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Antigen-specific human T-cell hybridomas with helper activity

(mutant T-cell lines/cell fusion/tetanus toxoid/interleukin 2)

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ABSTRACT **Human T cell hybridomas were produced by fusing the hypoxanthine phosphoribosyltransferase-deficient line of the human T cell lymphoma Jurkat with a continuous line of normal human T cells specific for tetanus toxoid (TeT). The hybridomas were selected for their ability to produce interleukin 2 after exposure to TeT on semiautologous monocytes and for their ability to bind to TeT-pulsed semiautologous monocytes. These antigen-specific T hybridomas demonstrated potent helper activity for semiautologous B cells as determined by the production of high levels of anti-TeT antibody *in vitro*.**

The induction and regulation of antigen-specific human antibody immune responses *in vitro* has been hampered by the heterogeneity of lymphocyte populations obtained from peripheral blood (1, 2). Dissection of these events has been greatly improved since the discovery of interleukin 2 (IL 2) (3) which supports the *in vitro* growth of T cells with maintenance of specificity and function (4). As documented with murine T cell lines, human T cells require repeated stimulation with antigen and autologous monocytes to induce proliferation (5, 6). Because availability of human autologous monocytes is limited, development of functional T cell hybridomas with autonomous growth potential was attempted. These hybridomas may also be a potential source of monoclonal lymphokines which regulate a variety of lymphocyte functions (7). Several reports on the establishment of functional albeit nonspecific human T hybridomas confirmed this prediction (8–10).

The present report describes the establishment of *antigen-specific* human T hybridomas that induce human B cells to generate specific antibody to the homologous antigen.

MATERIALS AND METHODS

Cell Lines. The 6-thioguanine-resistant T cell line was selected from the human T cell lymphoma Jurkat obtained through the generosity of Robert Gallo (National Cancer Institute). Cultures of logarithmically growing cells were mutagenized with ethyl methanesulfonate and selected in 6-thioguanine at 30 μg/ml. After selection, three clones were found, picked, and expanded in media containing 6-thioguanine at 30 μg/ml. All clones were tested for their hypoxanthine/aminopterin/thymidine (HAT) sensitivity, surface phenotype, and ability to produce IL 2 after mitogen stimulation. All clones died in HAT medium but grew in 6-thioguanine and were found to be hypoxanthine phosphoribosyltransferase-negative (data not shown). One clone, Jurkat-6TG-3, produced optimal amounts (38 units/ml) of IL 2 when stimulated with concanavalin A (Con A) (Miles Yeda, Rehovot, Israel) at 0.5 μg/ml. This clone produced less IL 2 than reported for the parental Jurkat line (11) but was the highest producer of the three mutants obtained. The clone Jurkat-6TG-3 was used for all fusions described.

Human Tetanus Toxoid (TeT)-Specific T Cell Lines. Lines of normal TeT-specific T cells used for fusions were obtained by the following protocol shown to enhance the frequency of antigen-specific helper cells in the mouse (4). Donors who had been immunized with TeT within the previous 2 yr were bled and their peripheral blood lymphocytes (PBL) were purified by centrifugation on lymphocyte separation media (LSM; Litton Bionetics, Bethesda, MD). PBL at 2×10^6 cells per ml were seeded in 24-well plates (Costar, Cambridge, MA) in RPMI 1640/10% AB human serum/10 μM 2-mercaptoethanol/TeT at 2 μg/ml (Division of Biologics, Boston, MA). After 6 days, viable T cells were seeded at 1×10^5 cells per ml in a source of partially purified, lectin-free human IL 2 at 10 units/ml (Associated Biomedics, Buffalo, NY). Every 5–7 days, cultures were refed with fresh media containing antigen, IL 2, and 4,000 rad γ-irradiated autologous PBL or adherent cells (monocytes) (1 rad = 0.01 gray). This procedure was followed until the total number of viable T cells reached $10–20 \times 10^6$. These cells were then seeded at 1×10^5 cells per ml in fresh media with IL 2 and grown for 5–7 days in the absence of irradiated fillers prior to testing and fusion. Before fusion, each T cell line was screened for antigen specificity in two ways: (*i*) TeT-induced T cell proliferation on γ-irradiated autologous monocytes and (*ii*) TeT-induced helper activity on autologous B cells.

Production of Human T–T Hybrids. Fusions between Jurkat-6TG-3 and the TeT-specific helper T cell lines were performed as described by Kontianen *et al.* (12). Fused cells were seeded in media containing HAT (1×10^{-4} M hypoxanthine, 4×10^{-7} M aminopterin, and 1.6×10^{-5} M thymidine; Sigma) and fed weekly by replacing half the media volume. Although the unfused T cell blasts and the Jurkat-6TG-3 died within 1–2 wk, colonies of growing T cell hybrids were observed by 7 wk after fusion and grew exponentially thereafter.

Karyotype Analysis. Metaphase chromosomes of parental and hybrid cells were bombed according to a modification (13) of the trypsin/Giemsa method of Seabright (14). At least 15 metaphases of each analyzed hybrid clone were photographed and karyotyped.

Surface Phenotypes. The Jurkat-6TG-3 and T–T hybrids were screened for the OKT-defined surface antigens by double-antibody immunofluorescence (15). Aliquots of each cell type were incubated in RPMI 1640 with 10% normal goat serum in the presence of OKT-3, -4, or -8 mouse monoclonal antibody (final dilution 1:10) at 4°C for 60 min. The cells were washed and fluoresceinated-F(ab')$_2$ fragment of goat anti-mouse Ig

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Abbreviations: IL 1, interleukin 1; IL 2, interleukin 2; HAT, hypoxanthine/aminopterin/thymidine; Con A, concanavalin A; TeT, tetanus toxoid; PBL, peripheral blood lymphocytes; SKSD, streptokinase/streptodornase; HLA, human leukocyte antigen.

(Litton Bionetics) was added. After 30 min at 4°C, the cells were diluted to 5×10^5 cells per ml with media and were analyzed by flow cytometry by using the Ortho System 50 cytofluorograph. The controls included were normal mouse serum with the fluorescein isothiocyanate-conjugated second antibody and the second antibody alone. These were uniformly negative. The percentage of hybrids bearing the receptor for neuraminidase-treated sheep erythrocytes and the receptor for human Ig was determined by rosetting (16).

Human Leukocyte Antigen (HLA) Typing. HLA types were determined by using the standard two-stage microlymphocytotoxicity technique (17). The antisera used defined the majority of the HLA-A, -B, and -DR specificities and were obtained from the Serum Bank of the Transplantation Immunology Branch of the National Institute of Allergy and Infectious Diseases, from exchange with other investigators, and from our own collection of well-characterized reagents from multipara.

Assay for IL 2 Production. We evaluated the ability of the hybrids to produce IL 2 in the presence of varying concentrations of mitogens or antigens by using the protocol of Gillis *et al.* (18) which measures IL 2 activity in supernatants by their ability to support the growth of an IL 2-dependent mouse T cell line, CTLL, obtained from Kendall Smith (Dartmouth Medical School).

Assay for T Hybridoma Binding to Antigen-Pulsed Monocytes. T hybrids and Jurkat-6TG-3 were prelabeled with [^{3}H]dThd at 1 μCi/ml (1 Ci = 3.7×10^{10} becquerels) in RPMI 1640 and 15% fetal calf serum overnight at 37°C. The next day the cells were extensively washed, counted, and equal numbers of cells were added to microtiter plates that contained 4,000 rad γ-irradiated adherent cells from a suspension of 5×10^5 semisyngeneic PBL. These adherent cell cultures had been previously pulsed for 3 hr at 37°C with nothing (media), TeT (20 μg/ml), or streptokinase/streptodornase (SKSD) (20 μg/ml). After 3 hr at 37°C, the nonadherent cells were washed away and the adherent cells detached with 0.25% trypsin in 0.1% EDTA. The contents of the plate were harvested onto glass fiber paper with a Titertek, and the filters were dried and the radioactivity was counted in liquid scintillation cocktail.

Helper Activity of Human T Hybrids. T hybridomas and Jurkat-6TG-3 were harvested from logarithmically growing cultures, washed, resuspended in Mishell–Dutton media containing RPMI 1640 (19), and seeded into triplicate microwells at 1×10^3 to 1×10^5 cells per well. Semiautologous B cells and monocytes were prepared from PBL by neuraminidase-treated sheep erythrocyte-rosetting (20), OKT-3 antibody (Ortho Pharmaceuticals, Raritan, NJ), and rabbit complement (Pel-Freez) treatment to remove T cells. B cells and monocytes were seeded at 10^5 cells per microwell containing normal T cells, T cell hybrids, parental Jurkat-6TG-3, or media. Varying concentrations of TeT and 10% monocyte-conditioned media (21) were added. After 24 hr, 10% AB human serum was added (21), from which anti-TeT antibody had been adsorbed by affinity chromatography. After 4–6 days of culture, cells were collected, washed free of antigen, and resuspended in fresh media for an additional 2 days. Supernatants from all groups were assayed for anti-TeT IgG in a solid-phase radioimmunoassay (22).

RESULTS

Production of TeT-Specific T–T Hybridomas. The continuous line of IL 2-propagated normal T cells used for fusion was found to proliferate specifically to TeT only in the presence of autologous monocytes and to provide dose-dependent helper activity to autologous B cells resulting in anti-TeT antibody production (data not shown).

After a representative fusion of this TeT-specific line with Jurkat-6TG-3, five clones of cells grew in HAT medium. The karyotype and surface phenotype of four of these clones are presented in Table 1. The mean chromosome number in the hybrids was hypotetraploid ($n = 72$) compared to the nearly diploid number ($n = 46$) carried by the Jurkat-6TG-3 (Table 1). These karyotypes remained constant when tested after 1 and 4 months in culture.

The HLA antigens of the TeT-specific continuous T cell line used for fusion were A, 29,33; B, 12,14; DR, 7/1. The HLA phenotype of the Jurkat-6TG-3 was A, 1,3; B, 7,35; DR, 4. The T hybrids typed as follows: A, 1,3,29,33; B, 12,14,7,35; DR, 7,4,2. Taken together, these data indicate that the HAT-resistant clones are hybrids between Jurkat-6TG-3 and the TeT-specific T cell line. In addition, these hybrids appear to express a new DR haplotype, DR2, not detected on either parent.

Hybrids were examined for their expression of T cell markers and the results are shown in Table 1. More than 95% of all hybrids and Jurkat-6TG-3 expressed the pan-T cell receptor for sheep erythrocytes, the Fc receptor for Ig, and the pan-T cell OKT-3-defined antigen. The helper (OKT-4) and suppressor/cytotoxic (OKT-8) determinations showed that no hybrid was exclusively helper or suppressor phenotype. In fact, at least one hybrid (SH5) contained cells that expressed both helper and suppressor phenotypes simultaneously. Moreover, Jurkat-6TG-3 appeared to contain a population of OKT-4- and OKT-8-negative cells. In contrast, the TeT-specific continuous T cell line uniformly expressed the OKT-3 marker and was enriched for the OKT-4 (helper) phenotype (data not shown).

Antigen Specificity of T–T Hybrids. Two approaches were used to evaluate the antigen specificity of these hybrids: (*i*) the ability to produce IL 2 after antigenic stimulation; (*ii*) the binding of T–T hybrids to semiautologous monocytes prepulsed with antigen.

In the first case, the hybrids were evaluated for their ability to produce IL 2 after antigenic stimulation in the presence of semiautologous γ-irradiated adherent cells (monocytes) from semiautologous PBL. The results are presented in Table 2. Clones SH1 and SH3 responded minimally to TeT by exhibiting a 3-fold increase in IL 2 production when exposed to antigen. SH2 showed a 5-fold increase in IL 2 production with TeT at 10 μg/ml. SH5, in contrast, was totally unresponsive as was the parental line Jurkat-6TG-3. Irradiated peripheral blood monocytes alone were also nonproductive. The presence of irradiated monocytes appeared to augment the ability of all hybrids and Jurkat-6TG-3 to respond to mitogens. This effect may be due to the production of interleukin 1 (IL 1) by the monocytes which has been shown to be the second signal in the induction of T cell-derived IL 2 by mitogens (23).

Table 1. Genotypic and phenotypic characteristics of T–T hybrids

Cell type	Genotype: Chromosomes, no.†	Phenotype*: SE‡ receptor	FC‡ receptor	OKT§ 3	OKT§ 4	OKT§ 8
SH1	72 (44–106)	>95	>99	86	28	46
SH2		>95	>99	58	46	67
SH3		>95	>99	50	55	59
SH5		>95	>99	72	83	83
Jurkat-6TG-3 (parent)	46 (45–47)	>95	>99	98	28	13

* Percent positive.
† Values are given as the mean (range in parentheses).
‡ Determined by rosetting assay. A minimum of 200 cells was counted.
§ Determined by quantitative flow cytometry.

Table 2. Recognition of TeT by human T–T hybrids by IL 2 production in the presence of semiautologous monocytes

Hybrids tested†	Induction of IL 2 with TeT, μg/ml* 0	0.2	1	5	10	PHA-P‡	Con A
SH1	1,365	1,328	3,105	2,929	1,806	14,527	20,327
SH2	2,614	3,916	4,380	4,873	11,277	22,112	6,028
SH3	2,201	1,636	7,373	1,517	1,181	7,476	12,616
SH5	719	443	588	1,077	461	15,879	15,538
Jurkat-6TG-3 (parent)	887	328	316	416	402	13,911	35,189
MΦ alone	1,107	804	785	333	361	1,128	2,724

MΦ, macrophage.

* Results are expressed as the cpm of [^{3}H]dThd incorporated by mouse CTLL cells after a 24-hr incubation in the presence of supernatants (30% final volume) from the sources indicated.

† T hybrids, washed free of HAT media, were seeded at 2×10^6 cells per ml in RPMI 1640 and 10% fetal calf serum on 3-hr adherent monolayers from peripheral blood cells that had been rosetted with neuraminidase-treated sheep erythrocytes to remove T cells and irradiated with 5,000 rad from a cesium source. Microcultures were then stimulated with antigen or mitogen for 24 hr. Supernatants from each group were collected and tested for their ability to support growth of a continuous line of IL 2-dependent mouse CTLL as described.

‡ PHA-P, phytohemagglutinin-P; optimal concentrations of each mitogen were used to obtain maximal IL 2 production.

The second approach to determine antigen recognition by these T hybrids utilized the observations that antigen-specific T lymphocytes from guinea pigs bound specifically to homologous antigen-pulsed autologous macrophages *in vitro* (24). By prelabeling the hybrids with [^{3}H]dThd and performing cell counts, we determined the percentage of hybrids bound to monocyte cultures in the presence and absence of specific antigen. The results in Table 3 show that twice as many SH1 and SH3 cells attached to the TeT-pulsed monocytes compared to SKSD-pulsed monocytes. The majority (57%) of SH2 cells were bound to TeT-pulsed monocytes. As was seen in the induction of IL 2, SH5 and Jurkat-6TG-3 were totally negative for binding. Less than 1% of all hybrids attached to wells without monocytes or to empty wells prepulsed with TeT at 20 μg/ml (data not shown).

Table 3. Recognition of TeT by human T–T hybrids: Binding of hybrids to antigen-pulsed semiautologous monocytes

Hybrids tested†	Binding to autologous monocytes prepulsed with* Media	TeT	SKSD
SH1	564 (13)	1,097 (26)	604 (14)
SH2	500 (12)	2,386 (57)	469 (11)
SH3	429 (13)	814 (26)	405 (13)
SH5	325 (7)	298 (7)	216 (5)
Jurkat-6TG-3 (parent)	108 (1)	292 (2)	146 (1)

* Results are expressed as the mean cpm of [^{3}H]dThd collected on filter strips from the cells binding to the adherent cell population. The SEM was <10% of the mean. The number in parentheses is the percentage of T cells that adhered to the attached monocytes out of the total number added to the adherent cell monolayer.

† T hybrids and Jurkat-6TG-3 cells were prelabeled with [^{3}H]dThd and washed and the radioactivity was counted; equal numbers of all clones were added to 24-hr cultures of autologous monocytes prepulsed with nothing (media), TeT (20 μg/ml), or SKSD (20 μg/ml). After 3 hr at 37°C, the nonadherent cells were washed away and the adherent cells were harvested.

These findings correlate with the IL 2 induction experiments that also show that SH1 and SH3 are marginally recognitive of TeT with monocytes, whereas SH2 demonstrated significant antigen recognition. These experiments do not distinguish between the possibilities that a greater proportion of SH2 expresses the receptor for monocyte-associated TeT or that the SH2 receptor has a higher affinity for antigen. However, our goal of obtaining antigen-specific helper T hybrids seemed best accomplished by using clone SH2.

Antigen-Induced Helper Function of T–T Hybrids. Initial experiments demonstrated small but significant helper activity provided by SH2 for semiautologous B cells when stimulated with TeT in a Mishell–Dutton culture for *in vitro* antibody production (data not shown). Therefore, we subcloned SH2 at 0.3 cell per well in an effort to obtain lines with greater activity. Thirteen subclones were obtained from 96 wells and these were evaluated for their ability to induce semiautologous B cells to generate anti-TeT antibody *in vitro*. The results of one such experiment with three subclones are presented in Fig. 1. Whole PBL showed maximal specific antibody production when stimulated with TeT at 1 ng/ml. After depletion of T cells from PBL, no antibody production could be detected at any concentration of antigen. As a source of helper T cells, subclones of SH2 (SH2E5, SH2E6) or Jurkat-6TG-3 were added at 10^4 T hybrids per 10^5 semiautologous B cells. In the presence of both hybrids, semiautologous B cells were induced to secrete high levels of anti-TeT antibody over a wide range of antigen concentrations. Jurkat-6TG-3 demonstrated no helper activity for B cells at any concentration of antigen. However, in the absence of antigen, we observed higher "background" levels of antibody production than we did with the hybrids.

Several clones such as SH2E9 and SH2E12 produced undetectable amounts of IL 2 in the CTLL assay. These same clones were also negative for helper activity when assayed in our modified Mishell–Dutton cultures. We retested these negative subclones for helper function in the presence of exogenous

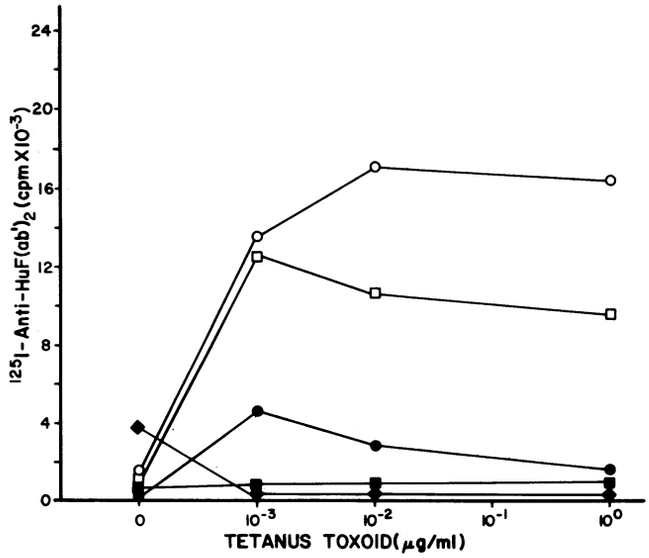

FIG. 1. Antigen-induced helper activity of T hybrids specific for TeT. PBL (○), B cells and monocytes alone (■), or B cells and monocytes and 10^4 SH2E5 (●), 10^4 SH2E6 (□), or 10^4 Jurkat-6TG-3 (◆) were cultured for 6 days in the presence of TeT. All cultures were then washed and recultured for 2 days without antigen. Supernatants were assayed for anti-TeT antibody in a solid-phase radioimmunoassay by using ^{125}I-labeled Ig fraction of goat anti-human F(ab')$_2$ fragments. The SEM was <10% of the mean. The nonspecific binding of supernatants to plates without TeT was <600 cpm.

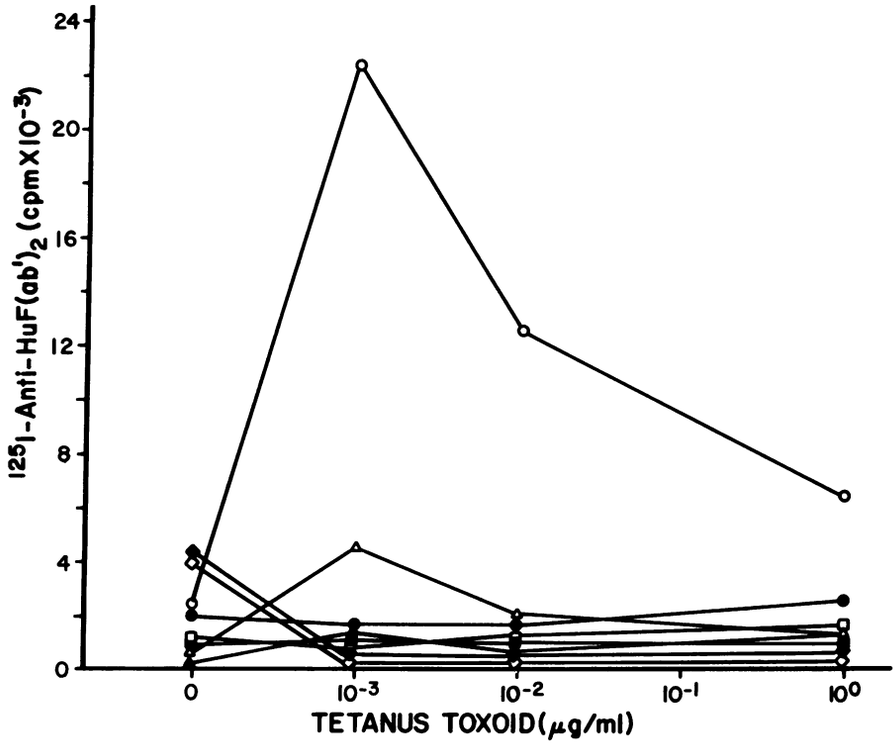

FIG. 2. Helper activity of human T–T hybrid is augmented by exogenous IL 2. Cultures of 1×10^5 semiautologous B cells and macrophages were cultured for 6 days in the presence of media (■), IL 2 at 2 units/ml (□), 10^4 SH2E12 (●), 10^4 SH2E12 and IL 2 (○), 10^4 Jurkat-6TG-3 (◆), 10^4 Jurkat-6TG-3 and IL 2 (◇), 10^4 normal T cells (▲), or 10^4 normal T cells and IL 2 (△). Cells were washed and resuspended in fresh media without antigen for 2 days. Supernatants were then assayed in a solid-phase radioimmunoassay for anti-TeT antibody by using ^{125}I-labeled Ig fraction of goat anti-human F(ab')$_2$ fragments. The SEM was <10% of the mean.

semipurified human IL 2 and compared the antibody produced with normal T cells as a source of help. The results of one such experiment with SH2E12 are presented in Fig. 2. Semiautologous B cells and monocytes alone produced no detectable anti-TeT antibody at any antigen concentration. Supplementing the B cells with 10^4 normal autologous T cells from PBL or human IL 2 (0.2–20 units/ml) resulted in no increase in antibody production. However, IL 2 at as little as 2 units/ml in cultures containing B cells and 10^4 normal T cells produced measurable anti-TeT antibody when the stimulating antigen concentration was optimal (2 ng/ml). These data indicate that: (*i*) signaling of B cells by normal T cells is required for specific antibody production for which IL 2 alone cannot substitute; and (*ii*) 10^4 normal peripheral T cells provide suboptimal levels of helper activity for B cells and the presence of exogenous IL 2 in these cultures results in optimal antibody production, presumably due to clonal expansion of specific helper T cells.

SH2E12 hybrid cells in numbers ranging from 1×10^3 to 5×10^4 demonstrated no helper function when assayed on semiautologous B cells. However, the addition of IL 2 at as little as 2 units/ml resulted in a dramatic increase of specific antibody production at antigen concentrations from 1 ng/ml to 10 μg/ml. Jurkat-6TG-3 was inactive in the absence or presence of IL 2.

Table 4 is a summary of the functional and phenotypic characteristics of several subclones of SH2. As can be seen, TeT-induced IL 2 production and binding to TeT-pulsed monocytes did not always correlate. However, helper function was observed with every clone that was positive for *either* IL 2 induction *or* binding. The OKT-4 and OKT-8 phenotypes of the subclones indicate that fewer cells were positive for these markers and "null" cells were present in each population. Percentages of OKT-4-positive cells could not be correlated with helper activity.

Table 4. Comparison of function and phenotype of subclones of SH2

Clone	IL 2 production*		Binding†	Helper activity‡	OKT-4	OKT-8
	Mitogen	Antigen				
SH2E3	+	−	−	NT	35	25
SH2E4	+	−	−	−	19	1
SH2E5	+	+	−	+	31	10
SH2E6	+	+	+	+	31	17
SH2E7	+	−	+	NT	25	6
SH2E9	+	−	+	+	31	7
SH2E12	+	−	+	+	13	1
SH2E13	+	−	−	−	12	1
SH2E15	+	+	−	NT	10	1

* Indicates the ability of T hybrids to produce IL 2 in response to mitogens in the absence of accessory cells or to antigen (TeT) in the presence of accessory cells.

† A + sign indicates the ability of 50% of T hybrids plated to adhere to TeT-pulsed monocytes.

‡ Indicates the helper activity of T hybrids in the induction of anti-TeT antibody by semiautologous B cells. A + sign represents >4-fold increase in specific antibody compared to cultures of B cells and antigen alone. NT, not tested.

DISCUSSION

We have described the production of antigen-specific human T cell hybridomas with functional helper activity. The advantages of using T cell hybrids versus normal antigen-reactive T cells to study lymphocyte–lymphokine interactions are several-fold. First, hybrids can be propagated without the requirement of autologous accessory cells and IL 2. Second, in our hands, the cloning efficiency is significantly better for hybrids than for normal continuous T cell lines (15–20% versus 1–5%, respectively). Third, due to the rate of proliferation (24-hr doubling time), large numbers of cells can be propagated to study secreted soluble products and membrane-bound receptors for lymphokines and antigen. Although the frequency of fusion products was low (5–10 hybrids per 10^6 lymphoma cells), we obtained at least one antigen-specific clone in every fusion performed. Currently, these clones still possess specificity and function after more than 6 months in culture.

The TeT-specific hybrids were shown to produce IL 2 after mitogen or homologous antigen stimulation as described in the murine system (7). Namely, induction of IL 2 by mitogens does not require accessory cells whereas its induction by homologous antigen does. In the latter case, the source of accessory cells was semiautologous and shared HLA determinants with only one genetic complement of the hybrids. Experiments must be undertaken to determine if the induction of IL 2 after antigen recognition is restricted to certain HLA-encoded products shown to be involved in antigen recognition by T cells (5, 25).

A more rapid assay for antigen specificity of T hybrids was also described—namely, the binding of T hybrids to homologous antigen-pulsed monocytes. These two assays appeared to correlate when performed immediately after hybrids were detected. In the initial experiments presented here, the data indicated that one clone (SH2) demonstrated the most significant level of antigen recognition in both assays. When both assays were performed on subclones of SH2, we found that IL 2 induction and antigen binding could be mutually exclusive properties. The advantage of using the binding assay was that it detected clones of helper hybrids that did not produce IL 2 after antigen stimulation.

Subclones of the TeT-specific clone SH2 showed a heterogenous pattern of function and phenotype. Our interpretation of these results is that during 3–4 months of subcloning and culture, SH2 experienced chromosome loss and that the abilities to bind accessory cell-bound antigen and to produce IL 2 after antigen stimulation are not encoded on the same chromosome. Chromosome loss analysis of these subclones may answer this question. Alternatively, SH2 may not have been a clone when isolated and the IL 2-producing and antigen-binding populations were mutually exclusive. We tend to discount this explanation owing to the low frequency of hybrids growing in HAT which argues against several hybrids appearing in the same well. However, it appears that the combination of both these assays detects antigen specificity because subclones of SH2 that were negative for binding *and* IL 2 production were also negative for helper function.

Potent helper activity of the TeT-specific T hybrids was demonstrated in an *in vitro* assay for antibody induction. Under these conditions, the addition of small numbers of T hybrids (10^4 per 1×10^5 B cells) induced dramatic production of specific antibody. The helper capacity of some clones (SH2E5 and SH2E6) was demonstrable in the absence of exogenous IL 2. These two clones produce IL 2 when stimulated with antigen and accessory cells, and therefore IL 2 was most likely generated endogenously in the Mishell–Dutton cultures. SH2E12, an IL 2 nonproducer, demonstrated helper activity only when exogenous IL 2 was added. Because these hybrids proliferate independently of IL 2, the action of this lymphokine is not as a T cell growth factor *per se*. IL 2 may be acting as a differentiation factor leading to the induction of antibody synthesis either at the level of the T cell or the B cell. Synergy between IL 2 and a T cell replacing factor produced by mouse T cells has been documented in several laboratories (26, 27).

The antigenic specificity of these hybrids has been confirmed vis-à-vis the afferent or recognitive arm of T cell function. It was interesting to note that, unlike normal T cells, these T hybrids responded to antigen over a broad concentration range—i.e., there was no high antigen dose inhibition of helper activity. It is feasible that once induced by specific antigen, these T hybrids will "help" antibody responses to other antigens provided that the B cell repertoire includes cells that recognize these antigens (28).

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