DETECTION, ISOLATION, AND FUNCTIONAL CHARACTERIZATION OF TWO HUMAN T-CELL SUBCLASSES BEARING UNIQUE DIFFERENTIATION ANTIGENS

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The majority of human peripheral blood lymphocytes differentiate under the influence of the thymus to express a variety of distinct immunologic functions. In vitro analyses of these functions have shown that highly purified human T cells are activated by specific antigens to proliferate and to elaborate mediators, are nonspecifically activated by polyclonal mitogens, and are triggered by alloantigen to proliferate and to differentiate into specifically cytotoxic killer cells (1-4). In addition, evidence is accumulating to suggest that human T cells manifest suppressor and helper functions important in the homeostatic regulation of the immune response (5-7).

While direct in vivo studies in syngeneic murine systems have shown that T cells mediate a spectrum of immune responses, including delayed hypersensitivity, graft rejection, and tumor immunity, more restricted in vivo studies in man have not clearly defined the role of human T cells in these activities. Moreover, the correlation between assays of T-cell functions in vivo and specific tests of T-cell activities in vitro remains poorly understood and controversial. The issue is further complicated by dissociations between different in vitro and in vivo T-cell functions in certain disease states (8-10), suggesting that functionally heterogeneous subpopulations of thymus-derived cells, and not a functionally pluripotential population of T cells, account for the wide range of human T-cell activities.

Recently, direct evidence that the pool of peripheral T cells is comprised of functionally heterogeneous subsets was provided by the demonstration that alloantisera directed against discrete thymus-dependent murine Ly antigens distinguish functionally unique T-cell subclasses (11-14, footnote 1). Although a variety of heterologous antisera have been developed that react specifically with human T cells (15-18), and in some cases with only a fraction of T cells (19, 20), the absence of antisera specific to functionally unique thymus-dependent differentiation antigens has limited studies of human T-cell heterogeneity.

To investigate the functional heterogeneity of human T-cell subpopulations, we utilized a rabbit antisera raised against highly purified human T cells and rendered T cell-specific by absorption with autologous Ig$^+$ lymphoblastoid cells. This antisera (anti-T$_{H1}$), although reactive in complement-dependent assays with 90% or more of human thymocytes, lysed only 50–60% of peripheral blood T cells and less than 1% of B cells. The data presented below provides evidence that anti-T$_{H1}$ distinguishes two functionally distinct subclasses of T cells. One subclass, resistant to lysis by anti-T$_{H1}$, proliferates in response to several soluble antigens (mumps, purified protein derivative [PPD],[2] tetanus toxoid) but does not elaborate lymphocyte mitogenic factor (LMF) or respond in mixed lymphocyte culture (MLC). The second subclass, sensitive to lysis by anti-T$_{H1}$ and complement, proliferates in MLC and elaborates LMF in response to tetanus toxoid but proliferates poorly in response to soluble antigens.

Materials and Methods

Isolation of Human T and B Lymphocytes from Unfractionated Peripheral Blood.   Human peripheral blood mononuclear cells were isolated from normal volunteers by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N. J.) density centrifugation. Unfractionated cells were separated into SIg$^+$ (greater than 98%) and SIg$^-$ (less than 2%) subpopulations by Sephadex G-200 antihuman Fab chromatography as previously described (1). T cells were further isolated from other SIg$^-$ cells by sequential sheep erythrocytes coated with antibody and complement (EAC) and nylon wool depletions, resulting in a T-cell population that was greater than 92% E rosette positive and less than 2% Ig or EAC positive (21).

Analysis of Surface Properties of Human Lymphocyte Subsets.   SIg was detected with flouresceinated rabbit antihuman Fab in a direct flouresceinated antibody technique (1). The percentage of lymphocytes forming spontaneous rosettes with sheep erythrocytes (E rosettes) or with erythrocytes coated with anti-SRBC antibody and complement (EAC rosettes) was determined as previously described (21).

Preparation of the Antisera.   The anti-T cell serum used in these studies was raised by intravenously injecting an adult New Zealand rabbit with 70 × 10^6 purified T cells (from normal donor R.E.) in saline on days 1 and 10. On day 17 the rabbit was bled and the resulting antiserum was heat inactivated at 56°C for 1/2 h. After absorption with equal volumes of human AB erythrocytes at 37°C for 1/2 h, the antiserum was absorbed with varying numbers of cells from an Ig$^+$ line, previously established from the peripheral lymphocytes of donor R.E. This autologous line, designated Laz 156, as well as other lymphoblastoid lines used in this study, were derived by standard techniques as described elsewhere (22). Gram quantities of Laz 156 cells were grown in large, screw cap Ehrlenmeyer flasks and harvested by centrifugation at 100 *g* for 20 min.

Fluorescence-Activated Cell Sorter (FACS) Analysis and Cell Separation.   Binding of anti-T$_{H1}$ was studied by indirect immunofluorescence using fluorescein-conjugated goat anti-rabbit Fc (G/R FITC) prepared as previously described (23). Cells were stained by resuspending 2–3 × 10^6 cells in 0.15 ml anti-T$_{H1}$ (diluted 1/10) and incubating at 4°C. After washing three times, the labeled cells were processed on a FACS I (Becton Dickinson Electronics Laboratories, Mountain View, Calif.) at 500–1,000 cells/s and the intensity of the pulse height was recorded for each individual cell on the pulse height analyzer. Background flourescence was determined by analyzing appropriate negative controls, including cells labeled with normal rabbit serum (NRS). Detailed methodology, analysis, and cell separation capabilities of the FACS I have been described (24–26).

For separation experiments 6 × 10^7 cells were labeled with anti-T$_{H1}$ (diluted 1/10) and G/R FITC (diluted 1/50) as described above, and then separated into populations falling within the upper and lower 25th percentiles of fluorescent binding.

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[2] *Abbreviations used in this paper:* ALS, antilymphocyte serum; CLL, chronic lymphatic leukemia; FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; LMF, lymphocyte mitogenic factor; MIF, migration inhibitory factor; MLC, mixed lymphocyte culture; NRS, normal rabbit serum; PPD, purified protein derivative; SIg, surface immunoglobulin.

Functional Studies

MLC. Standard one-way MLCs were established in round bottom microtiter plates (Linbro Chemical Co., New Haven, Conn.), using triplicate wells each containing 0.2×10^6 responding T cells and 0.2×10^6 mitomycin C-treated stimulating cells as previously described (4). All cultures were established in final media (media 199 containing 20% human AB serum, 1% penicillin-streptomycin, 200 mM L-glutamine, 25 mM Hepes buffer, and 0.5% sodium bicarbonate, Microbiological Associates, Bethesda, Md.). After 6 days cultures were pulsed with 0.2 μCi of [^{3}H]thymidine (1.9 Ci/mM; Schwarz-Mann, Division of Becton, Dickinson Co., Orangeburg, N. Y.), and [^{3}H]thymidine incorporation was measured after harvesting with a MASH II apparatus (Microbiological Associates).

SOLUBLE ANTIGEN-INDUCED PROLIFERATIVE RESPONSE. For soluble antigen-induced tritiated thymidine incorporation studies, 2×10^5 cells/0.1 ml and 0.1 ml of either media or antigen solutions were placed in round bottom microtiter plates as previously described (1). After 6 days of cell culture in a 37°C, 5% CO_2 humid atmosphere, cells were pulsed with 0.2 μCi of tritiated thymidine for 16 h and harvested as described above. All antigens were diluted in final media. PPD (Merck Sharp, & Dohme, West Point, Penna.) was used at a final concentration of 10 μg/ml. Mumps antigen (Microbiological Associates) and tetanus toxoid (kindly donated by Dr. Leo Levine, Massachusetts Public Health Biological Laboratories, Boston, Mass.) were used at final concentrations of 50 and 10 μg/ml, respectively.

LMF PRODUCTION. LMF was assayed as previously reported (2, 28). In brief, lymphocytes were adjusted to 3×10^6 cells/ml in final media and cultured in the presence of 10 μg/ml of tetanus toxoid for 48 h at 37°C in a 5% CO_2 humid atmosphere. Supernates obtained from the control cultures were reconstituted with antigen in the original concentration and designated as R. Antigen culture supernates were designated as P. Preincubated (P) and control (R) supernates were assayed for mitogenic activity on 2×10^5 lymphocytes from antigen-negative donors in microtiter plates. After 6 days, [^{3}H]thymidine was measured in triplicate as described above. Mitogenic activity was calculated as a stimulation index (SI = P/R) and a net increase in counts per minute (P-R).

Results

Analysis of the T Cell-Specificity of Anti-T$_{H1}$. To determine the effectiveness of selectively absorbing nonspecific heteroantibodies from the antihuman T-cell serum with cells from a B lymphoblastoid line (Laz 156) derived from the same donor, 3-cm^3 aliquots of the antiserum were absorbed with 0.5, 1.0, 2.0, and 5.0 $\times$ 10^8 Laz 156 cells and tested at a 1/10 dilution in a complement-dependent cytotoxic assay on thymocytes, peripheral blood T cells, peripheral blood B cells, and chronic lymphatic leukemia (CLL) cells (B cell leukemic cells). As shown in Fig. 1, the antisera killed nearly 100% of all cell populations before absorption, and lysed 80% of thymocytes and 60% of peripheral T cells after absorption with 200 $\times$ 10^6 Laz 156 cells. In contrast, the same number of absorbing cells abolished the lysis of both B cells and CLL cells. Serial dilution of anti-T$_{H1}$ demonstrated that maximal killing of peripheral T cells from five normal adults was 50–60%, while lysis of four different populations of fetal thymocytes exceeded 80% in all cases (Fig. 2). Attempts to increase the percent lysis of T cells by a second incubation with anti-T$_{H1}$ and complement resulted in no further cell kill, indicating that 40% of peripheral T lymphocytes were unreactive. The reactive 60% of peripheral T cells will be subsequently called T$_{H1^+}$ and the unreactive subpopulation will be designated T$_{H1^-}$. In contrast to the reactivity of T cells, peripheral B cells or CLL cells were not lysed at any dilution tested.

The specificity of anti-T$_{H1}$ for T-lymphoid cells was further established by similarly testing the antiserum with several lymphoblastoid lines bearing
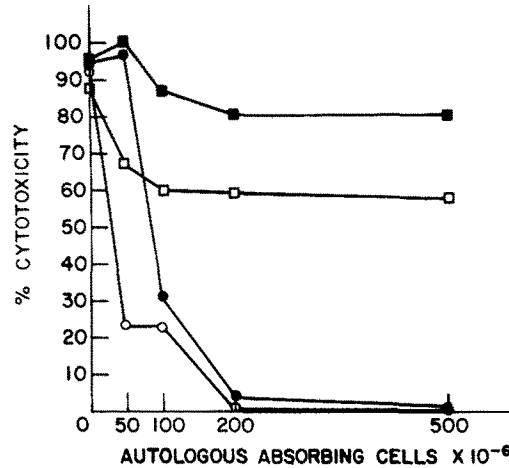
Fig. 1. Complement-dependent lysis of lymphoid subpopulations by anti-T_{H1} absorbed with increasing numbers of autologous Ig^+ lymphoblastoid (Laz 156) cells. Lytic activity of the antiserum at a 1/10 dilution against thymocytes (■), peripheral T cells (□), CLL cells (●), and peripheral B cells (○) is expressed as % cytotoxicity in a ^{51}Cr release assay.
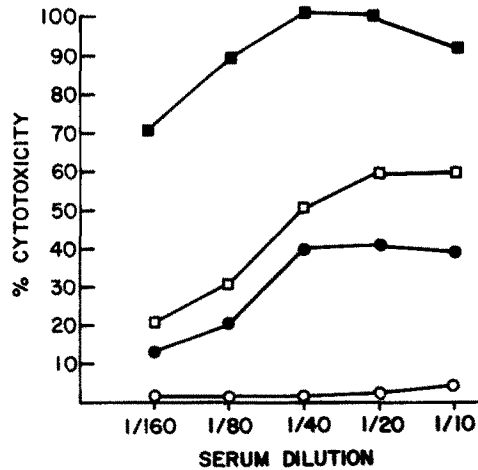

Fig. 2. Complement-dependent lysis of lymphoid subpopulations using serial dilutions of anti-T_{H1} absorbed with 1×10^9 autologous lymphoblastoid cells. Fetal thymocytes (■), peripheral T cells (□), unfractionated lymphocytes (●), and peripheral B cells (○).

either T- or B-cell markers (Table I). Like thymocytes, greater than 80% of cells from three lines carrying the receptor for SE were lysed at a 1/10 dilution, whereas cells from five lines bearing SIg were unreactive.

Functional Analysis of Subpopulations of T Cells Using Anti-T_{H1} Antibodies. Preliminary experiments indicated that when unfractionated lymphocytes were incubated with anti-T_{H1} and complement and then presented in vitro with either soluble antigen or mitomycin C-treated allogeneic cells (MLC), antigen-induced incorporation of [^{3}H]thymidine was not affected, whereas proliferation in MLC was abolished. This suggested that T_{H1}^+ T cells

TABLE I

Reactivity of Anti-T$_{H1}$ with T- or B-Lymphoblastoid Lines

Cell line	E rosette*	SIg*‡	Anti-T$_{H1}$ % Lysis§
Molt-4	+	−	95
HSB	+	−	85
CEM	+	−	100
Laz 67	−	+	<5
Laz 168	−	+	<5
SB	−	+	<5
Laz 007	−	+	<5
Laz 20	−	+	<5

Complement-dependent lysis of lymphoblastoid lines bearing either T(E rosette)- or B(SmIg)-cell markers by anti-T$_{H1}$.

* (+) indicates that >80% of cells were positive for the surface marker (E rosette or SmIg). (−) indicates that <2% of cells were positive.

‡ Determined by a direct fluoresceinated antibody technique using a fluoresceinated antihuman Fab.

§ Determined by a ^{51}Cr-release microcytotoxic assay.

were alloreactive, whereas T$_{H1}$- T cells were unresponsive to alloantigen but were able to recognize and respond to specific soluble antigen. To pursue this finding in more detail, purified T cells from individuals previously shown to be reactive in culture with mumps antigen, PPD, and tetanus toxoid were incubated with complement and either media alone, NRS, anti-T$_{H1}$, or an antilymphocyte serum (ALS). The ALS lyses greater than 90% of lymphocytes and was used as a positive control for complement-mediated lysis in this and subsequent experiments. After treatment, the cells were reconstituted to 2 × 10⁶ viable cells/ml and cultured either in the presence or absence of soluble antigen or equal numbers of mitomycin C-treated allogeneic cells. This experiment was repeated four times with cells from three different individuals. As shown in a representative experiment in Fig. 3, cells incubated with NRS or media alone mounted a good proliferative response to mumps, PPD, tetanus toxoid, and to allogeneic cells. Whereas T cells treated with anti-T$_{H1}$ and complement responded equally well to the battery of antigens, the same cell population did not proliferate in MLC. These results clearly indicated that the T$_{H1}$+ subset contained those cells responding in MLC as determined by the incorporation of [³H]thymidine. However, our data did not establish whether the cells proliferating in response to specific antigen were restricted to the T$_{H1}$- subset or were present in both the T$_{H1}$+ and T$_{H1}$- subsets.

To explore these two alternative possibilities, T cells most reactive with anti-T$_{H1}$ by indirect immunofluorescence staining were physically separated from less reactive T cells by fluorescence-activated cell sorting. Cells were first labeled with anti-T$_{H1}$ and then stained with FITC conjugated pepsin digested goat antirabbit Fab and analyzed on the cell sorter. Using this highly sensitive technique to analyze antibody binding, it was initially established that the T cell-specificity of anti-T$_{H1}$ was as demonstrable by indirect immunofluorescence as by complement-mediated lysis. A histogram plotting the fluorescence intensity vs. the cell number shown in Fig. 4 illustrates the binding of anti-T$_{H1}$ to purified peripheral T and B cells. While the binding of anti-T$_{H1}$ to B cells
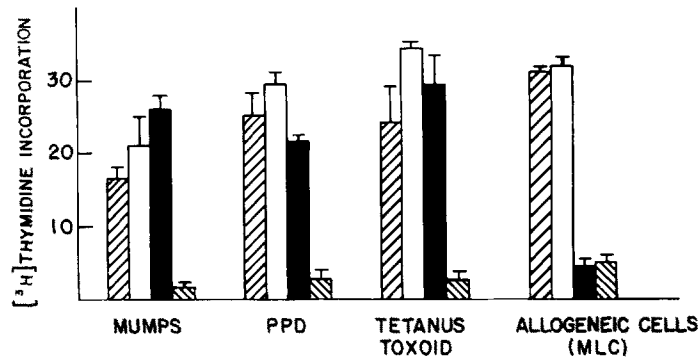
FIG. 3. The effect of anti-T_{H1} and complement on the proliferative response to soluble antigens and allogeneic cells. T cells were untreated (▨) or treated with a NRS (☐), anti-T_{H1} (■), or an ALS (▧), as a positive control.

FIG. 4. Histogram showing FACS analysis of fractionated peripheral blood lymphocytes (A), B lymphocytes, and (B), T lymphocytes. Cells were reacted with anti-T_{H1} and developed with goat Fab anti-rabbit Fc. The abscissa represents the relative intensity of fluorescence whereas the ordinate represents the number of cells counted per channel.

was no different from staining with a NRS, nearly all T cells were reactive, contrasting with the restricted number (50–60%) lysed by antibody and complement. It was therefore important to isolate low-density staining from high-density staining T cells to determine whether cells binding fewer antibody molecules would correspond functionally to the T_{H1}- subset. Thus, both low-density binding (lowest 25%) and high-density binding (highest 25%) T cells were collected and analyzed for their proliferative responses to both soluble antigen and to alloantigen in MLC. To avoid overlap, cells exhibiting intermediate-density binding (middle 50%) were discarded. As shown in Fig. 5, weakly stained T cells mounted a proliferative response exceeding the response by unfractionated T cells, while proliferating poorly in MLC. In contrast, the
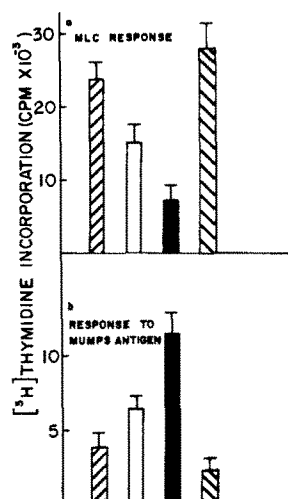
FIG. 5. The proliferative response to (a) allogeneic cells and (b) mumps antigen by T cells binding low and high amounts of anti-T_{H_1} as determined by the indirect immunofluorescent staining. T cells were untreated (▨), or stained with anti-T_{H_1} and a goat Fab anti-rabbit Fc (□), and separated on a FACS into subpopulations exhibiting low (lowest 25%) fluorescence (■), or high (highest 25%) fluorescence (▨).

more brightly stained cells did not react to mumps antigen but responded well in MLC, again in excess of the response by unfractionated T cells. These data strongly supported the concept that the antiserum was, in fact, dissecting two functionally unique subsets of human T lymphocytes; one $T_{H_1}+$ and reactive in MLC, the other $T_{H_1}-$ and responsive to soluble antigens.

To determine whether other functional distinctions between $T_{H_1}+$ and $T_{H_1}-$ could be demonstrated, we analyzed the effect of anti-T_{H_1} and complement on the production of LMF. Antigen-stimulated lymphocytes from sensitized donors are known to elaborate LMF which induces proliferation of nonsensitized T and B lymphocytes. In previous studies it was shown that purified T cells, but not B cells, were responsible for LMF production (3). Thus, in separate experiments, T cells purified from the peripheral blood of three different individuals sensitive to tetanus toxoid were first treated in the presence of complement with either media alone, anti-T_{H_1}, or ALS and then washed and incubated in culture with or without tetanus toxoid. After 48 h, cell culture supernates were examined for LMF activity on indicator cells from nonsensitized donors. As shown by a representative experiment depicted in Table II, incubation with anti-T_{H_1} and complement did not reduce the proliferative response to tetanus toxoid; however, it did abolish LMF activity from the supernates of those same cultures. In contrast, NRS and complement had no effect on either incorporation of tritiated thymidine or on production of LMF. These experiments therefore suggested that T lymphocytes which elaborate mediators in response to specific antigen are distinct from T cells which proliferate upon interaction with the same antigen.

Thus, the $T_{H_1}+$ subclass of human T lymphocytes is responsible for both the MLC response and mediator production, as distinct from the $T_{H_1}-$ subclass

TABLE II

Effect of Anti-T$_{H1}$ on Mitogenic Factor Production by T Cells

Cell cultures*	[³H]thymidine incorporation‡	Supernate effect on tetanus toxoid negative indicator cells [³H]thymidine incorporation§	Mitogenic stimulation index
T lymphocytes + media	2,476 ± 872	563 ± 32	
T lymphocytes + tetanus toxoid (10 µg/ml)	24,361 ± 2,571	4,566 ± 232	8.1
T lymphocytes (treated with NRS + C′) + media	3,334 ± 168	428 ± 132	
T lymphocytes (treated with NRS + C′) + tetanus toxoid (10 µg/ml)	27,312 ± 315	5,350 ± 358	12.5
T lymphocytes (treated with anti-T$_{H1}$ + C′) + media	436 ± 121	312 ± 75	
T lymphocytes (treated with anti-T$_{H1}$ + C′) + tetanus toxoid (10 µg/ml)	25,371 ± 3,121	843 ± 131	2.7

Effect of anti-T$_{H1}$ and complement on mitogenic factor production by T cells.

* T cells were purified by passage over Sephadex G-200 goat antihuman Fab columns followed by passage over nylon wool.

‡ Proliferation by T cells from sensitized donors after direct contact with tetanus toxoid in vitro.

§ Proliferation by T cells from donors not sensitized to tetanus toxoid after a 6-day incubation with supernates from cultures of sensitized T cells and antigen.

which contains the predominance of cells proliferating in response to specific antigen.

Discussion

These studies demonstrated that peripheral human T cells differentiate into at least two functionally discrete subclasses. One subclass, which is distinguished by its sensitivity to lysis by anti-T$_{H1}$ plus complement (T$_{H1}$+ cells), contains cells that are programmed to recognize and proliferate in response to alloantigens in MLC, and in addition, cells that are triggered by specific antigens to synthesize and secrete LMF. Interestingly, although the T$_{H1}$+ subclass elaborates mediators in response to specific antigens, it does not mount a measurable proliferative response to these same antigens. In contrast, the subclass resistant to lysis by anti-T$_{H1}$ (T$_{H1}$- cells) is comprised of cells that can be triggered by specific antigens to proliferate in [³H]thymidine incorporation assays, but not to secrete LMF or to respond in MLC. Of importance was the demonstration that after indirect immunofluorescent staining with anti-T$_{H1}$ sera, these two T-cell subclasses could be physically separated on a FACS to yield a high fluorescent population that effected exclusively T$_{H1}$+ functions and a low fluorescent population that expressed T$_{H1}$- functions.

The heterologous antisera which defined these two human T-cell subsets was prepared by immunizing a rabbit with highly purified T cells from a normal donor, and subsequently absorbing the resulting serum with an autol-

ogous Ig^+ lymphoblastoid cell line. We have shown previously that the development of an EBV-transformed line autologous to leukemic cells used for heteroimmunization provides an efficient and relatively simple means to selectively absorb antibodies raised against not only species- and tissue-specific determinants but also antibodies directed against MHC and B_2 microglobulin heteroantigens (23).

In the present studies, the absorbed antihuman T-cell sera (anti-T_{H1}) was shown by both complement-mediated lysis and indirect immunofluorescence to bind specifically to determinants on fresh peripheral T but not B cells derived from a variety of individuals. Moreover, anti-T_{H1} reacted specifically with lymphoblastoid lines bearing T-cell markers. Of greater significance was the demonstration that anti-T_{H1} lysed more than 90% of fetal thymocytes and adult thymocytes while killing 50–60% of peripheral blood T cells. Taken together, these results suggested that the antigenic distinction of the T_{H1}- subclass arose from the reduction of one or more thymus-specific antigens during its differentiation.

Similarly, certain thymus-dependent murine alloantigens (theta, Ly1, and Ly23) are reduced or lost in the periphery by subpopulations of T cells expressing distinct migratory and functional characteristics. For example, two subpopulations of mouse T cells have been defined in terms of their relative sensitivity to lysis by anti-theta serum (28, 29). One subpopulation, which is relatively resistant to anti-theta but sensitive to ALS, migrates to lymph nodes and survives long after adult thymectomy. The other T-cell subpopulation, which is highly sensitive to anti-theta but relatively resistant to ALS, migrates predominantly to the spleen and disappears soon after thymectomy. More recently, Cantor and Boyse (11–14) have dissected two functionally distinct murine T-cell subclasses by their expression of discrete thymus-dependent Ly alloantigens. The Ly1 phenotype is expressed by T cells responsible for helper activity, delayed hypersensitivity, and mixed lymphocyte reactivity to Ia determinants while the Ly23 phenotype is present on T cells that effect cell-mediated lympholysis and exert suppressor effects. Our data provides good evidence in man that functionally distinct subpopulations of T cells can also be defined by their expression of thymus-dependent antigens.

It is of theoretical and perhaps clinical interest that sensitivity to lysis by anti-T_{H1} and complement distinguishes subclasses of T cells that respond in MLC and elaborate LMF and MIF[3] in response to specific antigen from another subclass of T cells that incorporate [^{3}H]thymidine in response to specific antigen. The demonstration in vitro that specific antigen triggers T_{H1+} cells to secrete mediators on a background of low or negligible proliferative activity, while inducing T_{H1-} cells to proliferate in the absence of MIF or LMF production, suggests that the cellular immune response to foreign soluble antigens is mediated by at least two functional subclasses of human T lymphocytes. One subclass, a T_{H1+} mediator-secreting T cell, presumably serves to activate and to amplify the inflammatory response by recruiting nonsensitized immune cells responsive to factors such as MIF and LMF. The other subclass, the T_{H1-}

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[3] Chess, L., and R. Rocklin. Manuscript in preparation.

proliferating T cell, is triggered to effect in vivo functions that are as yet undetermined, but may include suppressor or cytotoxic activities. Although the relevance of in vitro T-cell activities to specific in vivo T-cell functions is controversial, this interpretation of our data is supported by the following considerations. (a) Both antigen-induced T-cell proliferation and mediator production are useful means of monitoring cellular immunity in the majority of acquired and genetic immunodeficient states, generally correlating well with delayed-type cutaneous hypersensitivity (30–32). (b) In certain immunodeficient states, these two activities are frequently dissociated (8–10), suggesting that each is effected by a distinct subclass of T cells (T_{H1^+} or T_{H1^-}), that can be selectively impaired by a specific disease process. (c) Finally, analysis of the in vitro and in vivo responses of murine Ly1 and Ly23 T cells shows that the Ly1 subclass is triggered to exert antigen-specific helper activities, while the Ly23 subclass contains the predominance of cells effecting antigen-specific suppression (33). Interestingly, like the T_{H1^+} subset, the Ly1 subclass also contains cells that are triggered by specific antigen to secrete MIF (H. Cantor, personal communication) and are responsive to Ia determinants in MLC (12). The possibility that the T_{H1^+} and the T_{H1^-} subclasses are similarly programmed to express corresponding helper and suppressor activities is therefore being actively investigated.

Finally, the demonstration that T_{H1^-} T cells proliferate in response to specific antigen but do not respond in MLC is of interest. Since Ia gene products are responsible for proliferation of the responder cell (T_{H1^+}) in MLC, and also play a major role in numerous immunologic phenomenon including genetic control of immune responsiveness and other cellular interactions in the immune system, the mechanisms by which T cells recognize and respond to Ia alloantigen as opposed to specific antigen may be quite different. Each of these two triggering mechanisms might therefore activate different subclasses of T cells (T_{H1^+} and T_{H1^-}) to proliferate and to express distinct immune functions. Alternatively, it is conceivable that the MLC response represents a primary immune response of a T-cell subset not requiring prior sensitization, while this is clearly required for the proliferative response to soluble antigen. Against this hypothesis is the fact that mediator production, which is also effected by T_{H1^+} T cells, represents an antigen-specific secondary immune response. However, it is possible that T_{H1^+} cells may be heterogeneous and further subdivided into mediator-secreting and MLC responsive cells.

Summary

A heterologous antihuman T-cell serum (anti-T_{H1}), raised against purified peripheral T cells, and absorbed with an autologous Ig^+ line, was shown to bind specifically to T- but not to B-lymphoid cells by both a complement-dependent cytotoxic assay and indirect immunofluorescence. Whereas 90% fetal thymocytes and thymocytes were killed by anti-T_{H1} and complement, a consistently restricted population (50–60%) of peripheral T cells from several normal donors were lysed, indicating that anti-T_{H1} is directed against one or more thymus-specific antigens which are lost or reduced on a subpopulation of human T cells in the periphery. Functional analysis of the unreactive (T_{H1^-})

and reactive ($T_{H1}+$) T-cell subclasses demonstrated that $T_{H1}-$ cells mounted a good proliferative response to a battery of specific soluble antigens (mumps, PPD, tetanus toxoid) but neither responded in MLC, nor elaborated LMF in response to tetanus toxoid. In contrast $T_{H1}+$ cells proliferated in MLC and elaborated LMF but did not respond by ^{3}H-incorporation to soluble antigens.

The relevance of these findings to human T-cell functions in vivo and to previously described functional subclasses of murine T cells is discussed.

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