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Immunology

Monoclonal antibodies against the antigen receptor on a cloned T-cell hybrid

(T-cell clones/T-cell receptor/interleukin 2/heterodimer/hybridoma)

LAWRENCE E. SAMELSON, RONALD N. GERMAIN, AND RONALD H. SCHWARTZ

The Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20205

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ABSTRACT **A pigeon cytochrome *c*-specific and Ia molecule-restricted T-cell hybrid was used as an immunogen in order to obtain monoclonal anti-antigen receptor antibodies. Two antibodies were isolated that specifically bound to and inhibited interleukin (IL) 2 release from only the immunizing clone. Lectin-induced IL 2 release was not affected by these antibodies. Binding assays with purified and iodinated monoclonal antibody indicated that there were ≈25,000 binding sites on the T-cell hybrid. Immunoprecipitation and $NaDodSO_4$/polyacrylamide gel electrophoresis of detergent lysates from surface-labeled hybrid cells revealed a heterodimeric structure composed of chains of apparent M_rs 45,000–50,000 and 40,000–44,000. The chains were linked by intermolecular disulfide bonds, and the difference in migration of the isolated chains under reducing and nonreducing conditions was consistent with the presence of intramolecular disulfide bonds. The molecule that has been identified is a candidate for the antigen-specific receptor on the immunizing T-cell clone.**

The structure of the antigen receptor on major histocompatibility complex (MHC)-restricted T cells remains one of the most important questions in current immunological research. Much understanding has come from the functional analysis of T-cell activation, but ultimately such issues as the nature of the interaction between the T-cell receptor, antigen, and MHC-encoded molecules will be resolved by biochemical investigations.

Attempts have been made over the last decade to define T-cell receptor material by the generation and use of anti-idiotypic reagents prepared against B-cell products or subsets of T cells (1). An improvement in this approach has become possible recently because of advances in cellular cloning techniques. Normal T-cell lines and clones with defined antigen reactivity can be produced (2). Moreover, the use of somatic cell fusion techniques allows for the generation of T-cell clones in large numbers and in the absence of contaminating cells, antigen, or growth factors (3). These cloned T-cell hybrids can be used as immunizing reagents, thus presenting large amounts of homogeneous receptor material to a recipient animal. Recently, several laboratories have taken these approaches to obtain monoclonal antibodies that bind T-cell clones or hybrids. These antibodies have been used to isolate molecules that may be the antigen receptor on the immunizing cell (4–6).

We have described a series of interleukin (IL) 2-producing T-cell hybrids specific for pigeon cytochrome *c* (7, 8). An extensive characterization of their fine specificity patterns for antigen and Ia molecules has resulted in a further understanding of the interaction between the T-cell receptor, the MHC-encoded restriction element, and the antigen. Two of these well-characterized hybrids have been used for immunization, and antisera have been generated that specifically perturb the antigen-induced IL 2 release of the immunizing clones (9). In this report we describe the use of this immunization protocol to generate monoclonal antibodies that specifically bind a cell surface structure that is a candidate for the antigen-specific T-cell receptor.

MATERIALS AND METHODS

Animals. B10.A/SgSn and (AKR/J × BALB/cJ)F_1 mice were obtained from The Jackson Laboratory. B10.A mice also were obtained from Harlan Sprague Dawley Laboratories (Madison, WI). The mice were immunized at 4 months of age.

Antigens. The carboxyl-terminal (residues 81–104) cyanogen bromide cleavage fragment from purified pigeon cytochrome *c* was prepared as described (10).

T-Cell Hybrids. Long-term lines of pigeon cytochrome *c*-specific T cells were prepared by the method of Kimoto and Fathman (11) as described by Matis *et al.* (12). These lines were used to generate T-cell hybrids by the method of Kappler *et al.* (3) as described by Hedrick *et al.* (8). Hybrids used in these studies have been cloned by limiting dilution at least twice.

The pigeon cytochrome *c*-specific T-cell hybrids 2B4 and 2C2 are B10.A-derived clones with similar fine specificities for cytochrome *c* species variants. 2H10, also a pigeon cytochrome *c*-specific hybrid, differs in certain features, such as the ability to be activated by pigeon cytochrome *c* on the allogeneic B10.S(9R) APC (8, 9).

Assay for Antigen-Specific Responses. T-cell hybrids were selected and subsequently assayed on the basis of IL 2 release when cultured in the presence of the appropriate antigen-presenting cell and antigen, as originally described by Kappler *et al.* (3). Supernatants were assayed for the ability to support the growth of the IL 2-dependent line HT-2, originally produced by J. Watson (University of Auckland, New Zealand) and kindly provided by P. Marrack (National Jewish Hospital, Denver, CO). Fifty microliters of IL 2 containing supernatants was added to 70 μl of medium containing 4,000 HT-2 cells. After a period of ≈24 hr, when HT-2 cells cultured without IL 2 appeared dead by phase microscopy, 1 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine was added. Cultures were harvested 12–18 hr later. Concentrations of antigens were used that reproducibly yielded IL 2 release resulting in values of HT-2 proliferation below plateau level.

Immunization and Cell Fusion Technique. A modification of a technique utilizing adoptive transfer of B-cell precursors to increase the frequency of desired hybrids was used (13). (AKR/J × BALB/cJ)F_1 female mice were primed intraperitoneally with 10^7 2B4 cells emulsified in complete Freund's adjuvant and boosted intraperitoneally with 2B4 in incomplete Freund's

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Abbreviations: IL, interleukin; MHC, major histocompatibility complex; FACS, fluorescence-activated cell sorter.

adjuvant 4 wk later. Six days before adoptive transfer, a second group of syngeneic F_1 females were primed intravenously with 10^7 2B4 cells irradiated with 10,000 R. This second group of animals was itself irradiated with 550 R on the day before reconstitution. The majority of the animals primed and boosted with 2B4 cells in complete and incomplete Freund's adjuvant had a marked ascites 2 wk after boosting. On the day of adoptive transfer, this ascites was harvested by inserting a 19-gauge needle into the peritoneal cavity. The cells in the draining fluid were washed in Hanks' balanced salt solution and mixed with viable 2B4 cells. A total of 10^8 ascitic cells and 2×10^7 2B4 cells were injected intravenously into the primed and irradiated recipients. Four days later, spleens of the recipients were harvested and fused with the hybridoma SP2/0 (14) by standard techniques (15). Medium in wells containing growing hybrids was replaced repeatedly to remove residual aminopterin and thymidine. Culture supernatants were tested for specific antibody by adding aliquots into the assay for antigen-induced IL 2 release and screening for inhibition. Hybrids that scored positive on this test were cloned three times by limiting dilution.

Flow Microfluorometry. Analyses by flow microfluorometry were performed on a fluorescence-activated cell sorter (FACS II; Becton Dickinson). Data are presented as histograms of fluorescence intensity in fluorescence units vs. cell frequency. Indirect staining was performed as described by using a fluorescein-conjugated Fab fragment from affinity-purified goat anti-mouse immunoglobulin (kindly provided by B. J. Fowlkes, National Institute of Allergy and Infectious Diseases) (9).

Binding Assay. The monoclonal antibody was purified from ascites by ion-exchange (DEAE Affi-Gel Blue; Bio-Rad) and affinity chromatography (protein A-Sepharose; Pharmacia) with 3.5 M $MgCl_2$ elution. It was labeled with diiodo ^{125}I-labeled Bolton–Hunter reagent (16) (New England Nuclear) to a specific activity of 2.8×10^6 cpm/μg.

T-cell hybrids were washed in Hanks' balanced salt solution containing 10% fetal calf serum and 0.02% sodium azide. Cells (5×10^5) were dispensed in polyvinyl chloride microtiter plates. Increasing amounts of ^{125}I-labeled monoclonal antibody were titered in duplicate onto the cells. The cells were incubated for 1 hr at 4°C on a microtiter plate shaker. After three washes with 10% fetal calf serum in Hanks' balanced salt solution, the wells of the plate were cut out and assayed for radioactivity.

Cell Surface Labeling, Immunoprecipitation, and NaDodSO$_4$/Polyacrylamide Gel Electrophoresis. T-cell hybrids were labeled with Na^{125}I by lactoperoxidase-catalyzed cell surface iodination (17). Cells were lysed at a concentration of 10^8 cells per ml in lysis buffer containing 0.5% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS; Calbiochem) (18), 0.05 M Tris, 0.3 M NaCl, 0.005 M EDTA buffer (pH 7.6), 0.02% sodium azide, 1 mM phenylmethylsulfonyl fluoride, and 10 μg of aprotinin per ml (19). The detergent extracts were precleared by serial incubation with 20 μl of the IgG2a monoclonal antibody 14-4-4S in ascites form (kindly provided by D. Sachs, National Cancer Institute) and 100 μl of protein A-Sepharose, followed by 20 μl of SP2/0-induced ascites and protein A-Sepharose, followed by protein A-Sepharose alone. At the same time, separate protein A-Sepharose beads (10 μl) were incubated with 200 μl of the culture supernatant of monoclonal antibody A2B4-2 on ice for 2 hr and washed three times with phosphate-buffered saline. Aliquots of the precleared cell lysate equivalent to 12×10^6 cells were then incubated with the protein A-Sepharose–monoclonal antibody complex for 30 min at 4°C. The immunoprecipitates on the beads were washed once with the lysis buffer containing in addition 0.2% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, 0.2% deoxycholate, and 0.1% NaDodSO$_4$, twice through this wash buffer containing 30% glycerol, and once more with buffer without glycerol. The samples were boiled for 5 min in sample buffer containing 1% NaDodSO$_4$, 0.05 M Tris (pH 6.8), and 20% glycerol with or without 1% 2-mercaptoethanol and then run on NaDodSO$_4$/polyacrylamide gel electrophoresis with 12.5% polyacrylamide. Radiolabeled protein markers (Bethesda Research Laboratories) were run in parallel with the immunoprecipitates. Autoradiography was performed with a DuPont Cronex Lightning Plus intensifying screen at −70°C for 1 wk.

RESULTS

Production of Monoclonal Antibodies. A method for reproducibly generating alloantisera against IL 2-producing antigen-specific T-cell hybrids has been reported (9). T-cell hybrids were emulsified in adjuvant and injected intraperitoneally into selected F_1 recipient animals. The resulting malignant ascites contained antibodies that inhibited or, in one case, stimulated IL 2 release only from the immunizing clone. The antibodies could be purified by conventional means and could be specifically adsorbed by the immunizing clone.

Multiple initial attempts to obtain a hybridoma cell producing a monoclonal antibody with similar properties failed. The use of the *in vivo* enrichment techniques of Fox *et al.* (13), when modified as described in detail in *Materials and Methods*, resulted in the generation of two monoclonal antibodies, which, on initial screening, completely inhibited the IL 2 release from the immunizing clone 2B4. These hybrids were cloned three times by limiting dilution. The two monoclonal antibodies, A2B4-1 and A2B4-2, were determined to be IgG1 and IgG2a, respectively, by radioimmunoassay with isotype-specific reagents. Cross-competition studies with purified iodinated antibodies demonstrated that A2B4-1 and A2B4-2 were directed at closely spaced or identical epitopes on the cell surface target molecule (data not shown).

Initial Characterization. Three pigeon cytochrome *c*-specific hybrids, 2B4, 2C2, and 2H10, were cultured with B10.A-irradiated spleen cells as antigen-presenting cells and with 2.4 μM pigeon cytochrome *c* carboxyl-terminal peptide (Fig. 1). Culture supernatant containing the A2B4-2 monoclonal antibody was titrated into culture. The monoclonal antibody inhibited the IL 2 release only from 2B4, the cell used as an immunogen in the fusion experiment. There was no inhibition of the other two pigeon cytochrome *c*-specific hybrids even though the fine specificity of one, 2C2, appeared to be similar to that of 2B4. Subsequent studies with antibody purified by affinity chromatography on protein A-Sepharose demonstrated that 50% inhibition of IL 2 in this assay can be achieved with 10 ng of antibody per ml. There is no difference in the specific inhibitory activity between the two monoclonal antibodies A2B4-1 and A2B4-2.

The binding of the monoclonal antibodies to the cell was analyzed with the FACS (Fig. 2). The T-cell hybrids 2B4 and 2H10 were incubated with culture supernatant containing monoclonal antibody or with control supernatant. After appropriate washing, the cells were incubated with fluorescein-labeled Fab fragments from affinity-purified goat anti-mouse immunoglobulin. The monoclonal antibody was shown to bind only to 2B4, the immunizing clone, and not to 2H10 cells (Fig. 2) or to 2C2 cells in other experiments (data not shown). Again, A2B4-1 had similar binding properties when assayed on the FACS. Thus, binding to and inhibition of the IL 2 release from the 2B4 clone was highly specific.

These data are consistent with the binding of the monoclonal antibody to a private epitope on a 2B4 cell surface structure involved in the stimulation of the cell and subsequent release

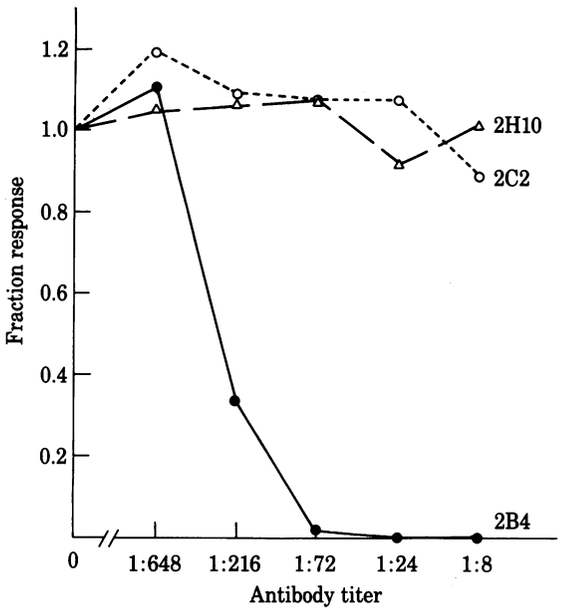

FIG. 1. Effect of the monoclonal antibody A2B4-2 on T-cell antigen-induced IL 2 release. In the absence of antibody, the supernatant from 1×10^5 2B4 cells cultured with 5×10^5 irradiated B10.A spleen cells and 2.4 μM pigeon cytochrome *c* fragment 81-104, when transferred onto HT-2 cells, generated 184,612 cpm of HT-2 proliferation. Supernatants from similar cultures of 2C2 cells generated 184,962 cpm, and supernatants from 2H10 cells generated 192,845 cpm. These values were normalized to 1.0. In the presence of increasing amounts of monoclonal antibody, the IL 2 release from the 2B4 hybrid (●) was inhibited, but not the IL 2 release from the 2C2 (○) or 2H10 (△) hybrids.

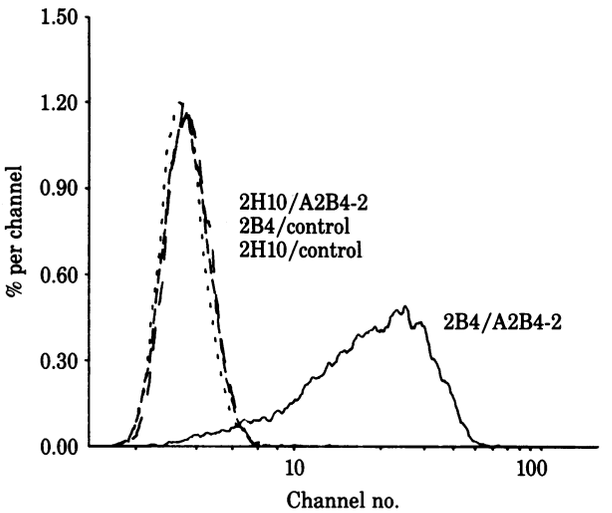

FIG. 2. FACS profile of the A2B4-2 monoclonal antibody. 2B4 and 2H10 cells were incubated with a 1:25 dilution of culture supernatant containing A2B4-2 in 3% fetal calf serum in Hanks' balanced salt solution with 0.02% sodium azide. After washing, the cells were stained with fluorescein isothiocyanate-conjugated Fab fragments from goat anti-mouse immunoglobulin and analyzed on the FACS. A2B4-2 binds to 2B4 cells specifically.

of the lymphokine IL 2. To demonstrate that the monoclonal antibody does not inhibit release of IL 2 from the clone when the cell is activated by means other than specific antigen, A2B4-1 in the form of a high-titer ascites was added into culture with 2B4 cells and the stimulating lectin concanavalin A at 20 μg/ml (Table 1). There was no inhibition of IL 2 release from these lectin-activated cells in the presence of high concentrations of antibody. However, the release of IL 2 by the same cells, when activated by antigen in the presence of syngeneic antigen-presenting cells, was markedly inhibited by the antibody. Therefore the monoclonal antibody, when bound to its cell surface antigen, does not give a nonspecific inhibitory signal. Only the stimulatory effect of specific antigen is inhibited by the antibody. This pattern of binding and inhibition is consistent with the hypothesis that the antigen receptor is the target for the monoclonal antibody. However, the possibility that the antibody reacts with a clone-specific, mutated variant (somatic mutation) of another molecule in the antigen-specific activation pathway has not been eliminated.

Quantitation of Cell Surface Antigen. The monoclonal antibody was purified from ascites by conventional ion-exchange and affinity chromatography and iodinated by the Bolton–Hunter method. Increasing amounts of this purified radiolabeled antibody were added in a cell surface binding assay onto both 2B4 and 2H10 cells (Fig. 3). With the addition of increasing amounts of antibody, more antibody was bound to the 2B4 hybrids until a plateau level, 9,597 cpm, was attained at 10^5 cpm added. Approximately 1% of the added antibody was nonspecifically bound to 2H10 cells at all doses (e.g., 911 cpm at 10^5 cpm added). If one assumes normal recovery of the antibody after iodination and gel filtration and assumes that the trichloroacetic acid-precipitable iodinated protein represented nondenatured monoclonal antibody, one obtains a value of 2.8×10^6 cpm/μg as the specific activity of the preparation. If one assumes also that binding to the cell surface target was univalent and that binding to the 2H10 cell was nonspecific, an estimate can be made that there are ≈25,000 binding sites on the 2B4 hybrid. In parallel experiments it was shown that the binding of the iodinated antibody could be completely and specifically inhibited by excess cold antibody.

Immunoprecipitation and NaDodSO$_4$/Polyacrylamide Gel Electrophoresis Analysis of the Molecule Bound by A2B4-2. The structure of the molecule bound by A2B4-2 was further analyzed by standard protein analytical techniques. The cell

Table 1. The effect of the monoclonal antibody A2B4-1 on antigen and concanavalin A-induced activation

Activation by	Proliferation of HT-2 cells, cpm*					
	0	1:10^6	1:10^5	1:10^4	1:10^3	1:10^2
Antigen,† 2.4 μM pigeon cytochrome *c* fragment 81-104	89,125	37,889	8,862	335	217	205
Concanavalin A,‡ 20 μg/ml	257,238	248,365	238,418	279,028	247,390	266,203

* After culture for 24 hr, 50 μl of supernatant was transferred onto 4,000 HT-2 cells. After an additional 24 hr, the HT-2 cells were pulsed for 18 hr with 1 μCi of [^{3}H]thymidine.

† 2B4 cells (1×10^5) were cultured with 5×10^5 B10.A spleen cells irradiated with 3,300 R, the indicated dilutions of monoclonal antibody, and 2.4 μM pigeon cytochrome *c* carboxyl-terminal fragment 81-104.

‡ 2B4 cells (1×10^5) were cultured with the indicated dilutions of antibody and 20 μg of concanavalin A per ml.

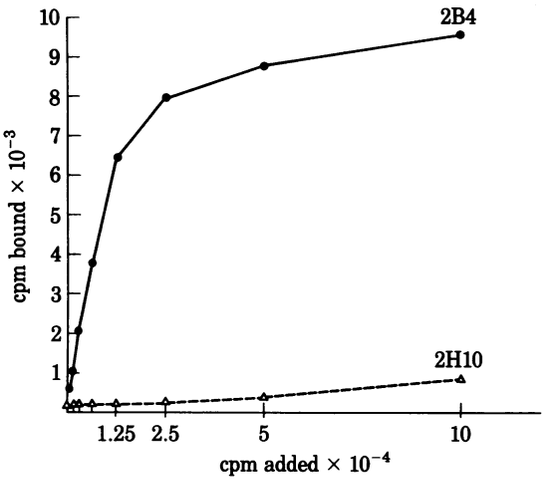

FIG. 3. Binding of ^{125}I-labeled A2B4-2 monoclonal antibody to T-cell hybrids. Monoclonal antibody was purified from ascites by ion-exchange and affinity chromatography and iodinated by the Bolton–Hunter method. Increasing amounts of labeled antibody were titered onto 5 × 10^5 2B4 or 2H10 cells in microtiter wells. After incubation at 4°C and washing, the wells were cut out and radioactivity was measured.

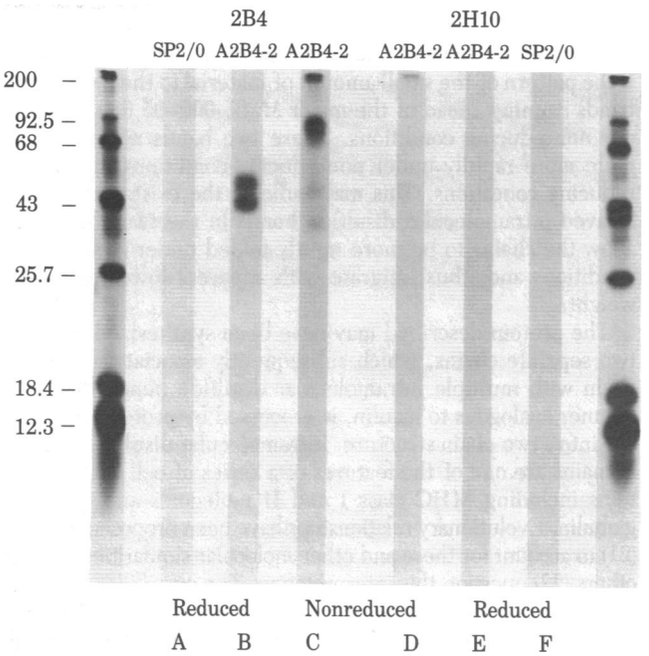

FIG. 4. NaDodSO$_4$/polyacrylamide gel electrophoresis analysis of a cell surface-labeled molecule immunoprecipitated from T-cell hybrids. 2B4 and 2H10 cells were iodinated with Na^{125}I by the lactoperoxidase method. Precleared lysates were immunoprecipitated with monoclonal antibody and protein A-Sepharose, eluted, and run on a 12.5% polyacrylamide gel. Lanes: A, B, E, and F, reducing conditions; C and D, nonreducing conditions; A–C, 3 × 10^6 cell equivalents of 2B4 cells; D–F, equivalent amounts from 2H10 cells. A2B4-2 monoclonal antibody precipitated bands from 2B4 cells specifically (lanes B and C) but not from 2H10 cells (lanes D and E). SP2/0 control supernatants did not immunoprecipitate material from 2B4 cells (lane A) or 2H10 cells (lane F). Approximate molecular weights (shown × 10^{-3}) were derived from the labeled markers.

surface of 2B4 was selectively labeled by Na^{125}I by using the lactoperoxidase-catalyzed reaction. Immunoprecipitation with the monoclonal antibody followed by NaDodSO$_4$/polyacrylamide gel electrophoresis under reducing conditions demonstrated two bands of M_rs 48,000–50,000 and 42,000–44,000 from 2B4 cells (Fig. 4, lane B), but nothing from 2H10 cells (Fig. 4, lane E). An analysis of the apparent molecular weights of these chains from a series of similar experiments indicated a range of M_r 45,000–50,000 for the heavy chain and M_r 40,000–44,000 for the light chain. Material from the same immunoprecipitation that was not reduced migrated on the gel in a band between M_rs 85,000 and 95,000 (lane C), the sum of the weights of the reduced chains. This band also was observed when iodoacetamide (60 mM) was present during membrane solubilization. Note also that a small amount of material in this lane migrated faster than the major M_r 85,000–95,000 component. This more rapidly migrating material, in the form of two separate bands, also migrated ahead of the totally reduced chains in lane B. This result suggested the possibility that these two chains, presumably derived by failure of assembly or by reduction of the interchain disulfide bond(s) during the isolation procedure, contained intramolecular disulfide bonds. These bonds, when not cleaved by stringent reducing conditions, would allow the chains to be more tightly folded and thus to migrate ahead of the totally reduced and opened chains. Similar data and conclusions have been reached concerning intrachain disulfide bonds in immunoglobulin and class I and class II MHC-encoded molecules (20). Overall, our data are thus compatible with the interpretation that the molecule bound by the antibody and, for reasons outlined above, thought to be the antigen recognition structure on the cell is a disulfide-bonded heterodimer consisting of two chains, each containing intramolecular disulfide bonds.

DISCUSSION

Development of monoclonal antibodies to the antigen-specific receptor on T cells has been a difficult problem. The use of cloned T-cell hybrids, available in large number, has facilitated this work. The enrichment of B-cell precursors making antibodies of interest and the use of a sensitive assay for specific inhibition of IL 2 release, a functional property of these T-cell hybrids, also were critical in obtaining these monoclonal antibodies.

The antibodies have the property of binding only to the pigeon cytochrome *c*-specific clone against which they were made. This specific binding correlates with the specific inhibition of antigen-induced IL 2 release. The fact that the target molecule is involved in antigen, but not lectin, activation of the cell (Table 1), strongly suggests that it is the receptor for antigen on this T-cell clone. However, the possibility that the antibody is recognizing a clone-specific alteration or mutation in some other molecule in the antigen-activation pathway remains a possibility.

It may be surprising that these antibodies distinguish between two clones, 2B4 and 2C2, that respond similarly to a small peptide with limited antigenicity. Indeed these monoclonals have not shown appreciable binding to cells from two long-term pigeon cytochrome *c*-specific proliferating lines. These data may suggest that the antibody is recognizing a private idiotope on the "variable" region of the receptor that possibly is not involved in the antigen combining site. Perhaps these idiotopes have arisen from a single germ line-encoded sequence by some physiologic process of somatic mutation. Thus, the fact that the monoclonal antibodies, as well as the previously described anti-2B4 and anti-2C2 antisera (9), appear to recognize private determinants reinforces the concept that they bind a molecule, such as the T-cell receptor that is inherently pleiomorphic.

The material immunoprecipitated by the antibody has certain features of interest. It appears to be a heterodimer with two chains of apparent M_rs 45,000–50,000 and 40,000–44,000

as demonstrated by the comparison of the migration pattern under nonreducing and reducing conditions. Of particular note is the pattern of the small amount of material in the form of two bands running ahead of the major M_r 85,000–95,000 band under nonreducing conditions. These two bands appear to migrate more rapidly under nonreducing conditions than under reducing conditions. This may indicate the existence of noncleaved intramolecular disulfide bonds in the two chains that allow the chains to be more tightly folded under nonreducing conditions and, thus, migrate with apparent lower molecular weight.

The protein described may have been synthesized either as two separate chains, which subsequently associate, or as one chain with multiple intramolecular disulfide bonds that, in a manner analogous to insulin, is processed by proteolytic cleavage into a two-chain structure. Intramolecular disulfide-bonded domains are one of the features of a series of cell surface proteins including MHC class I and II molecules and immunoglobulin. Evolutionary relationships have been proposed by some (21) to account for these and other molecular similarities, though others (22) question this interpretation. The possibility that both intramolecular and, the more rarely observed, intermolecular disulfide bonds characterize the molecule described here allows for further speculation that the T-cell receptor also may be evolutionarily related to this group of cell surface proteins.

Recently there have been several reports characterizing monoclonal antibodies against T-cell clones. Meuer *et al.* (4) have described two monoclonal antibodies that bound an alloreactive human cytotoxic clone. Their antibodies were clonotypic and affected the function of the clone only when it was activated by specific antigen. Lancki *et al.* (23) described an antibody that has similar properties on a murine alloreactive clone. They demonstrated that the monoclonal antibody could bind to a subpopulation of cells from a serially enriched bulk population with the same specificity as the clone. Haskins *et al.* (5) described an antibody to a murine T-cell hybrid reactive with ovalbumin. Again this antibody specifically reacted only with the immunogen and not with clones of similar specificity or even subclones of the immunizing clone that had lost the capacity to release IL 2 in the presence of antigen. This antibody also inhibited the binding of the T cell to antigen-pulsed presenting cells. The first reported example of a monoclonal antibody specifically binding a cloned T cell was from Allison *et al.* (6) who used a T-cell tumor as an immunogen. The antigen specificity and functional properties of this cell could not be determined. The data from all these laboratories were remarkably consistent in that there was specific binding of the antibodies to the immunizing T-cell clone. In cases where a T-cell function could be analyzed, the antibodies also were specific for the immunogen. Also where structure of the cell surface material could be analyzed, similar data have been reported. Consistently, heterodimers linked by disulfide bonds have been observed on $NaDodSO_4$/polyacrylamide gel electrophoresis. If there is any controversy, it is in the degree of size heterogeneity in the individual chains. In this regard, Allison *et al.* (6) and Haskins *et al.* (5) described two bands of nearly equal molecular weight, whereas the molecule identified in this report is most similar to that described by Meuer *et al.* (4), who defined chains of M_rs 49,000 and 43,000. These early reports, including our own, serve to reinforce each other and represent the exciting beginning of studies that will eventually characterize structures that appear to be antigen-specific receptors on MHC-restricted T cells.

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