe complexed T cell interactions in immunoregulation in vitro (45, 46), and provide a clue for analysis of molecular basis of immunoregulation in parasitic diseases. This methodology shall also enable us to begin to manipulate the critical cellular response in hopes of developing new therapeutic strategies.

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