CLONOTYPIC STRUCTURES INVOLVED IN ANTIGEN-SPECIFIC HUMAN T CELL FUNCTION
Relationship to the T3 Molecular Complex*

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Multiple lineage-specific surface molecules have recently been defined on human T lymphocytes. Although some of these appear during late intrathymic ontogeny and are maintained on all peripheral T cells (T3), others (T4, T8) arise earlier in differentiation and are selectively expressed on functional subpopulations of human T lymphocytes (1–3). In the case of the 20,000-mol wt T3 surface molecule, both its appearance in intrathymic ontogeny at the time of acquisition of immunologic competence (1, 4) and its critical role in T lymphocyte function suggested that it was closely linked to a recognition receptor for antigen. Thus, antibodies directed against T3 were able to block both the induction and the effector phase of cell-mediated lympholysis, inhibit T lymphocyte proliferative responses to soluble antigen, and be mitogenic for T lymphocytes (5–8).

Anti-T4 and anti-T8 monoclonals also blocked cell-mediated lympholysis (CML)1 (9, 10). However, whereas anti-T3 inhibited all cytotolytic effector clones, anti-T4 and anti-T8 selectively abrogated killing of T4+ or T8+ cytotoxic T lymphocytes (CTL), and this inhibition occurred on the level of target cell recognition and/or binding (8, 11). Moreover, unlike T3, they did not inhibit antigen-induced proliferation. Furthermore, the correlation of T4 and T8 surface expression on CTL cells with differential recognition of class II vs. class I molecules on target cells, respectively (9, 12), implied that these T cell surface structures might be functioning in associative recognition.

Given that T lymphocytes recognize antigen in a precise fashion (13–17), there must exist, in addition, discriminative surface recognition structures that are unique to individual antigen-responsive T cell clones (clonotypic). To delineate such molecules, we produced monoclonal antibodies in the present study against a human cytotytic T cell clone, CT8III (target specificity: HLA-A3), and developed a screening

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1 Abbreviations used in this paper: CML, cell-mediated lympholysis; Con A, concanavalin A; CTL, cytotoxic T lymphocyte; EBV, Epstein-Barr virus; E/T, effector/target; FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; [3H]TdR, tritiated thymidine; IL-2, interleukin 2; MHC, major histocompatibility complex; MLC, mixed lymphocyte culture; RIPA, radioimmune precipitation assay; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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strategy to select for anti-clonotypic antibodies. In the results reported below, we describe two such antibodies that block both the specific cytotoxic effector function and antigen-induced proliferation of CT8m. The molecules defined by anti-clonotypic antibodies are linked to the T3 surface structure but, in contrast to T3, they detect two associated glycoproteins of apparent molecular weights 49,000 and 43,000.

Materials and Methods

Derivation of Lymphocyte Populations. Lymphocytes were obtained by Ficoll-Hypaque density centrifugation of blood from a healthy donor. Epstein-Barr virus (EBV)-transformed B cell lines were kindly provided by Dr. Herbert Lazarus (Sidney Farber Cancer Institute).

Interleukin 2 (IL-2). IL-2-containing supernatants were produced by stimulating whole peripheral blood mononuclear cells with phytohemagglutinin and phorbol myristate acetate in the presence of irradiated Laz 156 cells as described (9).

Generation and Culture of Human T Cell Clones. All T cell clones used in this study were derived from a single individual's mononuclear cells stimulated in mixed lymphocyte cultures (MLC) with the allogeneic EBV-transformed lymphoblastoid cell line, Laz 156. Cloning and recloning were performed as described (9). Several alloreactive human T cell clones (CT8m, CT4n, and CT4n) were grown in continuous culture for >15 mo, maintaining initial phenotype and functions. Optimal growth of T cell clones was obtained in the presence of a feeder cell suspension (irradiated autologous peripheral blood mononuclear cells plus irradiated Laz 156 cells) in IL-2-conditioned media, although proliferation also occurred in the presence of either irradiated Laz 156 or IL-2-conditioned medium.

Cytotoxic T Cell Clone CT8m. The cytotoxic T cell clone CT8m used in the present study was generated and propagated in culture as described above (9). The phenotype of CT8m as determined with a panel of monoclonal antibodies by means of indirect immunofluorescence was T1+, T3+, T4+, T6+, TS+, T11+, T12+, Ia+. The target specificity as analyzed on a panel of HLA-typed target cells was HLA-A3. Moreover, the cytotoxic effector function of CT8m could be blocked by anti-HLA but not by anti-Ia antibodies on the target cell level.

Production and Characterization of Monoclonal Antibodies. Monoclonal antibodies anti-T3A, anti-T4A, anti-T6, anti-T8A-e, and anti-T12 were produced as previously described (5, 10, 18) and used in ascites form. Monoclonal antibodies directed at human Ia-like antigens and β2-microglobulin have been described (19). To produce clonotypic antibodies, BALB/cJ mice were initially immunized intraperitoneally with 5 × 10^6 CT8m cells in phosphate-buffered saline. 10 d later, a booster injection with 5 × 10^6 CT8m cells i.p. was performed. 3 d before somatic fusion of immune splenocytes with NS-1 myeloma cells, two mice were injected intravenously and intraperitoneally with a total of 5 × 10^6 CTSni cells. After the animals were killed, immune splenocytes were obtained and fusion was performed as previously described (5, 10, 18). Hybridoma growth was evident by 2 wk, and supernatants of hybridoma clones were subsequently screened for reactivity on CT8m by means of indirect immunofluorescence. Hybridomas that failed to produce antibodies reactive with CT8m were discarded. Reactive supernatants were then screened on a large panel of cell types. These included autologous and allogeneic resting peripheral blood cells (T cells, B cells, macrophages, granulocytes, and platelets); thymocytes; autologous and allogeneic activated T cells (mixed leukocyte reaction lymphoblasts, concanavalin A [Con A] lymphoblasts, T cell lines [2–4 mo old]); autologous and allogeneic B cell lines (Laz 509, Laz 156, and Laz 471); T cell tumor lines (CEM, Molt 4, and HSB); and tumor lines of non-T cell lineage (K562, HL-60, Laz 221, and KG1). Hybridomas producing antibody of interest were then cloned by limiting dilution, and individual hybrids were injected into pristine-rimed BALB/cJ mice. The resulting ascitic fluid was used as a source of antibody.

Phenotypic Analysis of T Cell Surface Antigens. Phenotype analysis of T lymphocytes was performed by means of indirect immunofluorescence with a panel of monoclonal antibodies and goat anti-mouse F(ab')2 fluorescein isothiocyanate (FITC) on an Epics V cell sorter (Coulter Electronics Inc., Hialeah, FL) (9). Quantitative histogram comparisons were performed with a Coulter Easy System Immunoprogram on the basis of mean channel fluorescence.

Modulation of Surface Antigens by Monoclonal Antibodies. CT8m cells at 1 × 10^6/ml were incubated with saturating amounts of one or another monoclonal antibody for 18 h at 37°C in
final culture medium RPMI 1640 plus 12% human AB serum. Subsequently, the cells were washed three times and analyzed for expression of various cell surface antigens by means of indirect immunofluorescence on a fluorescence-activated cell sorter (FACS) before assessment of their functional properties (5, 11).

**Competitive Antibody Binding Inhibition Studies.** For these studies, directly FITC-labeled, purified monoclonal antibodies (anti-T3A, anti-T1A, and anti-T1B) were prepared. All experiments were performed at 4°C to prevent modulation of cell surface antigens. In the first incubation step, CT8M cells were incubated with saturating amounts of one or another unlabeled monoclonal antibody for 30 min. Then the cells were washed twice and incubated with directly FITC-labeled monoclonal antibody (30 min) before FACS analysis.

**Investigation of Cell-Mediated Lympholysis.** To examine functional effects of monoclonal antibodies to T cell surface antigens on the cytotoxic effector function, clones were incubated for various periods (30 min–18 h) with monoclonal antibodies at several dilutions or medium prior to addition of 51Cr-labeled targets. These experiments were performed at an effector/target (E/T) ratio of 20:1 in V-bottomed microtiter plates (Falcon Labware, Oxnard, CA) according to a standard method (20).

**Proliferative Studies.** To investigate the effects of monoclonal antibody treatment on proliferative capacities, CT8M cells were incubated for various periods (30 min–18 h) with monoclonal antibodies at several dilutions or media. Subsequently, untreated or antibody-treated cells were plated at 15,000 cells/well into round-bottomed microtiter plates (Costar, Data Packaging, Cambridge, MA) along with either medium (RPMI 1640 supplemented with 10% human AB serum), irradiated Laz 156 cells (15,000 cells/well), IL-2-conditioned medium (final concentration 5%), or Laz 156 cells plus IL-2. After a 24-h incubation at 37°C, the various cultures were pulsed with 0.2 µCi of tritiated thymidine ([3H]Tdr) (Schwarz/Mann, Div. Becton Dickinson & Co., Orangeburg, NY) and harvested 18 h later on a Mash II apparatus (M. A. Bioproducts, Walkersville, MD). [3H]Tdr incorporation was then measured in a scintillation counter (Packard Instrument Co., Inc., Downers Grove, IL). Each value represents the mean of triplicates.

**Radiolabeling and Immunoprecipitation of T Cell Surface Antigens.** Cell surface iodination of T cell clones was performed with Na125I (New England Nuclear, Boston, MA) and catalyzed by lactoperoxidase. To 20 × 10^6 cells suspended in 1 ml PBS were added successively 10 µl glucose (0.5 mol/liter), 5 µl NaI (5 × 10^4 mol/liter), 10 µl lactoperoxidase (2 mg/ml), 1 mCi Na125I, and 20 µl glucose oxidase (7.5 mU/ml). This mixture was incubated for 15 min at room temperature followed by addition of 100 µl NaI (1 mol/liter). After an additional 2 min of incubation, cells were washed four times in Hanks’ balanced salt solution. The final pellet was lysed in 500 µl 1:5 diluted radioimmune precipitation assay (RIPA) stock solution containing 1% Triton X-100 (RIPA stock solution: 0.1 mol/liter NaH2PO4, 1 mmol/liter phenylmethylsulfonyl fluoride, 10 mmol/liter EDTA, 10 mmol/liter EGTA, 10 mMol NaF, 1% deoxycholate sodium salt, 200 KIU/ml aprotinin, pH 7.2). The suspension was centrifuged for 5 min at 1,120 g and the resulting supernatant was precleared twice using monoclonal antibody anti-T6 covalently linked to CnBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden), each for 60 min at 4°C, and subsequently centrifuged 5 min at 1,120 g. Precleared lysates were incubated with monoclonal antibodies bound to CnBr-activated Sepharose 4B for 2 h at 4°C. The resulting precipitate was subsequently washed five times in RIPA solution (stock 1:10 diluted) plus 1% Triton X-100 suspended in gel buffer (0.125 mol/liter Tris-HCl, pH 6.8, containing 10% glycerol, 3% sodium dodecyl sulfate [SDS], and 5% 2-mercaptoethanol) and boiled for 5 min. SDS polyacrylamide gel electrophoresis (PAGE) was performed on a continuous vertical slab gel (12.5% polyacrylamide) for 14 h according to a modification of the Laemmli procedure (21).

**Results**

To produce monoclonal antibodies to clonally restricted antigen recognition structures on human T lymphocytes, BALB/cJ mice were immunized with the human cytotoxic T cell clone CT8M (phenotype: T1-, T3+, T4+, T6+, T8+, T11+, T12+, Ia+), which was previously shown to be specific for the HLA-A3 antigen. Subsequently,
immune splenocytes were fused with the NS-1 mouse myeloma line by standard hybridization technique (3). Of 221 individual hybridoma clones that were established in hypoxanthine, aminopterin, thymidine medium, 59 secreted monoclonal antibodies reactive with the immunizing clone CT8III. However, 43 of these 59 antibodies were also reactive with the autologous B lymphoblastoid cell line LAZ 509 and were therefore most likely directed at either alloantigens or other broadly distributed cell surface molecules. In contrast, the remaining 16 antibodies were reactive with the immunizing clone but lacked reactivity with B cells, macrophages, granulocytes, platelets, B lymphoblastoid lines, myeloid lines, and other hematopoietic lines (see Materials and Methods).

Within the latter group, seven antibodies were reactive with the 76KD T8 surface structure (anti-T8D\textsubscript{a}), one recognized the 20,000-mol wt T3 molecule (anti-T3B), and six were specific for activated but not resting T lymphocytes (anti-TA\textsubscript{A-6}). In addition, two antibodies, termed anti-T1\textsubscript{IA} and anti-T1\textsubscript{IB}, reacted exclusively with the CT8III clone and were of the IgG\textsubscript{1} and IgM isotype, respectively.

Unlike the other 14 antibodies, anti-T1\textsubscript{IA} and anti-T1\textsubscript{IB} did not react with human thymocytes or resting or activated human peripheral T cells (both autologous or allogeneic). More importantly, these were also nonreactive with 80 additional individual alloreactive T cell clones derived from the same donor as CT8III. The pattern of antibody binding to the alloreactive T1\textsubscript{IA}-T1\textsubscript{IB}\textsuperscript{+} clone CT8III, and CT8IV, a representative T1\textsubscript{IA}-T1\textsubscript{IB}\textsuperscript{+} clone, is shown in Fig. 1. All CT8III cells (panel A) are reactive with anti-T1\textsubscript{IA} and anti-T1\textsubscript{IB}, whereas CT8IV cells are nonreactive with either antibody as analyzed by indirect immunofluorescence on an Epics V cell sorter. In contrast, both CT8III and CT8IV express the 20,000-mol wt T3 molecule as defined by anti-T3 reactivity (shaded area). Similar results to those obtained with CT8IV were found for the other 79 autologous clones.

Given the unique reactivities of anti-T1\textsubscript{IA} and anti-T1\textsubscript{IB} monoclonal antibodies with
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Fig. 2. Influence of various monoclonal antibodies on CML function of T cell clone CT8m. CT8m cells were incubated with various monoclonal antibodies or media before assessment of their cytolytic activity directed at 51Cr-labeled Lazz 156 target cells. Final dilutions of monoclonal antibodies: anti-T3A, 1:300; anti-T3B, 1:500; anti-T12A, 1:500; anti-T12B, 1:500; anti-T12A, 1:500; anti-T12B, 1:500; anti-T12, 1:500. The E/T ratio was 20:1. Numbers give percent specific lysis and represent the means of triplicates (SD ≤ 10% of maximal lysis).

the antigen-specific cytotoxic T cell clone CT8m, it was important to determine whether the surface structures that they defined were involved in antigen recognition. To this end, anti-T12A and anti-T12B were incubated with CT8m cells for various periods before assay of the clone's cytolytic and proliferative capacities. In parallel, the effects of several other T cell-specific monoclonal antibodies (anti-T3A, -T3B, -T8A, -T8C, and -T12) were investigated. As shown in Fig. 2, at an E/T ratio of 20:1, CT8m cells were highly efficient (>50% specific lysis) in lysing the HLA A3+B lymphoblastoid line Lazz 156. Anti-T3A,B and anti-T8A both markedly inhibit CTL effector function of this clone (≤10% lysis), whereas anti-T8C and anti-T12 do not. These results are in keeping with earlier studies which indicated that both anti-T3 and anti-T8 monoclonal antibodies can inhibit T8+ CTL (5, 8, 11). In addition, they are consistent with the finding that not all anti-T8 antibodies mediate this effect (9, 10). More importantly, both anti-T12A and anti-T12B markedly inhibit the CT8m clone's cytotoxic effector capacity (<20 and <5% specific lysis, respectively). Although it is not shown, the fact that inhibitory effects of the monoclonal antibodies were observed at dilutions of ascites varying from 1:250 to 1:2,500 and were evident after <30 min of preincubation with CT8m should be noted. However, the magnitude of the inhibitory effect increased with the length of preincubation.

Although by indirect immunofluorescence anti-T12A and anti-T12B reactivity appeared to be restricted to the CT8m clone, we also determined whether the functional effects of these antibodies on cytolytic activity were restricted to CT8m. As shown in Table I, anti-T12A and anti-T12B exclusively inhibited killing of the CT8m clone among a panel of five CTL clones tested. In contrast, the effects of anti-T3, anti-T4,
and anti-T8 antibodies were not restricted to the CT8m clone. Anti-T3 monoclonal antibodies blocked all five CTL clones irregardless of their T4+ or T8+ subset derivation or specificity; anti-T4A inhibited cytolysis by the T4+ clones CT4I and CT4II and had no effect on killing by the T8+ clones CT8I, CT8II, and CT8m; and in a reciprocal fashion, anti-T8A and anti-T8B monoclonal antibodies inhibited killing of all three T8+ CTL but not the two T4 CTL clones, CT4I and CT4II.

Previous studies indicated that some human CTL clones not only specifically kill target cells but also proliferate to them in an antigen-specific fashion (5). To examine the effect of the anti-clonotypic antibodies on the antigen-specific proliferative capacity of clone CT8III, an additional series of experiments was performed. As shown in Table II, untreated CT8III cells proliferate to both IL-2-containing supernatants as well as the allogeneic cell line Laz 156, to which they had been originally stimulated. Under these experimental conditions, antigen (Laz 156) is the stronger stimulus for CT8III cell proliferation as judged by the greater \[^{3}H\]TdR incorporation into CT8III with the former (>12,000 cpm vs. 2,714 cpm). In addition, the induction of clonal proliferation by the combination of alloantigen and IL-2 is greater than with either alone (17,152 cpm). Pretreatment of CT8III with anti-TiA or anti-TiB markedly reduces antigen-specific proliferation of the CT8III clone. Thus, whereas the untreated CT8III clone proliferated with >12,000 counts of \[^{3}H\]TdR to Laz 156, CT8III preincubated with anti-TiA or anti-TiB proliferated with <2,500 cpm, a reduction

### Table I

Inhibitory Effects of Monoclonal Antibodies on CML by Various T Cell Clones

| Monoclonal antibody | CT4I | CT4II | CT8I | CT8II |
|---------------------|------|-------|------|-------|
| Anti-T3A,B          | +    | +     | +    | +     |
| Anti-T4A            | +    |       | -    | -     |
| Anti-T8A,B          | -    | -     | +    | +     |
| Anti-TiA            | -    | -     | -    | +     |

Clonally restricted functional activity of anti-TiA and anti-TiB. Various T cell clones, all cytotoxic for Laz 156 target cells, were incubated with monoclonal antibodies (final dilutions: anti-T3A, 1:300; anti-T3B, 1:500; anti-T8A, 1:500; anti-T8B, 1:500; anti-T4A, 1:500; anti-TiA, 1:300; anti-TiB, 1:500) before incubation with \[^{51}Cr\]-labeled Laz 156 cells at an E/T ratio of 20:1. +, inhibition of CML >60%; –, inhibition of CML <3%.

### Table II

Influences of Monoclonal Antibodies on Proliferative Responses of Clone CT8III

| Stimulus       | Untreated | Anti-TiA | Anti-TiB | Anti-T3A | Anti-T3B | Anti-T4A | Anti-T4B | Anti-T8A | Anti-T8B | Anti-TiA | Anti-TiB |
|----------------|-----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Medium         | 41 ± 23   | 139 ± 36 | 206 ± 54 | 92 ± 34  | 66 ± 27  | 23 ± 20  |
| IL-2           | 2,714 ± 135 | 10,927 ± 1,520 | 15,009 ± 1,049 | 13,417 ± 1,320 | 2,525 ± 327 | 2,459 ± 476 |
| Laz 156        | 12,395 ± 317 | 2,117 ± 416  | 1,169 ± 207 | 776 ± 99  | 17,756 ± 817 | 10,956 ± 357 |
| IL-2 plus Laz 156 | 17,152 ± 471 | 14,389 ± 1,005 | 15,393 ± 2,176 | 15,739 ± 1,737 | 15,389 ± 2,176 | 14,982 ± 590 |

Influence of various monoclonal antibodies on proliferative responses of CT8III to various stimuli. CT8III cells were incubated with monoclonal antibodies or media before assessment of their proliferative responses. Final dilutions of monoclonal antibodies were: anti-TiA, 1:300; anti-TiB, 1:500; anti-T3A, 1:1,500; anti-T3B, 1:500; anti-T4A, 1:500; anti-T4B, 1:500; anti-T8A, 1:500; anti-T8B, 1:500; anti-TiA, 1:500; anti-TiB, 1:500. Numbers are given as counts per minute of \[^{3}H\]TdR and represent the means of triplicates.
FIG. 3. Modulation and co-modulation of CT8m surface structures by anti-T3B treatment. CT8m cells were incubated with the monoclonal antibody anti-T3B (final dilution 1:300) for 18 h at 37°C and subsequently washed several times. Surface phenotype was determined by means of indirect immunofluorescence before (dark areas) and after (light areas) modulation with saturating concentrations of monoclonal antibodies. Panel A: anti-T3B (1:300); panel B: anti-T11A (1:500); panel C: anti-T11B (1:500); panel D: anti-T8A (1:500).

in proliferation of >80%. A similar reduction of proliferation was obtained with anti-T3B. That these effects were not simply due to an inactivation of the CT8m clone as a result of antibody treatment is clear from the fact that (a) anti-T12 and anti-T8A have no inhibitory effects in this system (Table II); and (b) the anti-T11A, anti-T11B- or anti-T3B-treated CT8m clone has an augmented proliferative capacity to IL-2-containing supernatant (>10,000 cpm vs. <3,000 cpm). It should be emphasized that anti-T8A, in contrast to its ability to profoundly block cytolytic effector function by T8+ CTL clones, lacks any inhibitory effect on antigen-specific proliferation.

The observation that anti-T3, anti-T11A, and anti-T11B all inhibited antigen-specific proliferation and CTL effector function and enhanced IL-2 responsiveness of CT8m suggested that there might be a relationship between the cell surface structures defined by these antibodies. In this regard, previous studies (5) indicated that binding of anti-T3 antibodies to T cells at 37°C resulted in selective modulation and external shedding of the T3 molecular complex without affecting cell viability or altering expression of other known T cell surface structures including T1, T11, T12, or T8 surface molecules. To determine whether anti-T3 induced modulation produced changes in surface expression of detectable T11A or T11B molecules, CT8m cells were first incubated with anti-T3B for 18 h at 37°C and washed to remove free monoclonal antibody; subsequently, cell reactivity was analyzed by indirect immunofluorescence on an Epics V cell sorter with a panel of monoclonal antibodies.

As shown in Fig. 3, before modulation, all CT8m cells were reactive with anti-T3A, anti-T11A, anti-T11B, and anti-T8A (panels A-D, dark curves). In contrast, after modulation with anti-T3B, T3 antigen was no longer detectable (panel A, light curve). More importantly, anti-T3B-induced modulation also resulted in loss of the anti-T11A and anti-T11B surface epitopes (panels B and C, respectively). That this was not a nonspecific effect is evident from the observation that the T8 antigen density (panel
Table III

Competitive Inhibition of Monoclonal Antibody Binding to Clone CT8m

| 1st incubation | 2nd incubation |
|----------------|----------------|
| Anti-T3A       | Anti-T3A-FITC  |
| Anti-T3B       | Anti-T3B-FITC  |
| Anti-T8A       | Anti-T8A-FITC  |
| Anti-TiA       | Anti-TiA-FITC  |
| Anti-TiB       | Anti-TiB-FITC  |

Competitive inhibition of monoclonal antibody binding to CT8m cells. 1st incubation: CT8m cells were incubated for 30 min at 4°C with an unlabeled monoclonal antibody (final dilution 1:100) and then washed twice in cold medium. 2nd incubation: to the antibody-treated CT8m cells was added an FITC-labeled monoclonal antibody (final dilution 1:50) followed by analysis on an Epics V cell sorter. +, blocking of binding of FITC-labeled monoclonal antibodies as judged by reduced fluorescence (>90% reduction, on the basis of mean channel fluorescence as compared with CT8m cells pretreated with negative control ascites). –, unchanged fluorescence (<5% reduction).

D) was not influenced by this process. Although not shown, incubation of CT8m cells with either anti-TiA or anti-TiB had identical effects: in all cases T3, TiA, and TiB molecules co-modulated. The above results would indicate that the molecules defined by anti-T3, anti-TiA, and anti-TiB are functionally and phenotypically linked on the cell surface of this clone.

To further investigate the relationship of the epitope defined by anti-T3 and those defined by anti-TiA and anti-TiB, both competitive antibody binding inhibition studies and immunoprecipitations were performed. As shown in Table III, unlabeled anti-T3A and anti-T3B inhibited subsequent binding of directly FITC-labeled anti-T3A, whereas anti-TiA and anti-TiB did not. In a reciprocal fashion, unlabeled anti-T3A or anti-T3B failed to inhibit subsequent binding of directly FITC-labeled anti-TiA or anti-TiB. In contrast, either unlabeled anti-Ti antibody was inhibitory for FITC-labeled anti-TiA or anti-TiB. Under no circumstances did unlabeled anti-T8A block binding of directly FITC-labeled anti-T3A, anti-TiA, or anti-TiB. Taken together, these findings would suggest that the surface epitopes recognized by anti-TiA and anti-TiB are spatially related (or identical) to one another but clearly distinct from those defined by anti-T3 or anti-T8 antibodies.

To define biochemically the surface molecules detected by anti-TiA and anti-TiB, CT8m cells were labeled by lactoperoxidase technique with $^{131}$I and immunoprecipitates from solubilized membranes subjected to SDS-PAGE. As a control, CT8iv, an additional autologous T8⁺ CTL clone nonreactive with anti-TiA and anti-TiB, was tested in parallel. Fig. 4 shows that under reducing conditions, anti-TiA precipitated two distinct bands of ~49,000 and 43,000 mol wt, respectively, from the CT8m clone (channel C), but not the irrelevant CT8iv clone (channel D). In a parallel fashion, anti-TiB precipitated identical structures from the same CT8m clone (panel E), but not the irrelevant CT8iv clone (channel F). Negative control precipitates from CT8m and CT8iv using monoclonal antibody T6 are shown in channels A and B, respectively. Both the inability of the anti-clonotypic antibodies to inhibit anti-T3 cell surface binding and the present biochemical data are consistent with the notion that these two sets of antibodies are directed at different glycoproteins than the major 20,000-mol wt glycoprotein defined by anti-T3 antibodies.
Discussion

In the present study, murine monoclonal antibodies were produced against a human T3⁺T8⁺ CTL clone, CT8m, with the view of defining components of its surface receptor structures for antigen. By first screening for reactivity on autologous B lymphoblastoid cells and other alloreactive clones using indirect immunofluorescence, antibodies to broadly distributed lymphoid lineage markers or allospecificities were eliminated. Only 2 of 221 B-B hybridomas resulting from a single fusion between CT8m-immune BALB/cJ splenocytes and NS-1 myeloma cells secreted antibodies that were restricted in their reactivity to CT8m; these were termed anti-TiA and anti-TiB. Anti-TiA and anti-TiB did not react with thymocytes, resting or activated T lymphocytes, B lymphocytes, macrophages, granulocytes, platelets, or any of 80 additional clones from the same individual donor as the CT8m clone. In contrast, anti-T3, anti-T4, and anti-T8 reacted with all mature T lymphocytes, inducer T lymphocytes, and suppressor T lymphocytes, respectively (1, 2). Moreover, all alloreactive human T cell clones were T3⁺ and expressed either the T4 or T8 surface...
molecules. Thus, unlike the previously defined antibodies, anti-TiA and anti-TiB recognized clonotypic structures.

Anti-TiA and anti-TiB had striking effects on CT8m cell function: both inhibited its antigen-specific proliferative responses and CTL effector function. Because similar effects were observed with anti-T3 antibodies (5-8, 11), we investigated the relationship of TiA, TiB, and T3 molecules in greater detail. It was found that all three surface structures were associated on CT8m because they co-modulated after binding either anti-TiA, anti-TiB, or anti-T3 antibodies. However, multiple lines of evidence indicated that these antibodies reacted with different surface epitopes: (a) the cellular distribution of anti-T3 reactivity was distinct from anti-TiA and anti-TiB, as noted above; (b) anti-T3 antibody blocked the killing function of all CTL clones tested, whereas the effect of anti-TiA and anti-TiB was restricted to CT8m; (c) in competitive binding studies with FITC-labeled and unlabeled antibodies, neither anti-TiA nor anti-TiB inhibited anti-T3 binding to CT8m cells and vice versa, whereas anti-TiA and/or anti-TiB inhibited each other’s binding to CT8m; and (d) unlike anti-T3A or anti-T3B, which precipitated a major 20,000-mol wt glycoprotein from all mature T cells and thymocytes (5, 22), anti-TiA and anti-TiB were found to immunoprecipitate two bands at 49,000 and 43,000 mol wt under reducing conditions from the 125I-radiolabeled CT8m clone alone. The specificity of anti-TiA and anti-TiB for epitopes unique to the CT8m clone was further supported by the absence of detectable material on SDS-PAGE analysis of immunoprecipitates from three other T8+ CTL clones and a heterogeneous T cell population (predominantly T8+) derived from the same donor as CT8m (Fig. 4 and data not shown).

More recent studies (5, 11) have indicated that preincubation of clones with anti-T3 antibodies abrogated antigen-specific proliferation and CTL effector function of every population tested. After anti-T3 antibody surface binding at 37°C, the 20,000-mol wt T3 molecules were externally shed in a modulation process that required 18-24 h. Loss of antigen-specific function was evident when surface T3 was bound by anti-T3 before modulation and persisted in the post-modulation period when neither surface antibody nor T3 molecules were detectable on the clone. More importantly, after anti-T3-induced modulation, CTL recovered effective cytolytic activity in parallel with reexpression of surface T3 molecules (5). That this effect was not simply due to the modulation process itself was evident from parallel studies in which a different antigen, T1, a 67,000-mol wt glycoprotein, was induced to modulate from T cell clones with anti-T1 antibody. In that case, no inhibition of T cell function was noted (5). Thus, both the appearance of T3 in late intrathymic ontogeny and its critical role in T lymphocyte function (1, 4) suggested that it was closely linked to an important recognition receptor. Hence, the association of the 20,000-mol wt T3 molecule with clonotypic structures such as TiA and TiB involved in antigen recognition was not entirely unexpected.

It is likely that anti-TiA and anti-TiB define variable regions of the human T cell antigen receptor on CT8m because they recognize clonotypic structures and inhibit antigen-specific function. In contrast, the wide distribution within the T lineage of the 20,000-mol wt T3 glycoprotein defined by the presently existing anti-T3 antibodies suggests that, unlike anti-TiA and anti-TiB, they define a constant region of the antigen receptor complex. This does not exclude the possibility, however, that
variability could exist within another portion of this structure or that it might possess antigen binding properties. In this regard, it is known that at least one other 25–28,000-mol wt structure is associated with the T3 molecular complex (22). Biochemical analysis at the protein and DNA level will resolve this issue.

Two major subsets of T lymphocytes have been defined on the basis of their differential expression of either the T4 or T8 surface molecules. These are glycoproteins of molecular weight 62,000 and 76,000, respectively, which are unrelated to T3 (2). Although cytotoxic effector T lymphocytes are derived from both of these populations, the target antigens they recognized are the products of different major histocompatibility complex (MHC) gene regions (9, 12). Specifically, allosensitized T4+ T cells kill class II MHC antigens, whereas T8+ T cells kill class I MHC antigens.

The association between surface phenotype of a CTL and the class of MHC molecule that it recognized implied that the same subset restricted structures might be required in the facilitation of selective lysis of target antigens. This hypothesis has now been substantiated by the finding that monoclonal antibodies to the T4 or T8 glycoproteins selectively inhibit cytolytic effector function of T4 or T8 clones, respectively. Because their inhibitory effects can be bypassed by agglutination of CTL and target cells with the lectin Con A, these structures are probably not involved in the lytic mechanism itself (11, 23). Similarly, it should be noted that Con A could reconstitute the capacity of the CT8m clone to kill the HLA-A3 alloantigen bearing target Laz 156, even in the presence of anti-T3, anti-T1A, or anti-T1B. However, in this case, killing specificity was lost because the CT8m clone killed HLA-A3 negative targets as well.

The effects of anti-T4 and anti-T8 antibodies are clearly distinct from those of anti-T3, or in the case of the CT8m clone, anti-T1A or anti-T1B, in one major respect. The former two antibodies, unlike the latter three, do not block antigen-specific proliferative responses by T cell clones. This suggests that the T4 and T8 glycoproteins may serve as subset-restricted association structures for nonpolymorphic determinants on classes of MHC gene products that may be required for efficient E/T interaction in CML rather than as components of the T cell antigen recognition receptor linked to T3, T1A, and T1B.

The observation that anti-T3, anti-T1A, or anti-T1B pretreatment of CT8m cells enhanced subsequent IL-2 responsiveness is important for several reasons. First, it provides strong evidence that the effects of these monoclonal antibodies on antigen-specific function were not due to trivial consequence of global diminution in clonal activity. Second, it suggests that in this one respect, the monoclonal antibodies triggered the clone in a similar fashion to specific alloantigen because both yield enhanced IL-2 responsiveness. Whether the basis of this effect is due to induction of endogenous IL-2 secretion by the CT8m clone itself, or alternatively, enhanced sensitivity to IL-2, for example, by means of alterations in the number or state of IL-2 receptors, is currently under investigation. In this regard, it is known that both major human T cell subsets produce IL-2 upon appropriate stimulation (24). Furthermore, the above findings may in part account for the mitogenic effect of anti-idiotypic heteroantisera on murine T cell clones, as previously reported (25).

Previous attempts to characterize T cell receptor molecules have been largely biased by the assumption that they share certain similarities with surface immunoglobulin on B lymphocytes (see ref. 26 for review). In fact, analysis of T cell idiotypic structures
has used antisera generated to variable regions of secreted immunoglobulin. Although the existence of such idiotypes on T cells has been suggested by a multiplicity of functional studies whereby antiidiotypes produced effects on immunoregulatory T cell populations (27-29), the precise nature of the T cell molecules defined by these antisera is still poorly understood. Furthermore, the lack of easily demonstrable Ig gene rearrangements in homogeneous murine T helper and T suppressor lines with JH and CH gene probes (30, 31) suggests that antigen recognition by T cells in all likelihood does not occur through structures closely analogous to those used by B lymphocytes.

The molecular weights of TiA and TiB in the human system appear to be strikingly similar to those of antigen-specific suppressor factors generated in a Xenopus laevis oocyte translation system using messenger RNA from a keyhole limpet hemocyanin-specific murine T-T hybridoma clone that consisted of two individual antigen binding molecules of molecular weights 29-30,000 and 45-62,000 (32, 33). However, others (28, 34-48) reported suppressor factors and antigen-binding material that appear to be of different molecular weight and characteristics. Whether the reported differences result from the use of functionally distinct T cell populations, alterations resulting from the fusion process in the case of T-T hybridomas and/or differences between secreted and surface forms of the T cell receptor, is not known at the present time, but clearly, all possibilities remain open.

The generation of monoclonal antibodies to cloned T cell populations may represent an advantageous approach to define T cell surface recognition structures because it makes no assumptions about the similarities between B and T cell receptor molecules. In addition, it allows for the development of probes to the cell surface receptor glycoproteins themselves as opposed to secreted antigen-specific products that may be significantly different from the intact membrane molecule. The use of cloned homogeneous T cells should furthermore provide the functional machinery to identify important biologic effects of anti-clonotypic antibodies on the immune response and yield the cellular material for precise biochemical and genetic studies.

**Summary**

Monoclonal antibodies were produced against a human cytotoxic T cell clone, CT8IIH (specificity: HLA-A3), with the view of defining clonally restricted (clonotypic) surface molecules involved in its antigen recognition function. Two individual antibodies, termed anti-TiA and anti-TiB, reacted exclusively with the CT8IIH clone when tested on a panel of 80 additional clones from the same donor, resting or activated T cells, B cells, macrophages, thymocytes, or other hematopoietic cells. More importantly, the two antibodies inhibited cell-mediated killing and antigen-specific proliferation of the CT8IIH clone but did not affect the functions of any other clone tested. This inhibition was not secondary to generalized abrogation of the CT8IIH clone's function, because interleukin 2 responsiveness was enhanced. To examine the relationship of the structures defined by anti-clonotypic antibodies with known T cell surface molecules, antibody-induced modulation studies and competitive binding assays were performed. The results indicated that the clonotypic structures were associated with, but distinct from, the 20,000-mol wt T3 molecule expressed on all mature T lymphocytes. Moreover, in contrast to anti-T3, anti-TiA and anti-TiB each immunoprecipitated two molecules of 49,000 and 43,000-mol wt from 125I-labeled
CT8HII cells under reducing conditions. The development of monoclonal antibodies to such polymorphic T cell surface structures should provide important probes to further define the surface receptor for antigen.

Note added in proof: Since submission of this manuscript, we have produced an anticonotypic monoclonal antibody, anti-Ti2A, to a surface structure on the T3+, T4+, T8- class II-specific CTL clone CT4HII. The Ti2A clonotype, like TiIA and TiIB on CT8HII, is membrane associated with T3. As expected, anti-Ti2A selectively blocks antigen-specific proliferation and CTL effector function of CT4HII and enhances its IL-2 responsiveness.

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