Functional characteristics of human T-cell subpopulations distinguished by a monoclonal antibody*

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EDGAR G. ENGLEMAN†, CLAUDIA BENIKE†, BARBARA OSBORNE‡, AND RICHARD GOLDSBY§

Departments of †Pathology and ‡Genetics, Stanford University School of Medicine, Stanford, California 94305; and §Department of Chemistry, University of Maryland, College Park, Maryland 20742

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ABSTRACT In animals and in man, diverse immunologic functions are mediated by specialized T-cell (thymus-derived lymphocyte) subsets that are distinguishable from one another on the basis of differences in cell surface determinants. Unfortunately, in humans, subset-specific antibodies have been difficult to generate. In this study, production of a murine monoclonal antibody specific for a subset of human T cells was achieved by fusing a sensitized B cell (bone marrow-derived cell) with a myeloma cell and isolating the antibody secreted by the resultant hybrid clone. This antibody binds 90–95% of peripheral T lymphocytes (T + cells) but fails to bind remaining T lymphocytes (T - cells), B lymphocytes, or monocytes. T + and T - subpopulations were separated with a fluorescence-activated cell sorter and their in vitro responses to various stimuli were assessed. T + and T - cells respond equally well to soluble antigens, allogeneic B cells, and autologous B cells, but only T + cells respond to concanavalin A. T + cells cultured in the presence of concanavalin A gradually lose the T + marker, an effect not observed after stimulation with phytohemagglutinin, soluble antigens, or alloantigens. These results suggest that the functional subpopulation of T cells defined by T + does not correspond to any previously described human T cell subset. Furthermore, somatic cell hybridization has been shown to be a feasible method for production of monoclonal antibodies specific for subpopulations of human lymphocytes.

A number of immunologic functions are attributed to T cells (thymus-derived lymphocytes), including proliferative responses to mitogens (1) and antigens (2), killing in cell-mediated lympholysis (3), and regulation of humoral and cell-mediated immunity via helper and suppressor functions (4–9). In mice, these diverse actions are mediated by specialized T-cell subsets distinguishable from one another on the basis of differences in cell surface determinants (10–12). Humans, too, have recently been shown to have functional T-cell subsets (13-15), but subset-specific antibodies have been difficult to generate. In general, such reagents must be produced in other species, and specificity can only be achieved after extensive absorption protocols (16).

To avoid this problem, we have utilized the technique of somatic cell hybridization (17) to produce a monoclonal antibody specific for a subset of human T cells. In a separate report (18) we describe the production of this antibody, establish that it reacted with peripheral human T cells but not B cells, and demonstrate that it precipitated its target antigen, which we have termed "T +", from preparations of purified T cells.

In this report, we demonstrate that one-third of normal peripheral T cells are T + and that T + but not T - cells proliferate vigorously in response to concanavalin A (Con A). These and other studies described herein suggest that monoclonal anti-T + antibody distinguishes a functional subset of human T cells.

METHODS AND MATERIALS

Isolation of Human T and Non-T Cells from Peripheral Blood. Peripheral blood mononuclear leukocytes (PBML) were obtained from normal volunteers by Ficoll/Hypaque density gradient centrifugation of fresh defibrinated blood (19). Populations enriched for T or non-T cells were prepared with a sheep erythrocyte rosetting technique (20). Briefly, 5 × 10⁶ cells per ml were suspended in 40% fetal calf serum in phosphate-buffered saline and mixed with an equal volume of 3% sheep erythrocytes in the same medium. This mixture was immediately sedimented over a second Ficoll/Hypaque gradient in order to separate the rosetted T cells (E-rosettes) from the nonrosetted cells. Cells recovered from the rosetted pellet by treatment with 0.155 M NH₄Cl were 96–100% E-rosette-positive and contained fewer than 3% surface-immunoglobulin-positive cells on the basis of a direct fluorescein-conjugated antibody binding assay (21). In addition, fewer than 2% of this T-cell enriched fraction were monocytes, based on staining with naphthyl acetate (22). Nonrosetting (non-T) cells included 82–91% surface-immunoglobulin-positive cells, including 21–40% monocytes.

Preparation of Monoclonal Anti-T + Antibody. The production of monoclonal anti-T + antibody will be published elsewhere (18). Briefly, a BALB/c mouse was injected intraperitoneally with 1 × 10⁷ human PBML and then, 2 weeks later, with 5 × 10⁶ cells. Four days after the second injection, spleen cells from the immunized mouse were fused in the presence of polyethylene glycol to a nonsecreting variant of the MOPC21 myeloma derivative, X63AG8. Of 93 initial hybrid populations, 60 produced antibodies that bound to PBML. A few of the initially positive hybrid populations were retained through several passages and were successfully cloned by limiting dilution in microtiter dishes. One of these hybrids secreted antibody that bound specifically to a subpopulation of peripheral human T cells. Studies of this antibody showed it to be an IgG, and gel electrophoresis of immunoprecipitates of T-cell extracts demonstrated that the antibody reacted with T +, a T-cell surface antigen that was part of or in firm association with a polypeptide chain.

Analysis and Cell Separation on the Fluorescence-Activated Cell Sorter (FACS). The binding specificity of monoclonal anti-T + was studied by indirect immunofluorescence with fluorescein-conjugated rabbit anti-mouse Ig (R/M-FITC) that had been adsorbed on a column of Sepharose-conjugated

Abbreviations: PBML, peripheral blood mononuclear leukocytes; FACS, fluorescence-activated cell sorter; R/M-FITC, fluorescein-conjugated rabbit anti-mouse Ig; Con A, concanavalin A; PHA, phytohemagglutinin; MLR, mixed lymphocyte reaction; T +, target antigen from T cells.

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human immunoglobulin. Cells were stained by resuspending 2 × 10^6 cells in 0.1 ml of anti-Ta to which sodium azide had been added to a level of 0.01% to prevent capping. The cells were incubated for 20 min at 25°C, washed twice in phosphate-buffered saline, and incubated for an additional 20 min at 25°C with 0.1 ml of R/M-FITC diluted 1:50 in phosphate-buffered saline. After washing, the labeled cells were processed in a FACS III (Becton Dickinson Electronic Laboratories, Mountain View, CA) at 1000 cells per sec, and the intensity of the pulse height for individual cells was recorded on a pulse analyzer (23, 24). Background fluorescence was determined by analyzing cells stained with an irrelevant murine monoclonal antibody (anti-trinitrophenyl) or normal mouse serum. To obtain populations enriched for Ta^+ or Ta^- cells, T cells were labeled as described and then separated aseptically by the FACS into populations falling into the upper and lower 30th percentiles of fluorescence intensity.

Proli erative Responses of T Cells to Mitogens and Antigens. All functional assays were performed as described (21) in RPMI 1640 medium supplemented with 25 mM Hepes buffer, 2 mM glutamine, 100 units of penicillin and 100 μg of streptomycin per ml, and 10% pooled human serum. Stimulation with concanavalin A (Pharmacia) or phytohemagglutinin (PHA, Burroughs Wellcome) was carried out in an atmosphere of 5% CO2/95% air for 4 days at 37°C. T cells (100,000) were incubated in round-bottom microtiter wells in 0.2 ml with 50 μg of Con A or 1 μg of PHA per ml unless otherwise indicated.

Assays were performed in triplicate and [3H]thymidine was added 18 hr before harvest (1 μCi per well; 1 Ci = 3.7 × 10^10 becquerels) with a MASH II apparatus (Microbiological Associates, Walkerville, MD).

Stimulation of 100,000 T cells with tetanus toxoid (0.5 LF unit/ml) or diphtheria toxoid (0.5 LF unit/ml) (Massachusetts State Department of Public Health) was carried out in the presence of 20,000 irradiated (1500 rads (150 grays)) autologous non-T cells. In the absence of non-T cells, no proliferation was detectable. Triplicate cultures were maintained for 6 days at 37°C before radiolabeling and harvesting as described.

Allogeneic mixed lymphocyte reactions (MLR) were carried out between 50,000 T responder cells and 50,000 irradiated (3000 rads) allogeneic non-T cells in a volume of 0.2 ml. Cultures were incubated in 5% CO2/95% air for 6 days at 37°C, and proliferation was measured as described above. Autologous MLRs were conducted by using 100,000 T cells and 100,000 irradiated autologous non-T cells in 0.2 ml.

Unseparated T cells as well as sorted populations of Ta^+ and Ta^- cells were tested in these assays. When mixtures of Ta^+ and Ta^- cells were tested, equal numbers of each population were included in the cultures.

RESULTS

Reactivity of Monoclonal Anti-Ta Antibody with Peripheral Blood Lymphocytes. Unseparated PBML as well as purified populations of T cells and non-T cells from a normal individual were incubated with anti-Ta and stained with R/M-FITC. Analysis of PBML or T cells in the FACS revealed a distinct shoulder of brightly stained cells (Ta^+) as well as a large number of unstained or weakly stained cells (Ta^-) (Fig. 1). This pattern has been reproduced in studies of 12 healthy persons, and the percentage of brightly stained cells has been 21–27% of PBML and 30–38% of T cells with the exception of two individuals with approximately 55% brightly stained T cells. The staining pattern was unchanged when the preparations of anti-Ta used had been concentrated 10-fold. Control experiments, in which PBML or T cells were incubated with an irrelevant murine monoclonal antibody or normal mouse serum, resulted in no significant staining. Although we previously had observed that some transformed B-cell lines bind anti-Ta antibody (18), neither B cells nor monocytes from healthy donors stained with anti-Ta and R/M-FITC.

FIG. 1. FACS profile of 1 × 10^6 peripheral blood T cells from a healthy donor when stained with monoclonal anti-Ta antibody and R/M-FITC. T cells comprising the brightly stained shoulder are designated Ta^+. Background fluorescence staining (—) was obtained by incubating T cells with normal mouse serum and R/M-FITC.

Proliferative Responses of Ta^+ and Ta^- Cells. To obtain purified populations of Ta^+ and Ta^- cells, T cells incubated with anti-Ta and R/M-FITC were separated by the FACS on the basis of relative fluorescence. As indicated by the brackets in Fig. 1, brightly stained Ta^+ cells were collected in one tube, unstained Ta^- cells were collected in another tube, and weakly stained cells were discarded. Analysis of these sorted populations by FACS confirmed that efficient separation of Ta^+ and Ta^- cells had been achieved (not shown).

To determine if Ta^+ and Ta^- cells were functionally distinct, the separate populations were tested for their capacity to proliferate in response to several known T-cell stimuli (Table 1). Binding with anti-Ta and R/M-FITC had no effect on the responses of unsorted T cells. Ta^+ and Ta^- cells separated by FACS responded equally well to alloantigens in the allogeneic MLR, to autoantigens in the autologous MLR, and to soluble antigens such as tetanus toxoid and diphtheria toxoid. Of note, however, is that Ta^+ cells responded well to Con A and PHA and that the response of Ta^- cells to Con A was significantly greater than the response of the same number of unsorted T cells. By contrast, Ta^- cells responded poorly to PHA and not detectably to Con A. Similar results have been obtained in studies of five additional healthy persons. The results, as measured by [3H]thymidine incorporation, were paralleled by the number of blast cells observed microscopically for each culture.

The results of Con A and PHA cultures shown in Table 1 were based on 4-day incubations with 50 μg of Con A or 1 μg of PHA per ml. To assess the possibility that the apparent lack of responsiveness of Ta^- cells at 4 days was due to altered kinetics, Ta^- cells were incubated with Con A or PHA and proliferation was measured after 2, 4, and 6 days. No early or late
response peaks of Ta- cells to Con A or PHA were observed (data not shown).

Additional experiments were performed to assess the responses of Ta+ and Ta- cells to varying concentrations of mitogens. Ta+ cells responded in a dose-dependent manner to Con A, whereas Ta- cells responded only weakly to concentrations of Con A as high as 200 µg/ml (Fig. 2). By contrast, the responses of Ta+ and Ta- cells to PHA were similar at higher doses of this mitogen (Fig. 3).

Responses of Mixtures of Ta+ and Ta- Cells. Equal numbers of Ta+ and Ta- cells were cocultured in the presence of mitogens, soluble antigens, or allogeneic B cells, and the responses of these mixtures were compared to the responses of unsorted T cells. The results (Table 1) indicate that Ta+ and Ta- cells are additive rather than synergistic in their effects. Ta+ cells did not enhance or inhibit responsiveness of the Ta- population, and Ta- cells neither helped nor suppressed the responses of Ta+ cells. Similarly, no change in reactivity was observed when irradiated Ta+ or Ta- cells were added to the sorted or unsorted populations (data not shown). These data rule out the possibilities that, prior to exposure to mitogens or antigens, Ta+ or Ta- cells can function as helper or suppressor cells. In addition, it is unlikely that either population is contaminated with significant numbers of monocytes.

Effect of Monocytes on the Responses of Ta+ and Ta- Cells. The initial studies of mitogen responsiveness were performed in the absence of monocytes. Because monocytes are known to augment these responses (25), Ta+ and Ta- cells were stimulated with Con A or PHA in the presence of 20% irradiated (1500 rads) autologous non-T cells (Table 2). This resulted in markedly enhanced responses by Ta+ cells to both mitogens.

The addition of accessory cells to Ta- cells also significantly increased the response of this population to PHA, but the response of Ta- cells to Con A was only slightly increased. We have tested the responses of Ta+ and Ta- cells in the presence of 10% and 40% non-T cells (not shown), and the results were similar in each case. Ta- cells gave negligible responses to Con A regardless of the number of monocytes. These data suggest that most T cells capable of proliferating in response to Con A are confined to the Ta+ subset.

Stability of the Ta Marker. To determine the stability of the Ta marker, unsorted T cells or sorted Ta+ and Ta- populations were incubated in medium with or without mitogen or antigen. After varying periods of incubation, the cells were washed, resuspended with anti-Ta and R/M-FITC, and analyzed in the FACS. When unsorted T cells were incubated in medium for up to 6 days (Fig. 4A), the proportions of Ta+ and Ta- cells were unchanged. Sorted Ta+ cells retained Ta+ positivity, and sorted Ta- cells did not become Ta+. However, under continuous exposure to Con A, a gradual but definite loss of the Ta marker occurred (Fig. 4B). For example, when cultures were initiated with Ta+ cells, only 20% of these cells remained Ta+ by day 6. Changes in Ta expression, such as that induced by Con A, were not detected after stimulation with PHA (Fig. 4C), tetanus toxoid (Fig. 4D), or allogeneic B cells (not shown). When cultures were initiated with Ta- cells, slight (5-10%) increases in the percentage of Ta+ cells were observed after stimulation with PHA, tetanus toxoid, or allogeneic cells. These changes could be accounted for by the presence of a small number of Ta+ blasts derived from cells weakly positive for Ta. Blasts with a relatively low density of Ta may appear as positive events on the FACS because of their increased surface area.

![Figure 2](image_url)  
**Fig. 2.** Responses of Ta+ cells, Ta- cells, and unsorted T cells (UT) to varying concentrations of Con A. Peripheral blood T cells were labeled with anti-Ta antibody and separated by FACS into Ta+ and Ta- populations. Then 10^6 Ta+ cells (●), Ta- cells (▲), or unsorted T cells (O) were incubated in the presence of varying concentrations of Con A, and [3H]thymidine uptake was measured after 4 days.

![Figure 3](image_url)  
**Fig. 3.** Responses of Ta+ cells, Ta- cells, and unsorted T cells to varying concentrations of PHA (see legend to Fig. 2).
Table 2. Influence of non-T cells on the responses of Ta⁺ and Ta⁻ cells to Con A and PHA

<table>
<thead>
<tr>
<th>Responder cells</th>
<th>Irradiated non-T cells*</th>
<th>Mean (± SEM) responses, cpm</th>
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<td></td>
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<td>Ta⁺</td>
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* These assays were performed in the presence (+) or absence (−) of 20,000 autologous non-T cells that had been irradiated (1500 rads).

DISCUSSION

These data demonstrate the existence of a cell surface marker, Ta, that distinguishes a subpopulation of human T lymphocytes which proliferate briskly when exposed to the mitogens Con A and PHA. By contrast, T cells lacking the Ta marker fail to respond to Con A and respond weakly to PHA. In the presence of monocytes, the Ta⁻ population recovers responsiveness to PHA but remains weakly reactive to Con A. Thus, most T lymphocytes capable of responding to Con A are included in the Ta⁺ population. Moreover, coculture of Ta⁺ and Ta⁻ cells did not reveal the presence of helper or suppressor cells in either population. Therefore, these differences in responsiveness appear to be due to differences in the capacity of each population to recognize or respond to mitogen.

The relatively fixed ratio of Ta⁺ and Ta⁻ cells in the peripheral blood of healthy persons suggests that the percentage of Ta⁺ cells may be constant in good health. The stability of the marker on unstimulated cells is indicated by its continued presence on Ta⁺ cells and absence from Ta⁻ cells over 6 days of culture. Stimuli such as PHA, soluble antigens, and allogeneic cells also failed to alter the expression of Ta. The exception was Con A, which caused a marked reduction in the density of Ta⁺ on Con A-responsive T cells. It is doubtful that this reduction is due to competition between Con A and anti-Ta for binding sites on T cells because detection of Ta⁻ was unchanged immediately after addition of Con A. It is equally unlikely that stimulation with Con A resulted in selective death of Ta⁺ cells because: (i) cell viability (based on exclusion of trypan blue)

![Diagram](image-url)

**Fig. 4.** Detection of Ta⁺ in cultured T cell populations. Peripheral T cells were labeled with anti-Ta antibody and either sorted into Ta⁺ and Ta⁻ fractions or left unsorted (UT). Then 5 × 10⁶ Ta⁺ cells (●), Ta⁻ cells (△), or unsorted T cells (○) were cultured in medium (A), Con A (B), PHA (C), or tetanus toxoid (D). On days 3 and 6, aliquots were collected, relabeled with anti-Ta, and analyzed in a FACS to determine the percentage of Ta⁺ cells.
exceeded 85% throughout the experiment; (ii) starting populations in these cultures consisted of at least 99% T\textsuperscript{a-} cells (after cell sorting); and (iii) many of the cells that were T\textsuperscript{a-} at 6 days were blasts, and such blasts probably did not arise from T\textsuperscript{a-} cells because T\textsuperscript{a-} cells could not be stimulated by Con A. It is possible that the loss of T\textsuperscript{a} may reflect the transition of T\textsuperscript{a+} cells into functional suppressor cells. Con A induction of suppressor cells has been extensively documented in mice (11, 26) and humans (27, 28), and it will be of interest to test the suppressive capacity of stimulated T\textsuperscript{a+} and T\textsuperscript{a-} populations.

Some functional comparisons can be made between the T\textsuperscript{a+} and T\textsuperscript{a-} subsets described here and human T cell subsets reported previously. Using absorbed heteroantisera, Schlossman and colleagues have found subsets of human T cells that are either responsive or unresponsive in the MLR (13) as well as subsets that either effect or fail to effect cell-mediated lympholysis (15). Investigators from the same laboratory have found that autoantibodies in the sera of patients with juvenile rheumatoid arthritis distinguish subsets of T cells that either enhance or fail to enhance secretion of Ig by B cells (29). Additionally, Moretta et al. (14) have demonstrated that populations of T cells that help or suppress the response of B cells to pokeweed mitogen have surface Fc receptors with specificity for different immunoglobulin isotypes. Although several of these subsets have distinct patterns of reactivity to mitogens (29, 30), the patterns do not coincide with those of T\textsuperscript{a+} or T\textsuperscript{a-} cells. Therefore, it is unlikely that any of these previously reported subsets corresponds precisely to the populations described in this report.

In the current studies, production of an antibody specific for a subset of human T cells was achieved by fusing an antibody-secreting B cell with a myeloma cell and isolating the resultant hybrid clone. Because antibodies secreted by such clones are highly specific and can be produced in large quantity, they should be extremely useful as discriminators of lymphocyte subpopulations. Although we have described only one monoclonal antibody, these data confirm the feasibility of the technique. Undoubtedly, additional monoclonal anti-T antibodies will soon be forthcoming, which will help to clarify the relationship between T cell surface structure and function as well as the nature of immunologic defects in human disease.

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