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Functional helper activity of monoclonal T cell populations: Antigen-specific and *H-2* restricted cloned T cells provide help for *in vitro* antibody responses to trinitrophenyl-poly(LTyr,Glu)-poly(DLAla)--poly(LLys)

(cell interactions/major histocompatibility complex/*Ir* genes)

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ABSTRACT **The ability of long-term cultured and monoclonal T cell populations to provide antigen-specific help was assessed in a system of *Ir* gene-controlled *in vitro* antibody responses to soluble antigens. T-cell colonies and monoclonal T-cell lines were generated which proliferated specifically in response to poly(LTyr, Glu)-poly(DLAla)--poly(LLys) [(T,G)-A--L] and were *I-A* restricted in these proliferative responses. These (T,G)-A--L-specific T-cell populations were evaluated for their ability to help unprimed and T-cell depleted spleen cell populations in the generation of antibody responses to trinitrophenyl (TNP)-(T,G)-A--L *in vitro*. It was found that long-term T-cell lines, including monoclonal T-cell populations derived by limiting dilution, were highly efficient helper cells for IgM responses to TNP-(T,G)-A--L. These helper T cells were both antigen-specific and *I-A* restricted in their ability to be activated and to cooperate with T-cell depleted spleen cell populations. Once specifically activated, however, these clones provided help that was antigen nonspecific. These studies have thus demonstrated the ability of antigen-specific and *H-2*-restricted monoclonal T-cell populations to provide help for responses to soluble antigens *in vitro*.**

A number of observations in recent years have contributed to an understanding of the mechanism by which helper T cells (T_H cells) function in T-cell-dependent antibody responses. It has been shown that T_H cell populations recognize both specific antigen and "self" major histocompatibility complex (MHC) products expressed on the accessory cells or B cells (1–6) with which they interact. The nature of the T-cell receptor or receptors that mediate these complex recognition events is not yet well defined. In addition, the potential complexity of T_H cell populations has been indicated by experimental findings which suggest that an interaction of two or more distinct T-cell subpopulations may be required for optimal T-cell-dependent antibody responses (7–10). Further studies of T_H cell function and receptor specificity would be facilitated by the ability to investigate the properties of functional monoclonal T_H cell populations.

Monoclonal T-cell populations have recently been generated which express a number of functional capacities. For example, monoclonal T-cell lines have been described which are both antigen-specific in their proliferative responses to soluble antigen and restricted by a requirement for the recognition of self MHC products expressed on the antigen-presenting cells involved in these responses (11–13). Cloned lines of cytotoxic T lymphocytes (14–15) and of alloantigen-specific proliferating T cells (16) have also been described. In addition, it has been reported recently that monoclonal T cells can provide antigen-specific help for antibody responses to sheep erythrocytes (SRBC) *in vitro* (15, 17).

The present studies were undertaken in an effort to extend these findings by characterizing the ability of cloned T-cell lines to function as T_H cells for genetically restricted antibody responses to soluble antigen. T-cell clones that proliferate in antigen-specific and *H-2* restricted responses to the polypeptide poly(LTyr,Glu)-poly(DLAla)--poly(LLys) [(T,G)-A--L] (13) were assayed for helper activity in the T-cell-dependent and *Ir*-gene-controlled *in vitro* antibody response to trinitrophenyl (TNP)-conjugated (T,G)-A--L (18, 19). It was demonstrated that antigen-specific cloned T cells were highly efficient in providing help for IgM responses to TNP-(T,G)-A--L *in vitro* and that these $H\text{-}2^b$ T_H cells were genetically restricted in their ability to cooperate only with (B + accessory) cells of the $I\text{-}A^b$ haplotype.

MATERIALS AND METHODS

Animals. C57BL/6 (B6), C57BL/10 (B10), B10.A, and B10.D2 mice were purchased from The Jackson Laboratory. B10.A(4R), B10.A(5R), and B10.MBR mice were generously provided by D. Sachs (National Institutes of Health).

Antigens. Keyhole limpet hemocyanin (KLH) (lot 530195, Calbiochem) and (T,G)-A--L (lot MC6,Yeda Research) were conjugated with 2,4,6-trinitrobenzenesulfonate (Pierce) as described (18).

Immunization and Cell Culture of (T,G)-A--L-Reactive T Cells. Immunization and cell culture procedures have been described in detail (13). Briefly, mice were immunized with 100 μg of (T,G)-A--L in complete Freund's adjuvant by injection at the base of the tail. Seven days later, cells from the draining lymph nodes were cultured with (T,G)-A--L 200 μg/ml in complete culture medium (13). After 4 days in culture, blast cells were enriched by Ficoll-Paque gradient and 2×10^5 interphase cells were recultured with 10×10^6 syngeneic irradiated [3300 rads (33 grays)] filler cells in 2 ml. (T,G)-A--L-reactive T cells were maintained by cycles of 4-day antigen restimulation alternating with 10-day periods of resting culture with syngeneic filler cells in the absence of (T,G)-A--L.

Derivation of (T,G)-A--L-Reactive T-Cell Colonies. Long-term-cultured (T,G)-A--L-reactive T cells were stimulated with

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Abbreviations: C, complement; KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex; PFC, plaque-forming cells; SRBC, sheep erythrocytes; (T,G)-A--L, poly(LTyr,Glu)-poly(DLAla)--poly(LLys); T_H cell, helper T cell; TNP, trinitrophenyl.

(T,G)-A--L at 200 μg/ml in the presence of syngeneic filler cells. After 36 hr of incubation, cells in the flask were harvested and the resulting 1-ml cell suspension mixed with 2 ml of 0.5% agar medium on a supporting layer of 0.5% agar as described (13). Colonies observed after 5–7 days were picked and expanded (13).

Cloning by Limiting Dilution. (T,G)-A--L-reactive T cells were plated in microtiter wells to give 0.3 viable cell per well with 1×10^6 fresh syngeneic filler cells, (T,G)-A--L at 200 μg/ml, and 25% concanavalin A supernatant in a total volume of 0.2 ml (13). Ten to 14 days later, wells showing positive growth were transferred to larger wells and expanded. Cloning efficiency by this limiting dilution technique ranged from 40% to 100%.

Assay of Proliferative Response. After 10–14 days of resting culture in the absence of (T,G)-A--L, 1×10^4 (T,G)-A--L-reactive T cells were restimulated with (T,G)-A--L at 200 μg/ml in the presence of 1×10^6 filler cells in 0.2 ml of complete medium per well in microtiter plates (Falcon, no. 2040) for 48 hr. [^{3}H]Thymidine (2 μCi; 1 Ci = 3.7×10^{10} becquerels) was added 16 hr before harvest; cells were harvested on filter paper and radioactivity was measured by using standard scintillation counting. Results were expressed as the mean of triplicate cultures. The standard deviation of each mean was ≤10%.

Preparation of Cells for *in Vitro* Antibody Responses. *T cells.* Conventional splenic T-cell populations were prepared by collection of cells nonadherent to nylon fiber columns (18). Long-term cultured T cells were assayed for helper activity after 10 days of resting culture in the absence of antigen.

(B + accessory) cells. T-cell-depleted spleen (B + accessory) cells were prepared by treatment with a monoclonal anti-Thy 1.2 reagent (20) + complement (C). In some experiments, further T-cell depletion was accomplished by positive selection of surface immunoglobulin-positive cells through adherence to rabbit anti-mouse Ig-coated plates (21). The T-cell specificity of these cytotoxic reagents and the completeness of T-cell elimination by these treatments were monitored by the abrogation of responses to the T-cell mitogens phytohemagglutinin and concanavalin A, without depletion of responses to lipopolysaccharide.

Culture Conditions for *in Vitro* Antibody Responses. All cultures were performed in a volume of 200 μl per flat-bottom well of microtiter plates as described (13) and incubated for 4 days at 37°C in a humidified 5% Co_2/95% air atmosphere. Cells were harvested, and individual cultures assayed for plaque-forming cells (PFC).

PFC Assay. SRBC were conjugated with TNP (TNP-SRBC) and direct (IgM) PFC were assayed as described (18). All data points shown represent the geometric mean responses of triplicate cultures.

RESULTS

Antigen-Specific Monoclonal T Cells Are Able to Provide Help for Antibody Responses to TNP-(T,G)-A--L. The T cells used in the present studies were originated by repeated *in vitro* antigen stimulation of lymph node cells from (T,G)-A--L primed C57BL/6 mice followed by the isolation of "colonies" in soft agar and by the subsequent isolation of "clones" by limiting dilution. The antigen-specific proliferative responses of T cells from the soft agar-derived colonies 1a and 1d and from the limiting dilution clones 1a36 and 1a39 are illustrated in Table 1. Each of these populations generated a strong proliferative response to (T,G)-A--L but little or no response to KLH in the presence of irradiated syngeneic $H\text{-}2^b$ spleen cells (Exp. 1). Moreover, the ability of T cells from both colonies and limiting dilution clones to respond to (T,G)-A--L was *H-2 (I-A)* restricted because proliferation was induced only by (T,G)-A--L in the presence of $H\text{-}2^b$ but not $H\text{-}2^d$ or $H\text{-}2^k$ antigen-presenting cells (Exp. 2). These populations were without detectable alloreactivity to $H\text{-}2^d$ or $H\text{-}2^k$ determinants.

The ability of these (T,G)-A--L-specific T cells to provide functional helper activity was examined for *in vitro* IgM PFC responses to TNP-(T,G)-A--L. The responses of unprimed B10 spleen cells to both TNP-(T,G)-A--L and TNP-KLH were abrogated by treatment with anti-Thy 1.2 plus C, and responses to both antigens were fully reconstituted by the addition of unprimed nylon-nonadherent B10 spleen (T) cells (Fig. 1), demonstrating the T-cell dependence of these responses. Moreover, these results demonstrated that a conventional $H\text{-}2^b$ T-cell population is competent to provide help for responses to both of these antigens.

The helper activity of (T,G)-A--L specific T cells from soft agar-derived colonies was similarly examined for *in vitro* IgM responses to TNP-(T,G)-A--L and TNP-KLH. T cells from colony 1d were sufficient to provide help for responses to TNP-(T,G)-A--L (Fig. 1). The help provided by these T cells was highly efficient: as few as $3\text{–}10 \times 10^2$ cells per culture gave significant responses and $1\text{–}3 \times 10^3$ cells per culture gave nearly maximal responses. The magnitudes of the responses generated by cells from these colonies were several fold greater than those generated by conventional T-cell populations from unprimed (Fig. 1) or (T,G)-A--L primed (data not shown) mice; and the help provided by titrated numbers of these T cells was 50- to 100-fold more efficient than that provided by conventional T cells. In addition, the activation of help mediated by T-cell colonies was highly antigen-specific in that no significant responses were generated to TNP-KLH (Fig. 1) or to SRBC (data not shown), whereas conventional T-cell populations supported responses to each of these antigens. Of a total of eight (T,G)-A--

Table 1. T-cell colonies and limiting dilution clones are antigen-specific and *I-A* restricted in their proliferative responses to (T,G)-A--L

Antigen-presenting cells	Antigen	Thymidine incorporation, cpm: Soft-agar colonies 1a	Soft-agar colonies 1d	Limiting dilution clones 1a36	Limiting dilution clones 1a39
		Exp. 1			
B10	(T,G)-A--L	13,867	22,516	5,035	11,690
	KLH	1,513	1,741	657	445
	—	164	708	274	293
		Exp. 2			
B10	(T,G)-A--L	5,947	15,808	28,049	
	—	204	1,123	449	
B10.A	(T,G)-A--L	212	448	501	
	—	69	304	495	
B10.A(4R)	(T,G)-A--L	230	2,004	275	
	—	143	602	152	
B10.A(5R)	(T,G)-A--L	4,006	12,501	ND	
	—	150	505	ND	
B10.MBR	(T,G)-A--L	212	569	198	
	—	87	192	290	
B10.D2	(T,G)-A--L	266	345	ND	
	—	224	343	ND	

Responding cells (1×10^4) of the indicated colony or limiting dilution clone were cultured for 2 days with 1×10^6 irradiated spleen cells of the indicated strain as antigen-presenting cells and without antigen or with (T,G)-A--L or KLH at 200 μg/ml. [^{3}H]Thymidine incorporation was measured over the last 16 hr of the culture period. ND, not done.

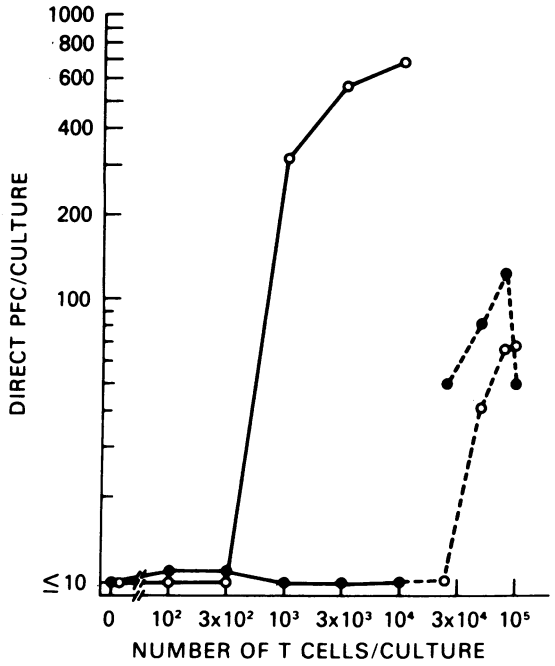

FIG. 1. T cells from a (T,G)-A--L specific colony provide antigen-specific help for *in vitro* antibody responses to TNP-(T,G)-A--L. Titrated numbers of nylon-nonadherent B10 spleen cells (broken lines) or T cells from the (T,G)-A--L specific colony 1d (solid lines) were cultured with 4×10^5 unprimed B10 spleen cells treated with anti-Thy 1.2 plus C and either TNP-KLH at 5 μg/ml (●) or TNP-(T,G)-A--L at 0.8 μg/ml (○). Responses in the absence of antigen were <10 PFC per culture.

L specific colonies, seven had helper activity that was qualitatively and quantitatively similar to that demonstrated for colony 1d (data not shown).

These findings suggested that a monoclonal T-cell population may be sufficient to provide help for antibody responses to the soluble polypeptide antigen TNP-(T,G)-A--L. In order to test this interpretation more rigorously, two additional experimental procedures were used. First, to determine whether rigorously monoclonal T-cell populations were competent to provide help, monoclonal populations were isolated by limiting dilution from the soft agar colonies characterized above. The populations studied for helper activity were derived at a limiting dilution of 0.3 cell plated per microtiter well. The apparent plating efficiency under these conditions ranged from 41% to 100%. Cells from two such clones, 1g31 and 1a36, were assayed for helper activity and were found to provide help indistinguishable from that provided by the soft-agar colonies from which they were derived (Fig. 2). In addition, an attempt was made to minimize the possibility that residual T cells in the unprimed "T cell-depleted" populations were contributing an essential helper activity for these responses. Rigorously T cell-depleted populations were prepared by sequentially enriching surface-Ig positive B10 spleen cells by adherence to anti-mouse Ig-coated plates followed by treatment with anti-Thy 1.2 plus C or by using B10 *nu/nu* spleen cells treated with anti-Thy 1.2 plus C. (T,G)-A--L specific colony 1a T cells were sufficient to help each of these T cell-depleted populations (Fig. 3). These results are consistent with the conclusion that a monoclonal antigen-specific T-cell population is sufficient to provide help for *in vitro* IgM responses to soluble TNP-(T,G)-A--L.

Antigen-Specific T Cells Are *I-A* Restricted in their Ability to Provide Help. Although conventional heterogeneous T_H cell populations have been shown to be both antigen-specific and MHC-restricted in their activity, it is not possible from such studies to demonstrate directly whether the same cells are both antigen and MHC specific. It therefore was of interest to determine whether the homogeneous helper T cells that had been shown to be (T,G)-A--L specific in their activation were also *H-2* restricted. When colony 1d T cells were tested for their ability to help B10 or B10.A (B + accessory) cells for responses to TNP-(T,G)-A--L it was observed that they generated strong responses in B10 populations but no significant responses in B10.A (Fig. 4). Thus, these (T,G)-A--L specific T_H cells were also *H-2* restricted in their ability to cooperate with (B + accessory) populations. In order to map the subregion of the *H-2* gene products restricting the helper activity of these T cells, intra-*H-2* recombinant strains were used as a source of (B + accessory) cells. Responses were generated in B10.A(5R) (B + accessory) cells which were equal in magnitude to those in B10 cells; B10.A(4R), B10.MBR, and B10.A were unresponsive (Fig. 4). These results demonstrate that a product of genes en-

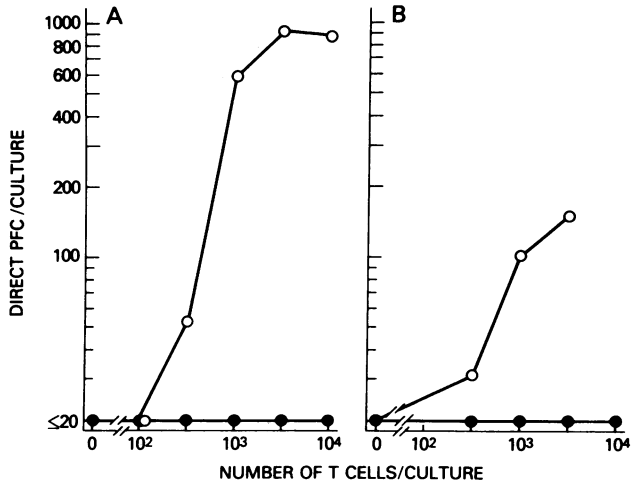

FIG. 2. T cells from (T,G)-A--L specific limiting-dilution clones provide antigen-specific help for *in vitro* antibody responses to TNP-(T,G)-A--L. Titrated numbers of T cells from the (T,G)-A--L specific limiting dilution clones 1g31 (*A*) and 1a36 (*B*) were cultured with 4×10^5 unprimed B10 spleen cells treated with anti-Thy 1.2 plus C and either TNP-KLH at 5μg/ml (●) or TNP-(T,G)-A--L at 0.8 μg/ml at (○). Responses in the absence of antigen were <10 PFC per culture.

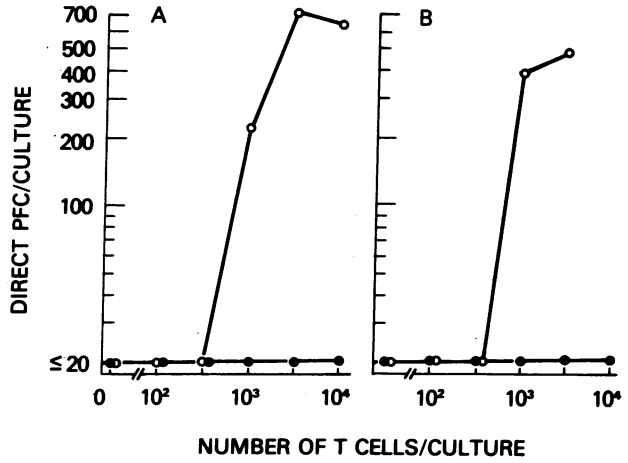

FIG. 3. T_H cells from colony 1d provide antigen-specific help for exhaustively T cell-depleted populations. Titrated numbers of T cells from (T,G)-A--L specific colony 1d were cultured with 4×10^5 surface Ig-positive B10 spleen cells (*A*) or B10 *nu/nu* spleen cells (*B*). In all cases the cells were treated with anti-Thy 1.2 plus C and TNP-KLH at 5 μg/ml (●) or TNP-(T,G)-A--L at 0.8 μg/ml (○).

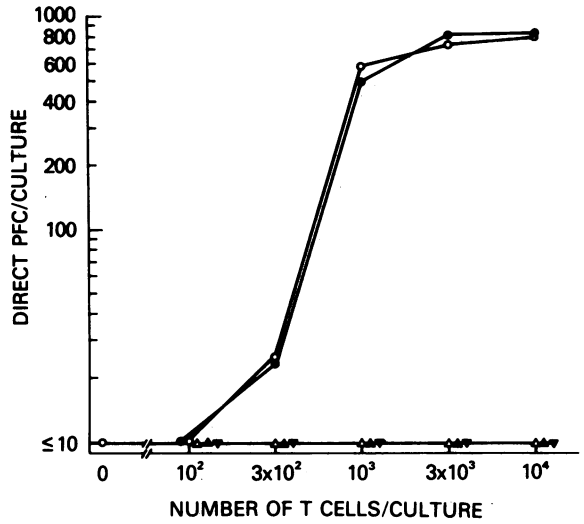

FIG. 4. The (T,G)-A--L specific helper activity of colony 1d T cells is *I-A* restricted. Titrated numbers of T cells from (T,G)-A--L specific colony 1d were cultured with 4×10^5 unprimed spleen cells of various strains treated with anti-Thy 1.2 plus C and TNP-(T,G)-A--L at 0.8 μg/ml. Responses in the presence of TNP-KLH or in the absence of antigen were <20 PFC per culture. See Table 2 for definition of symbols and strains.

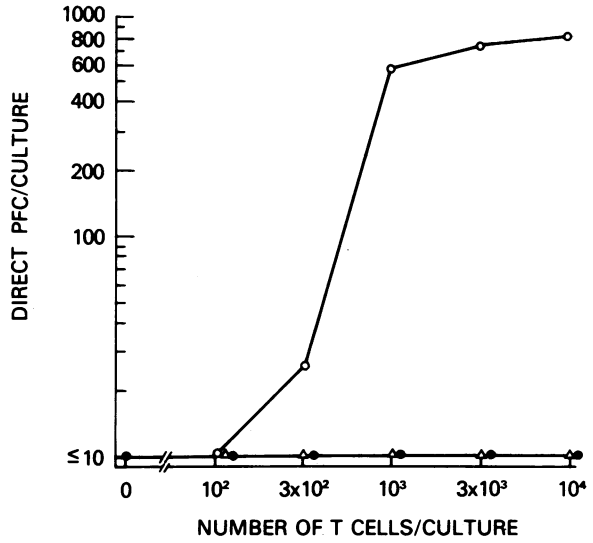

FIG. 5. The (T,G)-A--L specific helper activity of clone 1a36 is *H-2* restricted. Titrated numbers of T cells from the (T,G)-A--L specific limiting dilution clone 1a36 were cultured with 4×10^5 unprimed B10 (○) or B10.D2 (△) spleen cells treated with anti-Thy 1.2 plus C and TNP-(T,G)-A--L at 0.8 μg/ml or with treated B10 spleen cells and TNP-KLH (●) at 5. μg/ml. Responses in the absence of antigen were <10 PFC per culture.

coded in the *I-A* subregion restricts the ability of colony 1d T cells to function as helper cells and, therefore, that these T cells recognize both antigen and *I-A* product.

It had been shown (19, 22) that the function of accessory cells for *in vitro* responses to TNP-(T,G)-A--L is under the control of *Ir* gene(s) mapping to the *I-A* subregion and that $I\text{-}A^k$ is a nonresponder haplotype for this response. Therefore, the possibility was considered that the apparent *H-2* restriction of T_H cell activity observed in the present study actually reflected an intrinsic *Ir* gene defect in the $I\text{-}A^k$ (B + accessory) populations for responses to TNP-(T,G)-A--L and did not result from *H-2* restricted T-cell recognition. In order to evaluate the *H-2* restriction of T_H cell function independent of *Ir* gene responder status, clone 1a36 T cells were evaluated for their ability to cooperate with $H\text{-}2^d$ as well as $H\text{-}2^b$ spleen cells treated with anti-Thy 1.2 plus C, both of which are responder haplotypes for TNP-(T,G)-A--L. T cells from clone 1a36 cooperated with B10 and not with B10.D2 (B + accessory) cells, demonstrating that these T_H cells are indeed *H-2* restricted in their activity even when this restriction is assayed between responder haplotypes (Fig. 5).

After Specific Activation, T-Cell Clones Provide Antigen-Nonspecific Helper Activity. In order to characterize the mechanism by which T-cell clones provide help for the responses being studied, we determined whether a requirement exists in these responses for presentation of (T,G)-A--L physically linked to the hapten TNP. As noted above, titrated numbers of clone 1a36 T cells provided efficient help to T cell-depleted B10 spleen cells for TNP-specific responses to TNP-(T,G)-A--L but not to TNP-KLH (Fig. 6) or to unconjugated (T,G)-A--L (data not shown). In contrast, when (T,G)-A--L and TNP-KLH were simultaneously present in culture, responses were generated that were equal in magnitude to those induced by covalently coupled TNP-(T,G)-A--L. These results suggest that, although the activation of cloned T_H cells is both (T,G)-A--L specific and *H-2* restricted, once activated their helper function is in fact antigen non-specific.

Table 2. T cell-depleted populations used in Fig. 4

Symbol	Strain	*K*	*A*	*B*	*J*	*E*	*C*	*S*	*D*
○	B10	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*
△	B10.A	*k*	*k*	*k*	*k*	*k*	*d*	*d*	*d*
▲	B10.A(4R)	*k*	*k*	*b*	*b*	*b*	*b*	*b*	*b*
●	B10.A(5R)	*b*	*b*	*b*	*k*	*k*	*d*	*d*	*d*
▼	B10.MBR	*b*	*k*	*k*	*k*	*k*	*k*	*k*	*q*

DISCUSSION

In the present study, long-term cultured T-cell lines and T-cell clones derived by limiting dilution were evaluated for their ability to function as T_H cells for *in vitro* antibody responses to TNP-(T,G)-A--L. The results of this study demonstrated that antigen-specific monoclonal T cells were competent and highly efficient in providing help for IgM PFC responses to TNP-(T,G)-A--L.

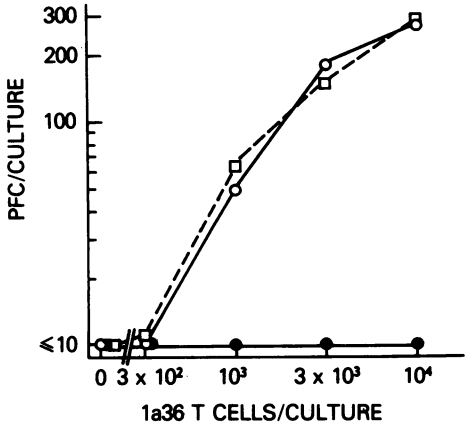

FIG. 6. Activation of unprimed TNP-specific B cells by (T,G)-A--L specific clone 1g31 T cells does not require carrier–hapten linkage. Titrated numbers of T cells from the (T,G)-A--L specific limiting dilution clone 1a36 were cultured with 4×10^5 unprimed B10 spleen cells treated with anti-Thy 1.2 plus C and TNP-KLH (●) at 5 μg/ml, TNP-(T,G)-A--L (○), or TNP-KLH at 5 μg/ml plus (T,G)-A--L at 0.8 μg/ml (□). Responses in the presence of only (T,G)-A--L at 0.8 μg/ml were <10 PFC per culture.

Moreover, these T_H cells were *H-2* restricted in their ability to help $H\text{-}2^b$ but not $H\text{-}2^d$ or $H\text{-}2^k$ (B + accessory) cells in these responses. It was also shown that, although the activation of these T-cell clones was both antigen-specific and *H-2* restricted, once activated, the help provided was in fact antigen nonspecific.

The ability of monoclonal T-cell populations to recognize both specific antigen and self-MHC product has been reported for proliferating T cells (11–13, 16), for cytotoxic T lymphocytes (14, 15), and for T_H cells active for antibody responses to SRBC (15, 17). The present studies have extended these observations to T_H cells functioning for *in vitro Ir* gene-controlled antibody responses to the soluble antigen TNP-(T,G)-A--L and have specifically demonstrated that the same monoclonal T-cell populations may be antigen specific and MHC restricted in both their proliferative responses and their ability to function as T_H cells. These findings confirm that monoclonal T cells with different functional capacities may be similar in their MHC-restricted recognition of antigenic signals.

The demonstration of helper activity in monoclonal T-cell populations provides some insights into the mechanisms by which T-cell help is effective in antibody responses. First, the findings reported here demonstrate that a single (monoclonally derived) antigen-specific T-cell population is sufficient to provide efficient help for responses to TNP-(T,G)-A--L, responses previously shown to be under the control of *I-A*-encoded *Ir* genes (19). This competence of monoclonal T_H cells is of particular interest in the context of suggestions, from a number of experimental systems, that the interaction of two or more different T-cell subpopulations is necessary for the generation of optimal T-cell help (7–10). In each of these systems, one T_H cell population is required that is both antigen specific and MHC restricted, properties consistent with those demonstrated for the monoclonal T_H cells characterized here. In addition, however, these systems have demonstrated a requirement for a second T-cell subpopulation for the generation of optimal help. This second population has been characterized as not MHC restricted (23) and, in several studies, has been specific for the recognition of idiotypic or immunoglobulin-linked determinants (8, 9, 23). The present report establishes that interactions between T-cell subpopulations are not necessary for the generation of efficient helper activity in responses to soluble antigens *in vitro*. These studies, however, do not exclude a role for additional T-cell populations in enhancing or qualitatively modifying these responses. In this respect, the determination of the ability of cloned T-cell populations to help IgG as well as IgM PFC will be of interest because it is possible that the T-cell requirements for these responses will differ.

The availability of cloned T_H cells will also allow further study of the antigen-specific T-cell receptor. A number of studies have demonstrated the expression on T cells of idiotopes crossreactive with those expressed on immunoglobulin molecules (24–26) and have led to the hypothesis that the same V_H gene products may function as antigen-specific receptors on both T cells and antibody. What has not yet been fully reconciled with this hypothesis is the possibility that such idiotype-positive T cells are also MHC restricted in their recognition of antigen. It therefore will be of interest to determine whether *H-2* restricted monoclonal T_H cells express the idiotypes shown to exist on antibody molecules such as those specific for (T,G)-A--L (26, 27).

It has been shown in the present studies that the monoclonal T_H cells studied are antigen specific in their activation but capable of providing antigen-nonspecific help once activated, consistent with a mechanism mediated by the antigen-nonspecific products of activated T_H cells. Although T_H function is shown to be *H-2* restricted in these responses, it has not yet been determined whether this restriction reflects a requirement for recognition by a single T_H cell of *H-2* products expressed on accessory cells or on B cells or on both. A number of studies have demonstrated that the activation requirements of B cells, including the requirement for direct T_H cell recognition of B-cell MHC products, may differ for distinct B-cell subpopulations (6, 28). It therefore remains to be determined whether the monoclonal populations characterized in the present studies are competent to activate each of these B-cell subpopulations or whether, in fact, the different activation requirements of these B-cell subsets are related to differences in the T_H populations interacting with each B-cell population.

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