Proc. Natl. Acad. Sci. USA
Vol. 76, No. 8, pp. 4061–4065, August 1979
Immunology

Separation of functional subsets of human T cells by a monoclonal antibody

(hybridoma/T cell antigens/helper T cells)

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Communicated by Philip Levine, May 18, 1979

ABSTRACT **A monoclonal antibody was produced to human peripheral blood T cells. This hybridoma antibody, termed OKT4, was reactive by indirect immunofluorescence with only 55–60% of the peripheral blood T cell population ($OKT4^+$) and unreactive with normal B cells, null cells, and macrophages. The $OKT4^-$ T cell population contained the previously described TH_2^+ subset that has been shown to contain cytotoxic/suppressor cells. With cell-sorter separation of $OKT4^+$ and $OKT4^-$ cells, it was shown that these T cell subsets were functionally discrete. Both gave proliferative responses with concanavalin A, alloantigens, and phytohemagglutinin, although $OKT4^+$ cells were much more responsive to the latter. $OKT4^+$ cells alone responded to soluble antigens whereas $OKT4^-$ cells alone were cytotoxic after alloantigenic sensitization of unfractionated T cells. However, both $OKT4^+$ and $OKT4^-$ cells were required during sensitization for optimal development of cytotoxicity. These data suggest that the $OKT4^+$ subset represents a helper population and that the $OKT4^-$ subset contains the cytotoxic effector population. OKT4 could be a valuable reagent for determining alterations of these functional subsets in human diseases.**

T cell heterogeneity has been shown to exist in several species including man (1–6). Prior studies utilizing differential Fc receptor binding, autoantibodies, and heteroantisera have facilitated the dissection of human T cell populations into distinct subsets with unique functional properties (4–6). By the use of a heteroantiserum termed anti-TH_2, approximately 20% of peripheral blood T cells were shown to be TH_2^+ (4). These TH_2^+ cells are phenotypically stable after activation and contain both the mature suppressor and cytotoxic effector cells (3, 4). The T cell population unreactive with anti-TH_2 (TH_2^-) was heterogeneous with respect to binding with an autoantibody found in serum of juvenile rheumatoid arthritis patients (anti-JRA) (7). The TH_2^- subset was therefore divided into JRA+ and JRA− subsets. In addition, the TH_2^- subset of cells was shown to contain a helper population and could also modulate the generation of concanavalin A (Con A)-inducible suppressor cells (4).

The recent development of monoclonal antibodies to cell surface antigens has provided an additional tool for the orderly dissection of subsets of T cells bearing distinct antigens (8–13). Recently, we described a hybridoma antibody that defined the entire human peripheral T cell population but was unreactive with lymphocytes of non-T lineage (14). In the present study, we have produced a hybridoma secreting monoclonal antibody reactive with a subset of human T cells. In the studies to be described, it will be shown that the hybridoma antibody, termed OKT4, reacts selectively with 55–60% of human peripheral blood T lymphocytes distinct from the TH_2^+ subset. Functional studies indicate that the $OKT4^+$ subset represents the equivalent of the murine Lyl inducer (helper) cell population (1).

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MATERIALS AND METHODS

Production of Monoclonal Antibodies. (*i*) *Immunization and somatic cell hybridization.* An 8-week-old female CAF_1 mouse (Jackson Laboratory) was immunized intraperitoneally with 2×10^7 purified human peripheral T cells in phosphate-buffered saline at 14-day intervals. Four days after the third immunization, the spleen was removed and a single-cell suspension was made. Cell fusion was carried out according to the procedure developed by Kohler and Milstein (8). Splenocytes (1×10^8) were fused by using 35% polyethylene glycol in 5% dimethyl sulfoxide and RPMI 1640 (GIBCO) with 2×10^7 P3X63Ag8U1 myeloma cells, kindly supplied by M. D. Scharff (Albert Einstein College of Medicine, Bronx, NY).

(*ii*) *Selection and growth of hybridoma.* After cell fusion, cells were cultured in hypoxanthine/aminopterin/thymidine medium at 37°C with 5% CO_2 in a humid atmosphere. Several weeks later, 40–100 μl of supernatant from cultures containing hybridomas were tested on 10^6 peripheral lymphocytes separated into E rosette-positive (E^+) and E rosette-negative (E^-) populations, which were prepared from the peripheral blood of healthy human donors as described by Mendes *et al.* (15). Detection of mouse hybridoma antibodies to these cells was determined by indirect immunofluorescence. Cells incubated with culture supernatants were stained with a fluorescent goat anti-mouse IgG (Meloy Laboratories, Springfield, VA) (fluorescein to protein ratio = 2.5), and fluorescent cells were subsequently analyzed on the Cytofluorograf FC200/4800A (Ortho Instruments, Westwood, MA) as described below. Hybridoma cultures containing antibodies reacting specifically with E^+ lymphocytes (T cells) were selected, cloned, and recloned by limiting dilution methods in the presence of feeder cells (13). Subsequently, the clones were transferred intraperitoneally by injecting 1×10^7 cells of a given clone into CAF_1 mice primed with pristine (Aldrich). The malignant ascites from these mice were then used to characterize lymphocytes as described below.

Characterization of OKT4 Reactivity: Isolation of Lymphocyte Populations. Human peripheral blood mononuclear cells were isolated from healthy volunteer donors (15 to 40 years old) by Ficoll/Hypaque density gradient centrifugation (Pharmacia) (16). Unfractionated mononuclear cells were separated into surface Ig^+ (B) and Ig^- (T plus null) populations

Abbreviations: FACS, fluorescence-activated cell sorter; Con A, concanavalin A; PHA, phytohemagglutinin; G/M FITC, fluorescein-conjugated goat anti-mouse IgG; CML, cell-mediated lympholysis; MLC, mixed lymphocyte culture.

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by Sephadex G-200 anti-F(ab')$_2$ column chromatography as described (17). T and null cells were separated from Ig$^-$ cell fraction as described (17). Normal human macrophages were obtained from the mononuclear population by adherence to plastic at 37°C overnight. The adherent population was detached by washing with cold serum-free medium containing 2.5 mM EDTA. Greater than 85% of these cells ingested latex particles.

Cytofluorographic Analysis and Cell Separation. Cytofluorographic analysis of all cell populations was performed by indirect immunofluorescence with fluorescein-conjugated goat anti-mouse IgG (G/M FITC) (Meloy Laboratories) on a Cytofluorograf FC200/4800A (Ortho instruments). In brief, 1–2 × 10^6 cells were treated with 0.15 ml of either of two monoclonal antibodies, termed OKT1 and OKT4, at a 1:1000 dilution, incubated at 4°C for 30 min, and washed twice. The cells were then mixed with 0.15 ml of a 1:40 dilution of G/M FITC for 30 min, centrifuged, and washed three times. These cells were then analyzed on the Cytofluorograf, and the intensity of fluorescence per cell was recorded on a pulse height analyzer. It should be noted that a similar pattern of reactivity with OKT4 was seen at a dilution of 1:50,000. OKT1 reactivity was seen at a dilution of 1:30,000 (unpublished results). Background staining was obtained by substituting a 0.15-ml aliquot of 1:1000 ascites from a BALB/c mouse injected intraperitoneally with a nonproducing clone.

In experiments designed to separate OKT4$^+$ and OKT4$^-$ T cells, 100 × 10^6 T cells were labeled with 4 ml of a 1:1000 dilution of OKT4 and developed with G/M FITC. T cells were separated by utilizing a fluorescence-activated cell sorter (FACS-I) (Becton, Dickinson, Mountain View, CA) (18, 19). Post-sort viability was >95% by trypan blue exclusion.

Analysis of FACS-Separated OKT4$^+$ and OKT4$^-$ Subsets with Equine Anti-TH$_2$. OKT4$^+$ and OKT4$^-$ T cells were separated on the FACS and placed in culture at 2 × 10^6 cells per ml in RPMI 1640 (GIBCO) containing 20% human AB serum, 1% penicillin/streptomycin, 200 mM L-glutamine, 25 mM Hepes buffer (Microbiological Associates, Bethesda, MD), and 0.5% sodium bicarbonate. After 24 hr in a 5% CO_2 humid atmosphere at 37°C, populations were mixed with equine anti-TH$_2$ and analyzed as described (4).

Functional Studies. (*i*) *Proliferative studies.* The mitogenic response of the unseparated and FACS-fractionated lymphoid cells was tested in microculture to optimal and suboptimal doses of Con A (Calbiochem) and phytohemagglutinin (PHA) (Burroughs Wellcome, Research Triangle Park, NC) as described (17). Alloantigen proliferative response was measured concurrently for these same populations by stimulating with mitomycin C-treated Laz 156, an Epstein–Barr virus-transformed human B lymphoblastoid cell line (4). Proliferation to tetanus toxoid (Massachusetts Department of Public Health Biological Laboratories, Boston, MA) was tested by utilizing 10 μg/ml final concentration. Herpes-zoster antigen was kindly provided by John Zaia (Harvard Medical School, Boston, MA) and utilized at a 1:6 dilution. Macrophages obtained as described above were added to all populations at 5% final concentration at the initiation of *in vitro* cultures.

(*ii*) *Cytotoxicity studies.* Sensitization cultures for cell-mediated lympholysis (CML) were established by placing unfractionated T cells, FACS-separated OKT4$^+$ and OKT4$^-$ T cell subsets, or different ratios of recombined OKT4$^+$ and OKT4$^-$ T cells with mitomycin-treated stimulator cells, all at 2 × 10^6 cells per ml in multiple microtiter plate wells as described (3). At the end of 5 days, these T cell populations were then added to ^{51}Cr sodium chromate-labeled target cells, and specific cytotoxicity was determined after a 6-hr cell incubation as described (20). In other experiments, unfractionated T cells were sensitized with mitomycin-treated stimulator cells as above and then fractionated into OKT4$^+$ and OKT4$^-$ T cell subsets on FACS after 5 days in mixed lymphocyte culture (MLC), and specific lysis was determined. All determinations were performed in triplicate, and the results are expressed as the mean ± SD.

RESULTS

Preliminary Characterization of the Antibody Reactivity of Hybridomas from the Cell Fusion. After polyethylene glycol-induced fusion, the cells were distributed in 384 culture wells, each containing 1 ml of hypoxanthine/aminopterin/thymidine medium. Supernatants from 34 wells reacted with human E$^+$ cells. Thirty-two of these supernatants also reacted with human E$^-$ cells; thus, two wells contained supernatants that reacted selectively with a fraction of human T cells. The hybridomas in these wells were cloned and recloned. The final clone derived from one of these wells was termed OKT4$^+$. The OKT4$^+$ hybridoma antibody was demonstrated to be of the IgG$_2$ subclass by the specificity of the staining with fluorescein-labeled goat anti-mouse IgG$_2$ and its failure to be stained by fluorescein-labeled antibodies directed against other subclasses of mouse immunoglobulin. OKT1 hybridoma antibody, obtained in a similar fashion, was shown to be of the IgG$_1$ subclass (14).

Characterization of OKT4 Antibody Reactivity on Normal Human Lymphoid Cells. Highly purified T, B, null, and macrophage populations were prepared from a given individual's peripheral blood mononuclear population. Fig. 1 shows the fluorescence pattern of 10,000 cells obtained on the Cytofluorograf after these populations had reacted with OKT1 or OKT4 at a 1:1000 dilution and G/M FITC. The entire T cell population (95% E rosetting cells) was reactive with OKT1 (14). In contrast, only approximately 60% of these T cells were reactive with OKT4, thus defining a subset of T cells binding monoclonal antibody. Furthermore, neither OKT1 nor OKT4 bound to normal human B cells, null cells, or macrophages from the

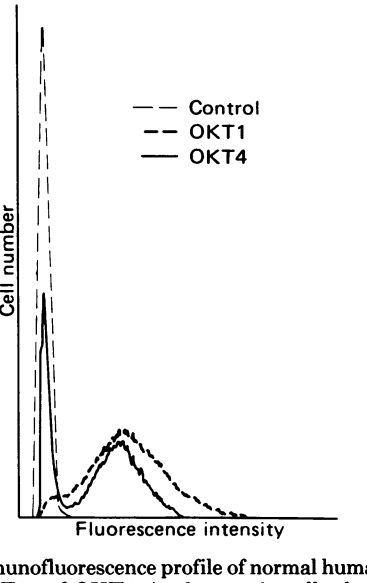

FIG. 1. Immunofluorescence profile of normal human peripheral T cells with OKT1 and OKT4. As shown, virtually the entire T cell population (95% E rosetting) is OKT1$^+$ (1:1000 dilution). In contrast, OKT4 reacts with only 55–60% (5,800 of 10,000 cells) of the same T cell population (1:1000 dilution). Background fluorescence staining was obtained by incubating each population with a 1:1000 dilution of ascitic fluid from a mouse injected with a nonproducing hybrid clone.

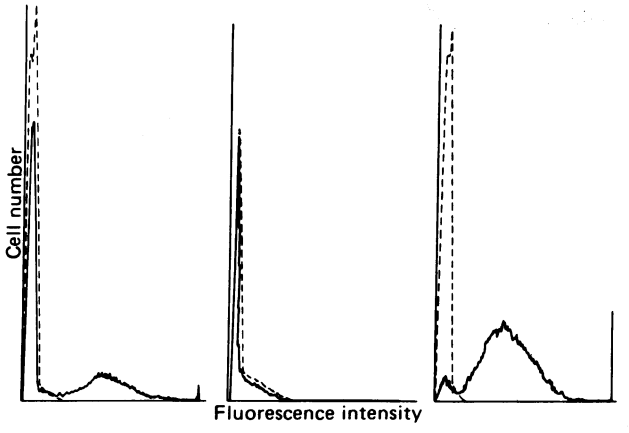

FIG. 2. Reactivity of OKT4-separated subsets with anti-TH_2. Unfractionated T cells or FACS-separated $OKT4^+$ and $OKT4^-$ T cell subsets were mixed with equine anti-TH_2 (1:125 dilution) and R/H FITC. As shown, the unfractionated T cell population (*Left*) is 25% TH_2^+ (2,500 of 10,000 cells reacted). In contrast, the $OKT4^+$ subset (*Center*) contains no TH_2^+ T cells, whereas the $OKT4^-$ T cell subset (*Right*) is predominantly TH_2^+ and contains the entire subpopulation of TH_2^+ T cells found in the unfractionated T cell population. Background fluorescence staining (dashed line) was obtained by incubating each population with a 1:125 dilution of normal horse IgG.

same individual at any dilution tested (data not shown). Similar results were obtained on analysis of lymphocyte subpopulations from 10 additional individuals (data not shown).

Because the OKT4 antibody appeared to define a human T cell subset, it was important to relate the antibody-reactive ($OKT4^+$) and antibody-unreactive ($OKT4^-$) T cells to the TH_2^+ subset that was previously shown to be functionally stable. To this end, a total peripheral T cell population was sorted on the FACS into $OKT4^+$ and $OKT4^-$ subsets, which were then mixed with equine anti-TH_2, stained with FITC-IgG fraction rabbit/horse IgG (Cappel Laboratories, Cochranville, PA), and subsequently reanalyzed on the FACS. As shown in Fig. 2, anti-TH_2 reacted with approximately 25% of the unfractionated T cell population. The $OKT4^+$ population was unreactive with TH_2, whereas approximately 65% of the $OKT4^-$ population was TH_2^+. These results indicate that TH_2^+ and $OKT4^+$ subsets are reciprocal and distinct from one another. The nature of the $OKT4^-$ TH_2^- subset, which accounts for 10–20% of the T cell population, remains to be determined.

Functional Characterization of T Cell Subsets Separated with OKT4 Monoclonal Antibody on FACS. (*i*) *Proliferative studies: Mitogen, soluble antigen, and cell surface antigen responses.* An unfractionated T cell population was treated with a 1:1000 dilution of OKT4 and G/M FITC and separated on the FACS into $OKT4^+$ and $OKT4^-$ subsets. Given the purity of the populations obtained (≥95%), the separated populations were supplemented with 5% macrophages prior to *in vitro* culture. The unfractionated T cell population and isolated $OKT4^+$ and $OKT4^-$ T cell subsets were then stimulated with PHA, Con A, soluble antigens, and alloantigens to assess their *in vitro* proliferative responses.

The proliferative response of the unfractionated T cell populations to PHA and Con A is shown in Table 1. A maximal proliferative response by the unfractionated T cell population was obtained with 1 μg of PHA per 10^6 cells with diminished responses occurring at 0.5 μg and 0.1 μg of PHA per 10^6 cells. Treatment of the unfractionated T cells with OKT4 and G/M FITC without subsequent fractionation did not alter the proliferative response. By contrast, differences in response to PHA were obtained with the separated $OKT4^+$ and $OKT4^-$ T cell subsets. The $OKT4^+$ population of cells responded to all doses of PHA in a fashion similar to the unseparated T cell population. However, the proliferative response of $OKT4^-$ cells was significantly less at all doses of PHA tested. Furthermore, at a dose of PHA of 0.1 μg per 10^6 cells, the $OKT4^-$ T cells did not proliferate at all, whereas the $OKT4^+$ T cell subset and unfractionated cells were still responsive. The proliferative response of these subsets to Con A, on the other hand, was similar, and the two subsets of cells could not be distinguished from one another or the unfractionated T cell population (Table 1).

The responses to alloantigen in MLC and to soluble antigens were next examined. As shown in Table 2, the unfractionated T cell population, the unfractionated T cell population treated with OKT4 and G/M FITC, and both the $OKT4^+$ and $OKT4^-$ T cell subsets responded in a similar fashion in MLC against Laz 156. It should be noted, however, that the $OKT4^-$ cells from two of six individuals tested, although proliferating significantly in MLC, incorporated less [^{3}H]thymidine than their respective $OKT4^+$ subset (data not shown). Proliferative responses to soluble antigens provided the clearest distinction between the subsets. In all cases tested, the $OKT4^+$ T cell subset proliferated well to the soluble antigens, tetanus toxoid, and Herpes-zoster, whereas the $OKT4^-$ T cell subset was virtually unresponsive (Table 2).

(*ii*) *Cell-mediated lympholysis: Effector capacity of hybridoma antibody-separated T cell subsets.* Previous studies have shown that specific cytotoxic effector cells are generated during the course of MLC (20). Although both $OKT4^+$ and $OKT4^-$ cells proliferated well to alloantigens, it remained to be determined whether one or both of these subsets mediated CML after sensitization in MLC. The unfractionated population of peripheral blood T cells was sensitized in MLC for 5 days against Laz 156 and then isolated into $OKT4^+$ and $OKT4^-$ T cell subsets. These unfractionated and isolated T cell subsets were placed in CML assay with ^{51}Cr-labeled Laz 156 targets, and specific lysis was determined at various effector-to-target

Table 1. Mitogen responsiveness of unfractionated and monoclonal antibody-separated T cell subsets

Proliferative stimulus*	T cells	T cells treated with OKT4 and G/M FITC	$OKT4^+$ T cells	$OKT4^-$ T cells
PHA (1 μg)	98,876 ± 3,061	99,780 ± 5,615	109,643 ± 11,043	23,841 ± 890
PHA (0.5 μg)	18,082 ± 3,588	17,423 ± 2,623	15,202 ± 603	3,185 ± 492
PHA (0.1 μg)	4,215 ± 386	4,317 ± 523	4,999 ± 677	72 ± 11
Con A (250 μg)	199,310 ± 14,317	192,158 ± 14,258	159,560 ± 2,619	174,992 ± 20,179
Con A (125 μg)	100,726 ± 8,864	92,633 ± 6,780	97,003 ± 9,089	82,199 ± 6,154
Con A (50 μg)	50,673 ± 6,155	52,317 ± 7,813	49,329 ± 4,110	38,842 ± 6,735
Media control	63 ± 2	57 ± 6	167 ± 8	69 ± 12

± refers to SD.
* Concentration per 10^6 cells.

Table 2. Proliferative response of unfractionated T cells and monoclonal antibody-separated T cell subsets to soluble antigens and alloantigens

Proliferative stimulus	T cells	T cells treated with OKT4 and G/M FITC	OKT4$^+$ T cells	OKT4$^-$ T cells
Exp. 1				
Laz 156	150,304 ± 6966	149,810 ± 7630	173,061 ± 4,336	167,087 ± 5228
Tetanus toxoid	18,046 ± 271	19,947 ± 3214	19,660 ± 2,348	516 ± 69
Herpes-zoster	47,413 ± 3208	40,774 ± 4305	55,785 ± 10,608	188 ± 129
Media	166 ± 22	180 ± 35	220 ± 10	106 ± 13
Exp. 2				
Laz 156	67,265 ± 3466	68,399 ± 6154	97,615 ± 4,361	67,483 ± 4792
Tetanus toxoid	16,853 ± 1053	18,597 ± 1643	17,222 ± 911	206 ± 23
Herpes-zoster	23,629 ± 3677	22,889 ± 1956	25,951 ± 2,609	169 ± 25
Media	167 ± 43	289 ± 57	298 ± 41	31 ± 8

± refers to SD.

cell ratios as shown in Fig. 3. These results demonstrated that the OKT4$^-$ T cell subset contains the effector population in CML. At effector-to-target ratios of 5:1, 10:1, and 20:1, this OKT4$^-$ T cell populations effected 32%, 44%, and 57% specific lysis, respectively. The lytic capacity of the isolated subset was significantly greater than that of the unfractionated T cell population. Perhaps of greater importance was the observation that the OKT4$^+$ T cell population was minimally cytotoxic at best. At an effector-to-target ratio of 20:1, the OKT4$^+$ population lysed only 10% of the target cells. Given our earlier observations that the TH_2^+ T cell subset in man effected CML and the present finding that the OKT4$^-$ population contains the entire TH_2^+ population, these results were not unexpected.

(*iii*) *Cytotoxicity: Helper capacity of hybridoma-separated T cell subsets in the generation of cytotoxic effectors.* The above studies demonstrated that the OKT4$^-$ subset contains the cytotoxic effector population after allosensitization of unfractionated T cells. Because prior murine and human studies (3, 21) suggested that the maximal cytotoxic response of the Ly2,3 subset in the mouse or the TH_2^+ subset in man required T cell help, the following studies were undertaken to determine whether OKT4$^+$ T cells help in the generation of the OKT4$^-$ cytotoxic cells. T cells were fractionated into OKT4$^+$ and OKT4$^-$ subsets prior to allosensitization in MLC, sensitized alone or recombined, and analyzed for specific cytotoxicity on day 6.

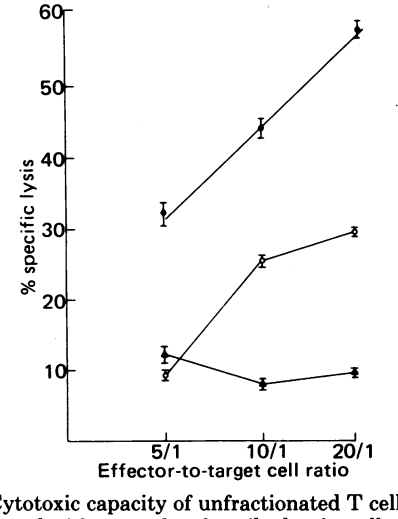

FIG. 3. Cytotoxic capacity of unfractionated T cells and T cell subsets separated with monoclonal antibody after allosensitization in MLC. As shown, the OKT4$^-$ T cell subset (●) effects CML and the degree of killing mediated by OKT4$^-$ T cells is greater than the unfractionated T cell population (○). In contrast, OKT4$^+$ T cells (▲) are minimally cytotoxic, even at high effector-to-target ratios. Bars represent SD for each group tested.

Table 3 shows that the OKT4$^-$ subset of T cells cannot generate much, if any, cytolysis when it is sensitized alone in MLC. Thus, although the OKT4$^-$ T cells became cytotoxic/effector cells in the unfractionated allosensitized T cell population and could respond in MLC, this subset alone could not be induced to mediate CML. Moreover, when the OKT4$^+$ population was sensitized in MLC in the absence of the OKT4$^-$ T cells, they could mediate a moderate, but significant, lysis in MLC. However, the recombined mixture of OKT4$^+$ and OKT4$^-$ T cells effected a maximal cytolysis, not unlike that of the unseparated T cell population. These findings demonstrate that the OKT4$^-$ subset cannot effect a maximal cytotoxic response alone, but requires help from the OKT4$^+$ population.

DISCUSSION

This study describes the production and characterization of a monoclonal antibody directed at a cell surface antigen on a subpopulation of human peripheral blood T cells. The hybridoma antibody, termed OKT4, resulted from the fusion between a myeloma line and splenocytes from a mouse immunized to human peripheral T cells. Analysis by immunofluorescence revealed that OKT4 reacted specifically with approximately 55–60% of peripheral blood T cells and was unreactive with normal B cells, null cells, and macrophages at any dilution tested. Purified T cells were separated into OKT4$^+$ and OKT4$^-$ T cell subsets on a FACS and tested for reactivity with TH_2 antibody. It was shown that the OKT4$^-$ T cell subset

Table 3. Evidence for T–T interactions in the generation of maximal cytotoxicity in CML

Responder population sensitized to Laz 156	% specific lysis* Exp. 1† 20:1	Exp. 1† 5:1	Exp. 2† 20:1	Exp. 2† 5:1
Unfractionated T cells	40	—	23	12
Unfractionated T cells treated with OKT4 + G/M FITC	44	24	25	13
OKT4$^-$ T cells	6	3	11	7
OKT4$^+$ T cells	26	14	18	12
OKT4$^+$ and OKT4$^-$ T cells‡	48	31	31	16

* Spontaneous release <20% in all cases and SD ≤10% at all effector-to-target cell ratios. Lysis by unsensitized T cells ≤3% for all populations tested.

† Effector-to-target cell ratios.

‡ OKT4$^+$ and OKT4$^-$ recombined at 1:1 ratios.

contained all of the TH_2^+ T cells, whereas the $OKT4^+$ population was unreactive with TH_2 antiserum. Thus, the $OKT4^+$ and TH_2^+ subsets are distinct and nonoverlapping when defined by specific cell surface markers.

The $OKT4^+$ and $OKT4^-$ subsets had functional differences as shown by their proliferative responses. The $OKT4^+$ subset proliferated maximally to soluble antigen and was comparable in its activity to the unfractionated T cell population. By contrast, the $OKT4^-$ population was virtually unresponsive to soluble antigen. The extent of responsiveness to PHA was also distinguishable between the subsets. It was shown, for example, that the $OKT4^+$ population proliferated maximally to PHA and was comparable to the unfractionated population at all doses of mitogen tested. However, the $OKT4^-$ population gave a suboptimal proliferative response to PHA at optimal concentrations of mitogen and was unresponsive at suboptimal concentrations. Both the $OKT4^+$ and $OKT4^-$ subsets proliferated significantly in response to Con A and alloantigen in MLC. These studies are in full agreement with previous studies (3, 4) defining T cell heterogeneity by use of T cell heteroantisera, wherein it was shown that both the TH_2^+ and TH_2^- subsets responsed well to alloantigen in MLC and Con A. In addition, the TH_2^- subset was shown to proliferate to soluble antigen, whereas the TH_2^+ subset did not (3). The observation that the TH_2^+ population is contained entirely within the $OKT4^-$, but not within the $OKT4^+$, population supports the above findings with a highly specific monoclonal antibody reagent, which now positively defines a subset of cells included in the TH_2^- subset.

It has been demonstrated in both human and murine systems that cytotoxic T cells are generated after allosensitization and that these cells are confined to distinct T cell subsets (3, 21). In the present study, only the $OKT4^-$ T cell subset was cytotoxic after alloactivation of the unfractionated population, in keeping with previous studies showing that this property resided in the TH_2^+ population. Thus, the $OKT4^-$ TH_2^+ T cell appears analogous to the $Ly2,3^+$ T cell in the murine system. The results obtained in CML when T cell subsets were separated prior to allosensitization in MLC were quite different from the above results in which the subsets were separated after allosensitization. The $OKT4^+$ subset, in the absence of the $OKT4^-$ population, known to contain regulatory cells, developed a measurable cytotoxic response that was considerably less than the cytotoxic response of the unfractionated population (4). The isolated $OKT4^-$ population became only minimally cytotoxic after alloactivation in the absence of the $OKT4^+$ cell. When $OKT4^+$ cells and $OKT4^-$ cells were recombined prior to initiation of the MLC, a maximal cytotoxic response occurred. These results are in keeping with the previous demonstration of T–T interactions in the generation of a cytotoxic cell's response. In the mouse, Ly1 cells induce the formation of Ly2,3 cytotoxic cells, and, in man, TH_2^+ and TH_2^- subsets collaborate in the generation of maximal cytotoxicity by the TH_2^+ subset (3, 21). These results help assign an inducer or helper role to the $OKT4^+$ population and provide additional evidence that a proliferative response to soluble antigen is restricted to the subset of cells with helper functions.

Additional studies are needed to determine whether the $OKT4^+$ T cell population is an inducer cell for B cell production of antibody and null cell differentiation into B cells and erythroid cells. The present study clearly supports the utility of this approach in defining T cell subsets, and the hybridoma antibody provides an exquisitely selective reagent for classifying functional subsets of human T cells according to their genetically programmed cell surface phenotype. Aberrations of suppressor cells have already been defined in human disease (22–24), and we would predict that abnormalities of $OKT4^+$ inducer cells will be found as well.

The authors thank Ms. Ann Penta, Ms. Carmeline O'Brien, Ms. Joan McDowell, Ms. Kathleen Singer, Ms. Mary Ann Talle, Ms. Mary DeMaria, and Ms. Georgia P. Lambrinos for skilled technical assistance. Thanks are also extended to Ms. L. M. Grappi and Ms. Cynthia Brain for secretarial assistance. This work was supported in part by National Institutes of Health Training Grant CA 09172 and Grants AI 12069, CA 19589 and CA 06516.

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