Immunology 1985 **54** 255

Immunoregulation by mouse T-cell clones

II. THE SAME H-Y-SPECIFIC T HELPER CLONE CAN PROVIDE HELP FOR THE GENERATION OF CYTOTOXIC LYMPHOCYTES AND ANTIBODY-SECRETING CELLS

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Summary. Mouse H-Y-specific and I-A^b restricted T-cell clones have been established and compared for their helper effects in the differentiation of both T and B lymphocytes. The results demonstrate that three individual T-cell clones and one subclone could help in the antigen-driven induction of cytotoxic lymphocytes (CTL) from their precursor cells (CTL-P), and were able to activate B cells to develop into antibody-secreting cells (PFC) in the presence of SRBC, provided the cloned T cells were restimulated by H-Y antigen on antigen-presenting cells. In addition, antigen or lectin could induce the same H-Y-specific T-cell clones to secrete factor(s) expressing helper activities similar to that of the cloned T cells. Furthermore, it is shown that the T cell-derived soluble mediator(s) was distinct from T-cell growth factor (TCGF) and from immune interferon (IFN-γ). The data reveal a new type of T cell with helper potential for the activation of CTL-P and B lymphocytes, and suggest the existence of distinct T helper cells which can provide help for both cytotoxic and antibody responses by virtue of different lymphokine activities.

Abbreviations: APC, antigen-presenting cell; BCGF, B-cell growth factor; CDF, T lymphocyte differentiation factor; Con A, concanavalin A; Con A-SN, supernatant of Con A-stimulated rat spleen cells; CTL, cytotoxic T lymphocytes; CTL-P, precursor of cytotoxic T lymphocytes; EL-4-SN, supernatant of PMA stimulated EL-4 tumour cells; IFN-γ, immune interferon; IL-2, interleukin-2; MLC-SN, supernatant of mixed lymphocyte culture; PFC, antibody-secreting cell; PMA, phorbol myristate acetate; rec. hIL-2, recombinant human interleukin-2; rec. IFN-γ, recombinant mouse immune interferon; SRBC, sheep red blood cells; TCGF, T-cell growth factor sources, including interleukin-2; TH, T helper cell.

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INTRODUCTION

Cell interactions between functionally distinct lymphocyte subsets are a necessary requirement for the generation of immune responses by immunocompetent T and B cells. It has been shown that T helper cells (TH) are required for the activation of B cells to secrete immunoglobulin (PFC; Cantor & Boyse, 1977) and for the development of cytotoxic effector cells (CTL) from their precursors (Alter *et al.*, 1973; Eijsvoogel *et al.*, 1973; Cantor & Boyse, 1975). In the past, both antigen-specific (Taussig, Mozes & Isac, 1974) and non-specific (Howard & Paul, 1983) soluble T helper products have been described which are able to partially or completely replace T helper cells (TH) in humoral responses. Studies on the mode of action of

these lymphokines have revealed that several distinct factors regulate growth and differentiation of B lymphocytes (Möller, 1982). Similarly, T-cell growth factor (TCGF, Gillis & Smith, 1977) and T-cell differentiation factors (CDF; Raulet & Bevan, 1982) have been described which act independently of antigen and are involved in the induction of CTL from their precursor cells (CTL-P).

Until recently, investigations into the cellular origin of the distinct helper activities, as well as into the question whether single T cells can participate in both humoral and cellular immune responses, were hampered by the fact that TH and their soluble mediators were derived from heterogeneous T-cell populations. With the help of two newly developed techniques, cloning of T lymphocytes and the production of lymphokines by DNA technology, these tasks can now be approached more readily. Studies on cloned mouse T cells have revealed that individual T cells may express multiple functions (Dennert, Weiss & Warner, 1981; Bianchi *et al.*, 1981; Simon *et al.*, 1984), possibly by their ability to secrete a variety of lymphokines (Glasebrook *et al.*, 1982). Moreover, cloned mouse TH cells for B cells have been shown to release TCGF upon antigen activation (Schreier, 1980; Glasebrook *et al.*, 1980; Crispe, Gascoigne & Owens, 1984), thereby indicating possible mechanisms by which individual TH cells can act on both T and B lymphocytes. The demonstration that recombinant human interleukin-2 (rec. hIL-2) provides growth and differentiation signals for B cells (Tsudo, Uchiyama & Uchino, 1984; Emmrich, Moll & Simon, 1984) suggests, in addition, that precursors of both T and B lymphocytes can respond to the same lymphokine.

In light of these findings, we have used cloned I-A-restricted T-cell lines and their soluble products to investigate the participation of homogeneous TH cells in the development of effector cells from both T and B lymphocytes. We report that H-Y-specific and I-A^b restricted clones are able to provide help for the generation of CTL and PFC from their precursor cells. The helper activity appears to be transmitted to T and B lymphocytes via a soluble mediator(s) which is distinct from TCGF and immune interferon (IFN-γ).

MATERIALS AND METHODS

Mice

Adult mice of the strain C57BL/6 (B6), BALB/c and DBA/2 were obtained from the animal colony maintained at the Max-Planck-Institut für Immunbiologie, Freiburg, West Germany. The original breeding pairs were obtained from the Institut für Versuchstierforschung, Hannover, West Germany. Mice from both sexes were used between 6 weeks and 6 months of age.

In vivo *immunization*

Femal B6 mice were injected i.p. with 3×10^7 male B6 spleen cells. Three weeks to 4 months later, spleen cells from these mice were used for *in vitro* cultures.

Medium and cultures

RPMI 1640 medium (Gibco, Karlsruhe, West Germany) was supplemented with L-glutamine (2 mM), kanamycin (100 μg/ml), tylosine (10 μg/ml), HEPES buffer (25 mM), 2-mercaptoethanol (2×10^{-5} M) and 10% selected fetal calf serum.

Cultures for the detection of PFC were set up in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2×10^{-5} M 2-mercaptoethanol, kanamycin (100 μg/ml), tylosin (10 μg/ml), glutamine (2 mM) and 10% selected fetal calf serum, and were kept without feeding or rocking in a 7% CO_2 incubator at 37°.

Preparation of lymphokine sources

(i) The preparation of supernatant from Concanavalin A (Con A)-induced rat spleen cells has been described in detail previously (Eichmann *et al.*, 1980). Briefly, Con A supernatant (Con A-SN) was prepared by stimulating spleen cells from Spraque Dawley rats with Con A (5 μg/ml) at 5×10^6 cells/ml in RPMI 1640 culture medium. Supernatant fluids were collected after 24 hr incubation and supplemented with 20 mg/ml of α-methyl-D-mannoside (α-MM; Sigma Chemical Co., Munich, West Germany).

(ii) El-4 supernatant (EL-4-SN) was obtained by stimulating a cloned subline of EL-4 thymoma with 10 ng/ml phorbol myristate acetate (PMA), as described elsewhere (Farrar *et al.*, 1982).

(iii) Secondary mixed lymphocyte culture supernatant (MLC-SN) was prepared as described by Ryser, Cerottini & Brunner (1978) by mixing 1×10^7 viable cells recovered from pools of 14-day-old primary BALB/c anti-DBA/2 MLC with 4×10^6 irradiated (2000 rads) DBA/2 spleen cells in 10 ml of RPMI culture medium in 50-ml tissue culture flasks (Nunc, 163371; Wiesbaden, West Germany). Supernatant was collected after 24 hr incubation at 37° and sterilized by filtration.

T-cell activation and cloning

Female B6 mice were injected subcutaneously at the

base of the tail with 3×10^7 male B6 spleen cells suspended in complete Freund's adjuvant (CFA) in a total volume of 100 μl as described by Corradin, Etlinger & Chiller (1977). Five days after *in vivo* immunization, draining inguinal and periaortic lymph nodes were removed and 2×10^7 lymph node cells were cultured with 4×10^7 irradiated (2200 rads) B6 male spleen cells in 15 ml RPMI 1640 culture medium. The cultures were set up in 50-ml tissue culture flasks (Nunc, 163371) and restimulated weekly with 3×10^7 irradiated male B6 spleen cells in 10 ml fresh culture medium. After a culture period of 4 months, the H-Y-specific cell line was selected for Lyt-2$^-$ T cells by treatment with anti-Lyt-2.2 antiserum and selected rabbit complement, as described elsewhere (Simon & Eichmann, 1980). H-Y-specific T-cell clones were obtained from the selected T-cell line by seeding one cell per well in round-bottomed microtitre plates (Nunc, 163320), together with 5×10^5 irradiated (2200 rads) male spleen cells and 10% Con A-SN. After 2–3 weeks, growing wells were picked and further expanded on antigen in a volume of 2 ml in macrotitre plates (Linbro 1624 TC), and subsequently in 50-ml tissue culture flasks by weekly restimulation with 3×10^7 irradiated male B6 spleen cells in 10 ml RPMI 1640 culture medium in the absence of Con A-SN. One of the clones (B5) was recloned at a dilution of 0·3 cells/well under similar conditions. The resulting subclone B 5.2 was also adjusted to grow on antigen in the absence of Con A-SN.

Helper assay for the generation of CTL

Graded numbers of cloned T cells were titrated into 1-ml cultures containing 3×10^6 spleen cells derived from female B6 mice previously immunized with male B6 spleen cells as a source for CTL-P and 5×10^6 irradiated (2200 rads) male B6 spleen cells. Alternatively, supernatant from cloned T cells sensitized with either antigen or mitogen was titrated into similar cultures. Control cultures contained the detection system alone or were supplemented with either Con A-SN or EL-4-SN. After 6 days of incubation, specific ^{51}Cr release was determined by incubating serial dilutions of effector cells with a fixed number (2×10^3 cells/well) of ^{51}Cr-labelled Con A-activated male B6 blasts as targets in a final volume of 0·2 ml for 4 hr. Percent specific lysis was calculated using the following equation:

$$\% \text{ specific } ^{51}\text{Cr release} = (X - Y/Z - Y) \times 100$$

in which X is c.p.m. in the supernatant of target cells mixed with effector cells, Y is c.p.m. in the supernatant of target cells incubated alone, and Z is c.p.m. after lysis of target cells in the presence of 1 M HCl.

Helper assay for PFC response

Graded numbers of cloned T cells were titrated into 1-ml cultures containing 5×10^5 sheep red blood cells (SRBC) and 3×10^6 splenic B cells enriched by treatment with anti-Thy-1.2 (Olac, Bicester, Oxon, U.K.), anti-Lyt-1.2 and anti-Lyt-2.2 antisera and selected rabbit complement. Lymphocytes selected by this protocol completely lacked proliferative responses to Con A. Alternatively, supernatant from cloned T cells sensitized with either antigen or mitogen was titrated into the same detection system. Control cultures consisted of the detection system in the presence or absence of EL-4-SN or MLC-SN, respectively. After 5 days of incubation, cultures were tested for direct SRBC-specific PFC as described elsewhere (Jerne & Nordin, 1963).

Preparation of supernatant from H-Y-specific T-cell clones

Mitogen-induced supernatant was prepared from H-Y-specific T-cell clones by incubating 1×10^6 cloned T cells per ml with 5 μg Con A/ml in RPMI 1640 culture medium. After 24 hr, supernatant was harvested and supplemented with α-MM (20 mg/ml). Supernatant from antigen-activated cultures was obtained by incubating $2–3 \times 10^6$ cloned T cells with 3×10^7 male B6 stimulator cells in a total volume of 10 ml for 7 days. Supernatants from cultures containing either male B6 stimulator cells alone or cloned T cells and female B6 stimulator cells served as controls.

Assays for lymphokine activity in culture supernatant of cloned T cells

TCGF activity. Culture supernatant was tested for TCGF activity by incubating serial dilutions of supernatant (50% initial concentration) with 2×10^3 cells of the lymphokine-dependent cytotoxic T-cell line CTLL-2 (kindly provided by Dr Gilles) in a total volume of 200 μl in round-bottom microtitre plates (Nunc, 163220). After 20 hr incubation, 1·25 μCi [^{3}H]TdR was added for the last 4 hr. Cells were harvested onto filter strips using an automatic cell harvester and counted in a liquid scintillation β-counter. A standard titration of recombinant human interleukin-2 (rec. hIL-2, kindly provided by Dr M. Wrann, Sandoz Forschungsinstitut, Vienna, Austria) at dilutions ranging from 100 ng–60 pg (1

U = 100 pg rec. hIL-2/0·2 ml which allows half maximal proliferation of 2×10^3 CTLL after 24 hr) was set up with each assay. The data were subjected to probit analysis, compared to the standard curve, and the activity of each sample was expressed in units of TCGF (Gillis *et al.*, 1978). This method shows a high sensitivity, with 0·005 U being easily detected.

Interferon activity. Culture supernatant was tested for IFN-γ activity by a plaque reduction assay in microtitre plates using vesicular stomatitis virus and L cells, as described (Landolfo, Kirchner & Simon, 1982). Titres are expressed in laboratory units using, as reference, recombinant mouse gamma-interferon (rec. IFN-γ, $1{\cdot}8 \times 10^6$ units/ml, was kindly provided by Dr P. Swetly, Ernst-Boehringer Institut für Arzneimittelforschung, Ingelheim, West Germany).

RESULTS

Characteristics of cloned T cells

H-Y-specific and I-A^b restricted T-cell clones were raised from female B6 mice primed with male spleen cells by limiting dilution procedures after serial restimulations of selected Lyt-2^- responder lymphocytes *in vitro*. All three clones, A3, A5, and B5, and one subclone, B5.2, chosen for the present experiments were Thy-1^+, but stained only weakly with anti-Lyt-1 antiserum when tested by flow microfluorometry (data not shown). The H-Y-reactive clones were tested for their ability to provide help for the generation of H-Y-specific CTL, and for the induction of PFC specific for SRBC from their precursor cells.

Helper activity for CTL-P

Graded numbers of individual T-cell clones A3, A5 and B5, and of subclone B5.2 were titrated into cultures containing spleen responder cells derived from female B6 mice previously primed to H-Y antigen and male B6 stimulator cells. The cytolytic activities generated were tested on day 5 on male B6 target cells. In general, under these conditions, the system develops marginal cytolytic activities and is dependent on exogenous help for the development of optimal cytotoxic responses. In this experiment, no significant H-Y-specific cytolytic activity was mounted by primed female B6 spleen cells, unless exogenous sources of helper factors (Con A-SN, EL-4-SN) were added (Fig. 1, panels 1 and 2). As shown in Fig. 1 (panels 3, 5, 8 and 10), titration of lymphocytes from either of the cloned T cells A3, A5, B5, and from the subclone B5.2, revealed that all T-cell clones can help in the generation of H-Y-specific CTL. The optimal cell concentration varied for the different T-cell clones (from 1×10^4 to 1×10^5), and higher numbers of lymphocytes from subclone B5.2 were even suppressive for the CTL response.

Similar experiments have shown that CTL with different specificities (for H-2^d or for trinitrophenyl coupled on syngeneic cells) are also activated by H-Y-specific T-cell clones, provided the male antigen on the relevant accessory cell was present in culture (data not shown). It was therefore tested whether soluble mediators derived from the same T-cell clones after stimulation with either H-Y antigen (SN_{Ag}) or mitogen ($SN_{Con\ A}$) could replace the lymphocytes in the induction of H-Y-specific CTL from their precursor cells. As seen in Fig. 1, both antigenic (panels 4, 6, 9 and 11) and/or mitogenic (panel 7) activation of the three T cell clones A3, A5, B5, and of the subclone B5.2, resulted in the secretion of soluble mediators which were able to provide help for CTL development. Again, as for the cloned T cells, different concentrations of supernatant of the individual clones were required for optimal CTL responses. No helper activity was detected in supernatant of cultures containing the male stimulator population alone (Fig. 1, x—x). It was found, in addition, that the H-Y antigen was required for the secretion of soluble mediators from the T-cell clones, since no helper factors were detected after their sensitization with female accessory cells (data not shown).

Helper activity for B lymphocytes

The three H-Y-specific T-cell clones and the subclone were also assayed for their potential to induce enriched male B6 B lymphocytes to mature into PFC in the presence of SRBC. As shown in Table 1, all three clones A3, A5, B5 and the subclone B5.2 provided help for the T-cell depleted B lymphocyte population to mount a SRBC-specific PFC response. The magnitude of the response was often comparable to that achieved in the presence of EL-4-SN (Table 1, Exp. 1). No PFC responses were detected in cultures containing B6 B lymphocytes and SRBC only (Table 1, line 1), or in any lymphocyte populations cultured in the absence of SRBC (data not shown). It is also shown in Table 1 (Exp. 2) that the helper activity provided by the T-cell clones was radioresistant.

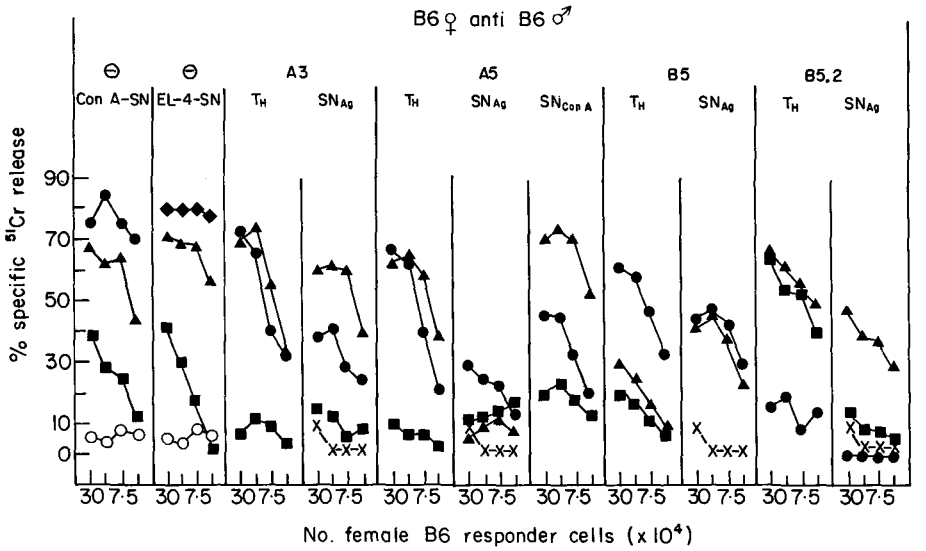

Figure 1. Helper activity of cloned H-Y-specific T cells and their soluble products on the generation of anti-H-Y-specific CTL *in vitro*. Cultures containing 3×10^6 primed female B6 responder cells and 5×10^6 irradiated male B6 stimulator cells in 1 ml were supplemented either with 1×10^5 (●), 1×10^4 (▲), or 1×10^3 (■) lymphocytes of the clones A3, A5, B5 and of subclone B5.2, or with 20% (●), 5% (▲) or 0·5% (■) of supernatants derived from the same clones by induction with either H-Y antigen or Con A, as described in Materials and Methods. Supernatant derived from cultures of male stimulator cells alone served as control and was added in a final concentration of 20% (x). For control of the detection system, the responder and the stimulator cells were incubated either alone (○) or were supplemented with 20% (●), 10% (♦), 5% (▲) or 0·5% (■) of either Con A-SN or EL-4-SN. After 6 days incubation, effector activities were tested on ^{51}Cr-labelled male B6 target cells. Numbers on the abscissa indicate the numbers of female B6 responder cells ($\times 10^4$) cultured on day 0, the descendants of which were tested on day 6. Percentage specific lysis was calculated as described in the Materials and Methods.

Furthermore, supernatants derived from either antigen- or mitogen-activated T-cell clones were able to promote the induction of PFC responses from enriched male B lymphocytes in the presence of antigen (Table 1). No helper activity for PFC responses was detected in supernatants of cultures containing either T-cell clones plus female stimulator cells, or the male stimulator population alone (data not shown). We therefore tested whether the recognition of H-Y antigen was required for the activation of T cells, for T-B cooperation, or for both. As shown in Fig. 2, antigen is required for the induction of helper activities from the H-Y-specific T-cell clones, since no PFC were detected in cultures containing both responder B lymphocytes and accessory cells from female B6 mice. However, in the presence of male B6 accessory cells, female B6 B cells are activated by H-Y-specific T-cell clones. This suggests that antigen is required for the induction of T-cell clones and not for T-B cooperation, an interpretation which is also substantiated by the finding that soluble helper factors are produced by the T-cell clones only in the presence of the appropriate antigen (see above).

Cloned H-Y-specific T cells do not secrete TCGF and IFN-γ

In order to find out whether the biological activities elicited by the H-Y-specific T-cell clones could be assigned entirely or partially to the lymphokines TCGF and/or IFN-γ, both activities were tested in T-cell clone-derived culture supernatants. The data in Table 2 show that none of the H-2I^b restricted T-cell clones secreted detectable levels of either TCGF or IFN-γ after stimulation with either H-Y antigen or Con A. For comparison, a H-2D^b restricted and H-Y-specific cytotoxic T- cell clone was found to be similarly deficient in producing TCGF, but secreted considerable amounts of IFN-γ when stimulated under similar conditions. Thus, the lymphokine(s) produced by the H-Y-specific and I-A^b restricted T-cell clones that effect the expansion and/or maturation of

Table 1. Effect of cloned H-Y-specific T cells and their soluble mediators on the SRBC-specific PFC response by male B6 B cells

Culture conditions					Anti-SRBC IgM PFC/10^6 B cells cultured		
Helper cells	No. cells ($\times 10^3$)	TH SN_{Ag} (%)	TH $SN_{Con\ A}$ (%)	EL-4-SN (%)	Exp. 1	Exp. 2	
–		–	–	–	0±0	3±3	
–		–	–	10	1495±135	3244±281	
				5	1363±112	ND	
				2·5	ND	1815±135	
A3	50	–	–	–	914±40	399±56	(439±26)*
	10	–	–	–	363±26	508±43	(261±10)
	5	–	–	–	ND	152±20	(92±10)
	–	10	–	–	ND	238±7	
	–	5	–	–	ND	152±36	
	–	2·5	–	–	ND	96±7	
A5	50	–	–	–		380±56	(337±17)
	10	–	–	–		469±43	(446±13)
	5	–	–	–		122±13	(116±17)
	–	10	–	–	ND	112±10	
	–	5	–	–		218±23	
	–	2·5	–	–		60±7	
	–	–	10	–		284±59	
	–	–	5	–		175±7	
	–	–	2·5	–		106±20	
B5	50	–	–	–	1486±135	330±20	(152±10)
	10	–	–	–	1122±92	498±50	(386±13)
	5	–	–	–	ND	327±26	(122±13)
	–	10	–	–	ND	261±40	
	–	5	–	–	ND	125±10	
	–	2·5	–	–	ND	106±7	
B5.2	50	–	–	–	1518±92	ND	
	10	–	–	–	1551±71	ND	
	5	–	–	–	ND	ND	
	–	10	–	–	ND	340±40	
	–	5	–	–	ND	406±30	
	–	2·5	–	–	ND	175±46	

Cultures containing 3×10^6 enriched male B6 B cells and 5×10^5 SRBC in 1 ml were supplemented with graded amounts of either cloned T cells or supernatants derived from the same clones after sensitization with either H-Y antigen or Con A as described in the Materials and Methods. Control cultures contained B cells and SRBC in the absence or presence of EL-4-SN. Direct anti-SRBC PFC responses were assayed 5 days later. Two experiments are shown.

* In Exp. 2, parallel cultures were set up with graded numbers of irradiated (1200 rads) cloned T cells.

T lymphocytes and of B lymphocytes are distinct from these two soluble mediators.

DISCUSSION

H-Y-specific and I-A^b restricted B6 T-cell clones have been established to investigate their helper potential for both T and B cells. By showing that the same T-cell clone and its soluble product(s) cause precursor cells of CTL and PFC to mature into effector cells, the data demonstrate that the same activated T cell can participate in cellular and humoral immune responses. The finding that the H-Y-specific cloned T cells could also provide help for CTL-P with irrelevant specificities and for female B lymphocytes specific for SRBC in the presence of H-Y-bearing APC indicates antigen specificity in the induction, but not in the effector phase of

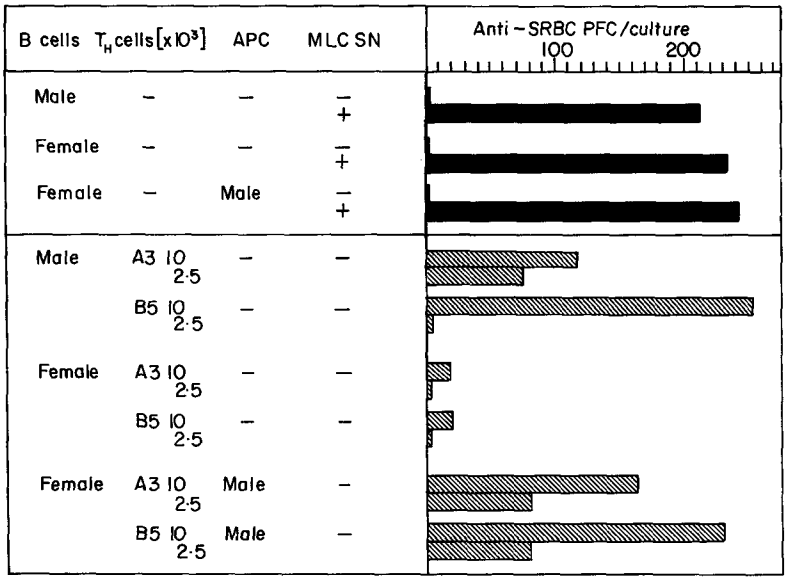

Figure 2. Antigen specificity of the induction of T helper activity in an anti-SRBC PFC response. 10×10^3 or $2{\cdot}5 \times 10^3$ cloned T cells were added to 1 ml cultures containing either 3×10^6 male or female T-cell deprived B6 spleen cells or 3×10^6 female T-cell deprived B6 spleen cells, together with 3×10^6 irradiated male B6 APC, in addition to 5×10^5 SRBC. In control cultures, the same B-cell populations were cultured, together with SRBC, in the absence or presence of 30% MLC-SN. Direct anti-SRBC PFC responses were measured 5 days later. The standard deviation was below 10%. Cultures lacking SRBC showed less than 35 PFC (data not shown).

TH. This was further supported by showing that only supernatants from H-Y-specific T-cell clones activated by either the relevant antigen or mitogen contained lymphokines with helper activity for the generation of CTL and PFC. It was found that the effector activities of both the lymphocytes and their induced soluble products varied to some extent in the different T-cell clones. Therefore, in order to reveal helper potential, cloned T lymphocytes and their soluble products have to be titrated carefully.

The soluble mediator(s) from the H-Y-specific cloned T cells with activity for both T and B cells, was shown to be distinct from TCGF and IFN-γ. Thus, the T-cell clones, as reported here, differ from those described previously in the mouse (Schreier, 1980; Glasebrook *et al.*, 1980; Crispe *et al.*, 1984) and in the human (Kaieda *et al.*, 1982) system. In these studies, it was demonstrated that T-cell clones with helper potential for B lymphocytes secreted IFN-γ and/or TCGF (Schreier, 1980; Glasebrook *et al.*, 1980; Kaieda *et al.*, 1982; Crispe *et al.*, 1984), thereby suggesting possible mechanisms by which one T cell can act on both T and B lymphocytes. Previous work has emphasized the participation of IL-2 (Wagner & Röllinghoff, 1978; Okada *et al.*, 1979; Gillis & Smith, 1977; Ryser *et al.*, 1978; Glasebrook *et al.*, 1980) and of IFN-γ (Simon, Farrar & Kindt, 1979; Kaieda *et al.*, 1982) in the development of CTL responses. Furthermore, it was recently shown with recombinant lymphokine sources that both rec. hIL-2 (Tsudo *et al.*, 1984; Emmrich *et al.*, 1984; Moll *et al.*, 1984) and rec. IFN-γ (Leibson *et al.*, 1984; Sidman *et al.*, 1984) also provide signals for the maturation of B cells. The experiments presented here clearly demonstrate that cloned T cells which do not produce detectable amounts of TCGF or IFN-γ nevertheless provide helper signals for both CTL-P and B cells. These findings challenge the concepts suggesting that IL-2 and IFN-γ are important lymphokines in T and B cell activation. There are at least two alternative explanations for this apparent discrepancy. Either the H-Y-specific cloned T cells induce IL-2 and/or IFN-γ in the indicator systems, or different subsets of T and B cells differentially respond to distinct lymphokines. The first possibility is rather unlikely for the B cell (but not the T cell) system, since the former population was shown to be totally devoid of functional T cells (data not shown). On the other hand, we know from B lymphocytes that different molecules may be involved in distinct stages of their maturation (Melchers &

Table 2. TCGF and IFN-γ activities in SN of antigen- or mitogen-activated H-Y-specific T-cell clones

T-cell line	Restricting specificity	SN	TCGF-activity units ($\times 10^2$)	IFN-γ titre
–	–	Con A-SN	5·5	3
–	–	EL-4-SN	16·5	<3
–	–	MLC-SN	0·35	27
A3	H-2I^b	Ag ind.	<0·005	<3
		Con A ind.	ND	<3
A5	H-2I^b	Ag ind.	<0·005	<3
		Con A ind.	<0·005	<3
B5	H-2I^b	Ag ind.	<0·005	<3
		Con A ind.	ND	<3
B5.2	H-2I^b	Ag ind.	<0·005	<3
		Con A ind.	ND	<3
2.C10.1	H-2D^b	Con A ind.	<0·005	243

Supernatants derived from cloned T cells by activation with either H-Y antigen or Con A were tested for their TCGF activity on CTLL-2 and for IFN-γ activity on mouse L cells as described in the Materials and Methods. For comparison, cultures supplemented with Con A-SN, EL-4-SN or MLC-SN were set up, and recombinant hIL-2 and recombinant IFN-γ sources were used in standard titrations. The titration curves obtained in the TCGF assay were subjected to probit analysis, compared to the standard curve, and the TCGF activity of each supernatant was expressed in units of TCGF (1 unit = 100 pg rec. hIL-2/0·2 ml, which allowed 50% of maximal proliferation in CTLL-2 after 24 hr). IFN-γ titres are expressed in laboratory units with the recombinant mouse IFN-γ as reference standard.

Andersson, 1984). It is therefore possible that at least some antigen-activated B cells do not require IL-2 and/or IFN-γ for maturation into PFC.

Comparison of helper activities for CTL-P in the TCGF-free lymphokine sources of the cloned T cells (TH-SN) with those in TCGF-rich supernatants (Con A-SN, EL-4-SN) revealed that there is no correlation between the TCGF content in the individual factor source and its ability to induce CTL responses from the responder populations described (Fig. 1, Table 2). These findings are consistent with previous studies showing that supernatants derived from mitogen-activated lymphocyte cultures contain helper factor(s) distinct from TCGF which independently promote differentiation of CTL-P (Raulet & Bevan, 1982; Wagner *et al.*, 1982). Moreover, the clonally derived helper factors for T-cell maturation described by Kaieda *et al.* (1982) could be separated by column separation into fractions containing either TCGF or IFN-γ activities, respectively, both of which showed similar helper effects in the development of cytotoxic responses from CTL-P. Therefore, because of the lack of both TCGF and IFN-γ in the supernatants of the H-Y-specific cloned T cells, our results indicate the existence of another CDF-like T-cell derived factor(s) involved in the maturation of CTL-P and stimulating them to mature into CTL in a hitherto unknown fashion. It is possible that the clonally derived lymphokine(s) acts directly on CTL-P, or that it promotes production of other lymphokines involved in the generation of CTL, as mentioned above. Another possibility is that our detection system is deficient of CDF (Raulet & Bevan, 1982; Wagner *et al.*, 1982), but not of TCGF, and is substituted by differentiation factors produced by the H-Y-specific T-cell clones.

The transmission of helper signals from H-Y-specific T-cell clones to B lymphocytes did not require direct T cell–B cell interaction, and was mediated by a soluble factor(s) present in supernatants of antigen- or mitogen-activated T-cell clones. The lymphokine-mediated PFC responses were also displayed by B cells of different H-2 genotypes (H. Moll, unpublished data). These data support previous studies showing that lymphocytes from long-term lines of antigen-spe-

cific T cells can induce resting and/or large B cells to differentiate into antibody-secreting cells, possibly by non-MHC-restricted factors (Glasebrook *et al.*, 1980; Asano *et al.*, 1982; DeFranco *et al.*, 1984; Julius, von Boehmer & Sidman, 1982). On the contrary, many other studies in which alloreactive cloned T cells (Pobor *et al.*, 1984) and H-Y-specific T-cell lines or clones (Pettersson, Pobor & Coutinho, 1982; Zubler & Kanagawa, 1982) were used have emphasized a strict MHC-dependent cell-cell interaction between T helper cells and B lymphocytes in antibody responses. The contradicting results may be due to the fact that the different B cell populations used in these studies have different requirements for TH signals (Asano *et al.*, 1982). Our data do not shed light on this discrepancy, since we used T-cell depleted spleen cell populations consisting of resting and large B cells, the latter of which have been shown to be receptive for non-MHC-restricted signals (Andersson, Schreier & Melchers, 1980; Schreier *et al.*, 1980). Thus, we do not know whether our clonally derived factor(s) acts on large B cells only, or also on resting B cells in a way similar to that described by DeFranco *et al.* (1984) for GAT-specific T-cell lines.

Our results show a strict requirement for SRBC in the induction of optimal IgM PFC responses from normal unselected B-cell populations; this is in line with some reports (Julius *et al.*, 1982; Zubler & Kanagawa, 1982) but not with others (DeFranco *et al.*, 1984; Pobor *et al.*, 1984). Again, these discrepancies may be due to differences in experimental design, as well as to the differential functional capacities of the individual T cell clones used. For this reason, it is also not clear whether the H-Y-specific T-cell clones reported here, and those of others (Julius *et al.*, 1982; Petterson *et al.*, 1982; Zubler & Kanagawa, 1982), are identical or different.

The conclusion that can be taken from the present study is that cloned H-Y- specific T cells can regulate both T and B cell responses via soluble mediators distinct from TCGF and IFN-γ. To our knowledge, TH of this type have not been previously reported. The combined results of this and other reports (Schreier, 1980; Glasebrook *et al.*, 1980; Kaieda *et al.*, 1982; Crispe *et al.*, 1984), showing that individual T cells secreting distinct arrays of lymphokines may express similar biological functions, suggest multiple ways in which individual T-cell clones can cause activation and/or differentiation of precursor cells of both the T- and B-cell lineage. The question as to whether one T cell is sufficient to trigger T and B lymphocytes in the presence of antigen for the entire process of maturation into effector cells remains to be answered.

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